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ASSESSMENT AND QUANTIFICATION OF THE QUALITY AND DYNAMICS OF ORGANIC MATTER IN AGRICULTURAL SOILS VIA PHYSICAL FRACTIONATION AND STABLE ISOTOPE TECHNIQUES

INSCHATTING EN BEGROTING VAN DE KWALITEIT EN DYNAMIEK VAN ORGANISCH MATERIAAL IN LANDBOUWGRONDEN DOOR MIDDEL VAN FYSISCHE FRACTIONATIE EN STABIELE ISOTOPENTECHNIEKEN

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GENERAL INTRODUCTION

Scope and research hypotheses

Soil organic matter (SOM) is a major factor in soil quality and fertility, as it sustains nutrient storage and supply, soil structure and biological activity. SOM, and more specifically soil organic carbon (SOC), is also receiving increased attention as a potential sink for atmospheric CO_2 (carbon sequestration). Maintenance and improvement in SOM content is thus generally accepted as being a major objective for any sustainable agroecosystem. Assessment of SOM quality, however, is complex, as it comprises an enormous array of compounds at various stages of decomposition, ranging from very active to recalcitrant. Physical fractionation of SOM and stable isotope techniques (e.g. ^{13}C natural abundance analysis and ^{15}N isotope dilution) are now more and more used in the research on quality and turnover of SOM.

Recently, physical fractionation techniques, like size and density fractionation of SOM, have yielded biologically meaningful SOM fractions or pools, which tend to differ in degradability and turnover. Moreover, it is generally observed that isotopic fractionation during the decomposition process may result in significant shifts in the ^{13}C isotopic signature ($^{13}C/^{12}C$ ratio) of SOM. A first research hypothesis was formulated as follows: *'Shifts in the ^{13}C isotopic signature can be used as an indicator of SOM quality, in terms of SOC degradability and turnover'.* In order to test this hypothesis, we studied shifts in the ^{13}C isotopic signature of SOM with increasing depth in soil profiles under permanent grassland (Chapter 2) and among different size and density fractions of SOM in the surface layer of cultivated and grassland soils (Chapter 3). It was evaluated to what extent these shifts in the ^{13}C isotopic signature were related to, or could serve as an indicator of the degradability and turnover of SOC at different depths in soil profiles (Chapter 2) or in different size and density fractions of SOM (Chapter 3).

The balance between potential gross N mineralization and gross N immobilization in soils is at an undefined equilibrium. This balance, and the potential N dynamics in general, tends to be affected by the quantity of SOM, the quality of SOM (availability for microbial degradation, C/N ratio) and soil type. It is generally observed that SOM in the clay- and silt-sized fraction (<50 µm) is less available for microbial degradation than SOM in the sand-sized fraction (>50 µm). In this way, the potential N availability in soils may be influenced by the distribution of SOM among different size and density fractions and by the clay and silt content (soil texture). Thus, a second research hypothesis was formulated as follows: *'Next to the total SOM content, the distribution of SOM among different size and density fractions and the soil texture are factors which largely affect the potential N dynamics in grassland soils'.* In order to verify this second hypothesis, we investigated (1) the influence of the total organic C and N content and the distribution of organic C and N among size and density fractions (Chapter 4), and (2) the influence of soil type (Chapter 5) on the potential N dynamics in permanent grassland soils.

Outline of the thesis

A first objective of this thesis was to investigate the quality of SOM, in terms of degradability and turnover, in cultivated and grassland soils by means of physical fractionation of the SOM and variations in its ^{13}C isotopic signature. A second objective of this thesis was to study the N dynamics in permanent grassland soils, as affected by the quantity and quality of SOM and soil type.

Chapter 1 is an introductory chapter, which focuses on (1) the role of organic matter in agricultural soils, (2) factors affecting organic matter content and organic matter turnover in agricultural soils, (3) assessment of the quality and turnover of SOM, and (4) the major N transformations that occur in agricultural soils.

In chapter 2 we investigated to what extent the variation of the ^{13}C isotopic signature in soil profiles under permanent grassland (C_3 vegetation) could be used as an indicator of the quality of SOM (in terms of degradability) at different depths in the profile.

In chapter 3 we compared the quantity and quality of SOM (in terms of turnover) in the surface layer of cultivated (C_4 vegetation) and non-cultivated soils (C_3 vegetation). This was achieved through separation of the SOM into different fractions (size and density fractions, microbial biomass, water soluble organic C), in combination with the analysis of their ^{13}C isotopic signature.

The accumulation of SOM upon conversion of arable land to permanent grassland, and the influence of quantity and quality of SOM on the gross N transformation rates in permanent grasslands of different age were studied in chapter 4.

In chapter 5 we quantified the gross N transformation rates and the potential N retention upon mineral fertilizer addition to permanent grassland soils of varying texture and SOM content.

Finally, the general conclusions and research perspectives of this thesis are formulated in Conclusions and perspectives, and a summary of the text is given in English and Dutch (Summary and Samenvatting).

CHAPTER 1

Introductory chapter

Content

1. Introductory chapter

1.1. The role of organic matter in agricultural soils

Soil organic matter (SOM) is a key component in the quality and fertility of agricultural soils, as it affects many of its biological, physical and chemical properties (Doran and Parkin, 1994). SOM is a primary source of, and a temporary sink for, plant nutrients and acts as an energy and nutrient source for the soil organisms. The essential plant nutrients (especially nitrogen, sulphur and phosphorous) are released through mineralization during the decomposition of the SOM, which is accompanied by the respiration of carbon as carbon dioxide (CO_2) (Brady, 1984). SOM also improves the aggregation of soil particles and thus contributes significantly to the formation and stabilization of the soil structure (Tisdall and Oades, 1982). This results in a better aeration and infiltration of water, and increases the water holding capacity of the soil (Gregorich et al., 1994). Furthermore, SOM increases the cation exchange capacity (CEC) of soils, which enhances the storage capacity of nutrients (Brady, 1984). Maintenance and improvement in SOM content is thus generally accepted as being a major objective for any sustainable agroecosystem (Haynes, 1999).

The SOM in agricultural soils, and more specifically the soil organic C (SOC), is also receiving increased attention as a potential sink for atmospheric CO_2 (C sequestration). The atmospheric concentration of CO_2, which is the most important anthropogenic greenhouse gas, has increased by about 32% (from approximately 280 ppm to approximately 370 ppm) since the onset of the industrial revolution (circa 1850) to the present, and is currently increasing at a rate of 0.5% yr^{-1} (Lal, 2001). Though the largest anthropogenic contribution to the increase in the atmospheric CO_2 concentration comes from fossil fuel combustion, a substantial release of CO_2 originates from terrestrial

vegetation and soils, caused by land use changes such as deforestation and cultivation (Schlesinger, 1997). Due to the concern about the increase in the atmospheric concentration of greenhouse gases (e.g. CO_2, CH_4 and N_2O) and their potential effects on global climate change (IPCC, 1995), the UN Framework Convention on Climate Change has imposed the industrialized countries to reduce their net emissions of greenhouse gases by 5% of the 1990 level by the year 2010 (Lal, 2001). A decrease in the atmospheric CO_2 concentration could thus be achieved by reducing the emissions, but also by sequestering and storing C in sinks. Carbon sequestration in terrestrial ecosystems is accomplished when C is stored in living plants through photosynthesis and then relocated to the soil and transformed into SOC (Follett et al., 2001). One possibility is to increase the C stored in agricultural soils, which can be accomplished through the adoption of reduced or no-till, use of cover crops, improved nutrition and yield enhancement, elimination of bare fallow, use of forages in crop rotations, use of improved varieties, and use of organic amendments (Bruce et al., 1999). When cultivated soils are converted to permanent grassland, SOM contents generally increase, which also involves increased C sequestration and in addition improves soil fertility (Robles and Burke, 1998).

1.2. Factors affecting organic matter content and organic matter turnover in agricultural soils

SOM originates from the organic residues (derived from plants, animals or micro-organisms) which enter the soil and undergo a gradual chemical and biological decomposition. The SOM pool thus encompasses plant, animal and microbial residues at various stages of decomposition and a diversity of heterogeneous, humified or nonhumified, organic compounds which are intimately associated with inorganic soil components (Christensen, 1992). The nonhumified organic compounds have been released

by decomposition of plant, animal and microbial tissue in their original or in a slightly modified form (mainly carbohydrates, amino acids, proteins, lipids, nucleic acids, lignins and a variety of organic acids). The humified organic compounds are products that have been derived from these nonhumified compounds (humification), and consist of complex substances (humic and fulvic acids), which are relatively resistant to microbial attack. The nonhumic and humic material are collectively called soil humus (Stevenson, 1967; Tan, 1994).

The SOM content in agricultural soils is mainly determined by the primary plant production, or more specifically the input rates and the quality of the plant residues, and the rate of SOM decomposition (Jastrow and Miller, 1998). Primary plant production is largely dependent on climatic factors (temperature and rainfall), vegetation type, soil type and management practices (Brady, 1984). The rate of SOM decomposition is strongly determined by the stability of the SOM against microbial degradation. The stability of the SOM against microbial degradation depends on (1) the biochemical stabilization of the SOM, (2) the degree of its association with the mineral components (mainly silt and clay particles), and (3) the physical protection of SOM within aggregate structures (Christensen, 1996). Biochemical stabilization of SOM is understood as the stabilization due to its own chemical composition (e.g. recalcitrant compounds such as lignin and polyphenols) and through chemical complexing processes (e.g. condensation reactions) (Heal et al., 1997; Six et al., 2002). It is generally observed that the association of SOM with silt and clay particles (by means of chemical or physicochemical binding), creates a physical protection against microbial decomposition (Christensen, 1996) and numerous studies have reported a positive correlation between the clay or silt plus clay content and the preservation of SOM in soils (Sorensen, 1971; Merckx et al., 1985; Christensen, 1992; Hassink, 1997). Soil aggregates physically protect SOM by forming physical barriers

between micro-organisms plus microbial enzymes and their substrates, by controlling food web interactions and by influencing microbial turnover (Elliott and Coleman, 1988).

Physical disturbance of the soil (e.g. tillage) is an important controlling factor in the process of aggregate formation and aggregate turnover in soils (Six et al., 2002), and consequently the SOM dynamics and SOM content in agricultural soils are also strongly dependent on the management practices (e.g. the frequency and intensity of tillage). It is generally accepted that no-tillage or cover crop systems, for example, have beneficial effects on soil fertility by decreasing erosion, increasing aggregation and potentially increasing SOM contents (Six et al., 2002). Crop sequence, rotation and management practice can affect the SOM content by influencing both the quantity and quality of crop residues which are returned to the soil and the rate of decomposition of added residues and native SOM (Gregorich et al., 1994; Haynes and Beare, 1996). Replacement of natural forests by agroecosystems generally increases the flux of terrestrial C to the atmosphere due to enhanced SOM decomposition, reduces levels of SOM, and thereby decreases soil fertility (Solomon et al., 2002). On the other hand, when arable land, which has been under long-term cultivation, is converted to permanent grassland, for example, the SOM content gradually tends to increase due to greater organic matter inputs, combined with a slower rate of SOM decomposition due to the absence of annual cultivation (Whitehead, 1995a; Haynes and Beare, 1996).

As SOM decomposition is mediated through microbial activity, which is influenced by temperature and soil moisture content, the rate of SOM decomposition is also strongly dependent on climatic factors (Zech et al., 1997). Therefore, it is generally observed that SOM in tropical soils has a faster turnover than in temperate soils, due to the enhanced decomposition under the higher moisture and temperature regimes of the tropics (Trumbore, 1993).

1.3. Assessment of the quality and turnover of soil organic matter

The quality of SOM could be broadly defined as its capability to sustain the soil quality in general, in terms of sustaining soil structure, nutrient storage and biological activity (Gregorich et al., 1994). Characterization of SOM quality is very complex as SOM comprises an enormous array of compounds, ranging from recent plant materials through a continuum of metabolic products of microorganisms, to components of stable humus (Zech et al., 1997). SOM can thus be considered to be composed of a series of fractions, ranging from very active to passive (Schimel et al., 1985).

Total C content, N content and the C/N ratio, in combination with the content of various classes of organic compounds, like lignin and polyphenols, can be considered as the classical chemical indicators of substrate quality (Haynes, 1986a). The microbial biomass content, carbohydrate content, the light fraction of SOM, water soluble C content and the mineralizable C and N content are assumed to represent or reflect the labile, active fractions of the SOM pool and are also often used as indicators of the quality of SOM (Gregorich et al., 1994). Microbial biomass is a key variable of SOM quality, functioning both as an agent for the transformation and cycling of SOM and plant nutrients within the soil, and as a sink (immobilization) or source (mineralization) of labile nutrients (Sparling et al., 1990). The potential C and N mineralization in soils, which are determined by means of incubation experiments, reflect the readily decomposable fraction of SOM and the capacity of the SOM to supply plant-available N, respectively (Gregorich et al., 1994).

In mathematical models, describing SOM dynamics, the continuum of SOM fractions in soil is conceptualized as kinetically defined pools with different turnover rates (Parnas, 1975; Jenkinson and Raynor, 1977; van Veen and Paul, 1981). However, a major problem related to predicting SOM dynamics by means of mathematical models is that these different pools usually can not be determined directly by chemical or physical

fractionation procedures (Paustian et al., 1992). Successful development of techniques for direct measurement of pool sizes would thus represent a major step forward towards appropriate verification of SOM models (Bonde et al., 1992). Historically, most scientists studying the nature of SOM have utilized chemical extractants to fractionate SOM (Stevenson and Elliott, 1989). However, chemical extracts of the soil are not clearly related to the dynamics of SOM because they extract SOM that may be physically protected from microorganisms and not readily available for decomposition (Cambardella and Elliott, 1994). Recently, biologically meaningful SOM fractions or pools have been obtained by methods based on physical fractionation of soil (according to particle size or density) without chemical treatments, which combined with biological and chemical analysis allows further insight into the functionality of the separated pools (Tiessen and Stewart, 1983; Bonde et al., 1992; Christensen, 1992; Solomon et al., 2002). Moreover, the use of the ^{13}C natural abundance technique coupled with particle size fractionation have further advanced process oriented SOM studies, since these methods are well suited to study soil organic carbon (SOC) dynamics over a time scale ranging from a few to several hundred years, and are relevant for understanding the consequences of natural and anthropogenic vegetation changes (Balesdent et al., 1987; Boutton, 1996; Shang and Tiessen, 2000).

1.3.1. Size and density fractionation of soil organic matter

The concept behind physical fractionation of soil emphasizes that the availability of SOM to decomposing organisms depends not only on its intrinsic biochemical nature, but also on the nature of its association with the soil mineral fraction (formation of organomineral complexes). Physical fractionation techniques, according to size or density of particles, are generally less destructive and the results obtained from physically

separated soil fractions may relate more to the structure and function of the SOM in situ (Christensen, 1992).

Christensen (1992) defined three levels of structural and functional complexity in the soil, which relate to the following experimentally identifiable SOM pools: the uncomplexed SOM, the primary organomineral complexes and the secondary organomineral complexes. The uncomplexed SOM consists mainly of particulate, partly decomposed plant or animal residues, and is a transitory pool between litter and mineral-associated SOM. Primary organomineral complexes can be divided into clay-sized (< 2 µm), silt-sized (2-20 µm or 2-50 µm according to the International Soil Science Society (ISSS) or United States Department of Agriculture (USDA) classification, respectively) and sand-sized (20-2000 µm or 50-2000 µm according to the ISSS or USDA classification, respectively) complexes of SOM with mineral particles of the corresponding size. Individual primary organomineral particles and particles of uncomplexed SOM can occur as discrete structural units in the soil, but in most soils most of the primary particles are incorporated into differently sized secondary organomineral complexes, or aggregates (Christensen, 1992). These secondary organomineral complexes can be further divided into micro-aggregates (< 250 µm) and macro-aggregates (> 250 µm). Micro-aggregates are composed from primary complexes and occluded SOM, and their stabilization involves both persistent and transient binding agents. Macro-aggregates are made up of micro-aggregates, primary complexes and uncomplexed SOM particles, held together by transient and temporary binding agents (Tisdall and Oades, 1982).

Size fractionation of soils is generally achieved through a preliminary dispersion of the soil, in order to break down the secondary organomineral complexes, followed by a combination of wet sieving and sedimentation to separate the primary organomineral complexes. A general observation on the composition of SOM in particle size fractions is that the C/N ratio tends to decrease from the coarser to the finer particle size fractions,

which indicates an increasing degree of humification (Tiessen and Stewart, 1983; Catroux and Schnitzer, 1987). Moreover, SOM in the sand-sized fraction is often more labile than SOM in the clay- and silt-sized fractions (Tiessen and Stewart, 1983), and considerable published evidence indicates that one of the principal factors responsible for physical protection of SOM is its tendency to associate with clay and silt particles (Tate and Theng, 1980). Density fractionation of SOM is based on the observation that during humification parts of SOM become more associated with the mineral fraction and thus occur in organomineral complexes of higher density (Barrios et al., 1996). By means of density separation, SOM is generally separated into a light fraction, and one or more heavy fractions. The so-called light fraction ("free" or uncomplexed SOM) is considered to be decomposing plant or animal residues with a relatively high C/N ratio, a rapid turnover rate, and a specific density considerably lower than that of soil minerals. The heavy fractions include the organomineral complexed SOM, which is assumed to be comparatively more processed material, with a lower C/N ratio, a slower turnover rate, and a higher specific density due to its intimate association with soil minerals (Greenland, 1965). The combination of size fractionation and density fractionation of SOM may thus enable us to identify both labile SOM fractions (sand-sized or light SOM), which may respond relatively faster to management changes in agroecosystems than the total SOM content, and more stable fractions (clay- and silt-sized SOM), which are more related to long-term SOM dynamics (Janzen et al., 1992; Barrios et al., 1996).

1.3.2. The ^{13}C isotopic signature of soil organic matter

C has two naturally occurring stable isotopes, ^{12}C and ^{13}C. Approximately 98.89 % of all C in nature is ^{12}C, and 1.11 % of all C is ^{13}C. The ratio of these two stable isotopes ($^{13}C/^{12}C$) in natural materials varies slightly around these average values as a result of isotopic fractionation (discrimination against ^{13}C) during physical, chemical and

biological processes (Boutton, 1996). $^{13}C/^{12}C$ ratios are usually expressed relative to a standard (carbonate from Pee Dee belemnite) as $\delta^{13}C$ values, and expressed in per mil (‰):

$$\delta^{13}C\ (‰) = \left(\frac{{}^{13}C/{}^{12}C_{\text{sample}} - {}^{13}C/{}^{12}C_{\text{standard}}}{{}^{13}C/{}^{12}C_{\text{standard}}} \right) * 1000$$

Atmospheric CO_2, plant material and SOM are depleted in ^{13}C relative to the standard and therefore have negative $\delta^{13}C$ values. The more depleted in ^{13}C a material is, the more negative the $\delta^{13}C$ value will be.

The $^{13}C/^{12}C$ ratio, or ^{13}C isotopic signature, of SOM is mainly determined by the $^{13}C/^{12}C$ ratio of the plant litter from which it is derived. Plants discriminate against $^{13}CO_2$ during photosynthesis and the extent of this discrimination is dependent on their photosynthetic pathway type. As C_3 plants, with the Calvin pathway, discriminate more against $^{13}CO_2$ than C_4 plants, with the Hatch and Slack pathway, C_3 plants have $\delta^{13}C$ values ranging from approximately -32‰ to -22‰, while C_4 plants have $\delta^{13}C$ values ranging from approximately -17‰ to -9‰ (Smith and Epstein, 1971). This difference in $^{13}C/^{12}C$ ratios can be used as a tracer for in situ labelling of newly incorporated SOM when the dominant vegetation type has changed from C_3 to C_4 species or vice-versa (Schwartz et al., 1986; Balesdent et al., 1987; Puget et al., 1995; Ryan et al., 1995).

The $^{13}C/^{12}C$ ratio in SOM remains close to the ratio in the original vegetation, but during decomposition of the plant residues and the SOM, significant changes in the $^{13}C/^{12}C$ ratio may occur due to isotopic fractionation (O'Brien and Stout, 1978; Melillo et al., 1989; Wedin et al., 1995). The magnitude and direction of the change in the $^{13}C/^{12}C$ ratio may vary with time and the prevailing environmental conditions (O'Brien and Stout, 1978). One source of alteration in the $^{13}C/^{12}C$ ratio of SOM is isotopic discrimination

against ^{13}C associated with microbial decomposition of SOM. In their metabolism, decomposing organisms would prefer ^{13}C-depleted molecules for respiration while ^{13}C-enriched molecules tend to be utilised in the production of biomass and the end-products of metabolism under aerobic conditions (Blair et al., 1985; Gleixner et al., 1993). As a result, SOM decomposition may lead to a progressive ^{13}C enrichment in the mixture of residual substrate and microbial products and metabolites (Balesdent and Mariotti, 1996). However, in anaerobic environments, the CH_4 evolved is generally very depleted in ^{13}C relative to the organic substrate, whereas the CO_2 evolved is enriched in ^{13}C (Games et al., 1978). A second possible source of alteration of the $^{13}C/^{12}C$ ratio may be the different decomposition rates of isotopically distinct biochemical components of plant litter (Stout et al., 1981; Melillo et al., 1989; Agren et al., 1996; Boutton, 1996). Due to isotopic fractionation during the biosynthesis of the major plant cell components, pectins, amino acids, hemicellulose and sugars tend to have a larger $^{13}C/^{12}C$ ratio than the bulk plant tissue, whereas cellulose, lignin and lipids usually have a smaller $^{13}C/^{12}C$ ratio (O'Brien and Stout, 1978; Stout et al., 1981; Benner et al., 1987). In particular, lignin is substantially depleted in ^{13}C (2-6 ‰) in relation to bulk plant tissue and decomposes at a significantly lower rate than the other biochemical fractions in the early stages of plant litter decomposition (Minderman, 1968; Benner et al., 1987). As such, ^{13}C enrichment of SOM due to microbial respiration would be more or less balanced by the slower decay of ^{13}C-depleted lignin in the early stages of plant litter decomposition (Balesdent and Mariotti, 1996). At more advanced stages of litter decay, lignin and other residual fractions would decompose at more similar rates (Berg et al., 1984; Nadelhoffer and Fry, 1988; Wedin et al., 1995) and microbial recycling of C would dominate (Balesdent and Mariotti, 1996), thus resulting in a gradual ^{13}C enrichment of the residual litter and the associated SOM pool in well drained, aerobic mineral soils. Thus, as the $^{13}C/^{12}C$ ratio in SOM tends to vary during the decomposition process, the ^{13}C isotopic signature may also be used as an indicator of SOM quality.

1.4. Nitrogen transformations in agricultural soils

Litter, originating from both above- and belowground plant parts, is the major pathway of supply of energy and N in most terrestrial ecosystems (Staaf and Berg, 1981) and decomposition constitutes the means by which N held in the structure of plant tissues is released into the soil for reuse by plants. In most soils, more than 95% of the total N is present in organic compounds, the remainder being in the inorganic form as ammonium (NH_4^+) and nitrate (NO_3^-). As all higher plants, except those depending on symbiotic N fixation, take up almost all their N as NO_3^- and NH_4^+, the transformation of organic N into inorganic N (mineralization) is a key process in the N cycle (Whitehead, 1995b).

1.4.1. Ammonification or mineralization

The biological process by which organic N compounds (proteins, amino acids, amino sugars and ureases) are converted into ammonium (NH_4^+) or ammonia (NH_3) during the decomposition of organic residues, is called ammonification (Tan, 1994). Ammonification, together with the subsequent oxidation of NH_4^+ to NO_3^- through nitrification, is generally called mineralization. Ammonification is carried out by a wide range of heterotrophic micro-organisms, most of which prefer aerobic conditions (Whitehead, 1995b). The process is an enzymatic reaction, and a wide variety of enzymes are involved, each acting on a specific type of N compound (Ladd and Jackson, 1982). The most important factors affecting the ammonification (and decomposition) process include the composition or quality of the decomposing material (particularly N content and C/N ratio, lignin and polyphenol content), climatic factors (particularly moisture content and temperature), soil pH and the availability of nutrients (Haynes, 1986a). A low C/N ratio (high N content) in litter facilitates ammonification by encouraging a high rate of decomposition, and ensuring that the release of mineral N exceeds the microbial

demand for mineral N (immobilization) during the decomposition process. In general, the higher the lignin and polyphenol content of the litter, the lower is the rate of decomposition and mineralization (Staaf and Berg, 1981; Melillo et al., 1989). Soil moisture content can influence the mineralization in three major ways: (1) moisture stress inhibits microbial growth directly, (2) as moisture content increases, aeration decreases and microbial growth is reduced, and (3) cycles of drying and rewetting tend to increase the amount of available substrate (Haynes, 1986a). When the moisture content is adequate for the soil micro-organisms, mineralization increases with increasing temperature over the range 5-30°C, and when the temperature is above about 5°C, mineralization increases with soil moisture content between permanent wilting point and field capacity (Stanford and Epstein, 1974). Since mineralization of soil organic N is carried out by a diverse range of micro-organisms, it is not greatly influenced by soil pH. However, when the conditions become strongly acid (pH < 4.5), there is a decline in the population and activity of bacteria, sometimes accompanied by an increase in soil fungi, resulting in a slower mineralization (Alexander, 1980).

Once the NH_4^+ is released through ammonification, it can be affected by several processes like plant uptake, volatilization (see 1.4.2.), nitrification (see 1.4.3.) and immobilization (through abiotic or biotic processes, see 1.4.4). Generally, the predominant form of mineral N available to plants is NO_3^--N since under most soil conditions NH_4^+-N is rapidly nitrified to NO_3^--N. However, under conditions that are unfavourable for the nitrification process to proceed (e.g. poor aeration and/or soil acidity) NH_4^+ is the major form of N available to plants (Haynes, 1986c).

1.4.2. Ammonia volatilization

Ammonia volatilization is the term commonly used to describe the physico-chemical process by which gaseous NH_3 is released from the soil surface to the atmosphere. A necessary prerequisite for NH_3 volatilization is a supply of free NH_3 (i.e. $NH_{3(aq)}$ and $NH_{3(g)}$) near the soil surface, which usually originates from soil NH_4^+ under alkaline conditions (pH >7) (Haynes and Sherlock, 1986). The quantities of NH_3 lost from a soil are highly variable, depending on such factors as rate, type and method of fertilizer N application, soil pH, and environmental factors including temperature, moisture and wind (Black et al., 1985; Hofman et al., 1995).

1.4.3. Nitrification

Nitrification is classically defined as the process whereby NH_4^+ is oxidized via NO_2^- to NO_3^-. The reactions are generally mediated in soil by two small groups of chemoautotrophic bacteria. These bacteria are obligate aerobes and synthesize all of their cell constituents from CO_2. The driving force for the reduction of CO_2 is the production of ATP during the oxidation of NH_4^+ or NO_2^-. A first group of chemoautotrophic bacteria, which includes *Nitrosomonas* and *Nitrosospira*, oxidizes NH_4^+ to NO_2^- as follows:

$$NH_4^+ + 3/2\ O_2 \rightarrow NO_2^- + 2H^+ + H_2O$$

The *Nitrobacter* bacteria oxidize NO_2^- to NO_3^- as follows:

$$NO_2^- + 1/2\ O_2 \rightarrow NO_3^-$$

NO_2^- is usually oxidized rapidly and accumulates in the soil only in conditions that combine a high concentration of NH_3 with high pH (Whitehead, 1985b). The biochemical pathway of the first stage of nitrification has not been fully elucidated yet (Hutchinson and

Davidson, 1993), but hydroxylamine (NH_2OH) is produced as intermediate, which may decompose chemically (by means of chemodenitrification) into nitric oxide (NO) and nitrous oxide (N_2O) (Stüven et al., 1992). NO and N_2O may then emit from the soil. As nitrification is mediated predominantly by a small group of autotrophic bacteria, it is generally more influenced by external factors (mainly temperature, moisture content and pH) than mineralization. The optimum temperature for nitrification in soils is usually between 25 and 35°C (Kowalenko and Cameron, 1976). Nitrification is inhibited by dry soil conditions, and the optimum soil moisture tension is normally between -0.1 and -1.5 Mpa (Davidson et al, 1990). Nitrification is curtailed when the pH is less than about 6.0, and the lower limit for autotrophic nitrification is generally found to be around pH 4.5. In soils of pH above 7.5, toxic levels of NH_3 may result in the inhibition of the activity of *Nitrobacter* and in the accumulation of NO_2^- (Paul and Clark, 1996).

Although the autotrophic nitrifiers are thought to be by far the most predominant agents of nitrification in the soil environment, several other minor pathways have been suggested (Haynes, 1986b). These include heterotrophic nitrification, carried out by bacteria or fungi (Focht and Verstraete, 1977; Paul and Clark, 1996), oxidation of NH_4^+ to NO_2^- by methylotrophic bacteria (Dalton, 1977), and the chemical oxidation of NO_2^- to NO_3^- (Bartlett, 1981).

Once the NO_3^- has been formed, it may also be affected by several processes, including plant uptake, leaching, immobilization (see 1.4.4.), denitrification (see 1.4.5.) or dissimilatory nitrate reduction to ammonium (see 1.4.6). As in most soil types NO_3^- is not retained on the clay and organic colloids of the soil, which are negatively charged, it is readily susceptible to leaching (in contrast with NH_4^+, which is better retained). Leaching is undesirable as it represents a loss of plant-available N from the soil, and has negative effects on surface and groundwater quality (Whitehead, 1995b).

1.4.4. Ammonium and nitrate immobilization

1.4.4.1 Abiotic immobilization

As clay and SOM have a predominantly negative charge, NH_4^+ may be adsorbed (exchangeable form) on clay and SOM by the process of cation exchange (Thomas, 1977). NH_4^+ may also be held by 2:1 clay minerals (e.g. vermiculites and montmorillonites) in a nonexchangeable "fixed" form (Cameron and Haynes, 1986). SOM can also fix considerable amounts of ammonia (NH_3) in nonexchangeable forms through polymerization reactions with aromatic humic compounds (e.g. phenols and quinones) (Broadbent and Stevenson, 1966).

NO_3^- can be adsorbed on positively charged sites on soil minerals like iron and aluminum oxides and hydroxides, 1:1 clay minerals (e.g. kaolinite) and allophane (mainly in tropical and/or volcanic soils) (Hingston et al., 1972; Cameron and Haynes, 1986). Davidson et al. (2003) recently described a mechanism of abiotic immobilization of NO_3^- via iron oxidation (the ferrous wheel hypothesis) as an important process in forest soils.

1.4.4.2 Biotic immobilization

Immobilization is the reverse of mineralization, i.e. the transformation of inorganic N into organic forms. One major route, next to uptake by plants, is through the assimilation of inorganic N by the soil micro-organisms. During the decomposition of plant residues or SOM in the soil, part of the mineralized C and N is assimilated (immobilized) into microbial tissue, and the rest is respired as CO_2 (which diffuses to the atmosphere) or released as NH_4^+ into the soil, respectively. The balance between mineralization and immobilization of mineral N in soils depends on the microbial demand

for N during the decomposition process, which is largely influenced by the C/N ratio of the material undergoing decomposition (Whitehead, 1995b). When organic residues with a high C/N ratio (>25 to 30) are incorporated into agricultural soils, net N immobilization commonly occurs during the initial stage of decomposition. During decomposition the C/N ratio progressively decreases, and at some critical point, where N becomes no longer limiting to microbial growth and activity, there is a switch from net immobilization to net mineralization. This critical point is generally considered to correspond with a C/N ratio <25 to 30. Nonetheless, in natural and agricultural ecosystems, the N level in litter at which net release of N occurs varies enormously (Haynes, 1986a). This balance between mineralization and immobilization is also affected by other aspects of the litter composition, like the lignin content. For example, highly lignified materials decompose slowly, and therefore tend to immobilize less N than would be expected on the basis of their C/N ratio (Fox et al., 1990). When both NH_4^+ and NO_3^- are present in soil, a preferential uptake of NH_4^+-N in relation to NO_3^--N is generally observed (Jansson et al., 1955; Rice and Tiedje, 1989).

1.4.5. Denitrification

Denitrification is the process by which NO_3^- or NO_2^- is reduced to NO, N_2O and N_2 (gaseous compounds), which then diffuse into the atmosphere. N_2O is a greenhouse gas contributing 5-6% to the enhanced greenhouse effect (Lal, 2001). In the stratosphere, N_2O may be converted to NO and thus contribute to the destruction of stratospheric ozone, which protects the earth from biologically harmful ultraviolet radiation from the sun (Crutzen, 1976). The process is mainly carried out by facultative anaerobic bacteria that have the capacity to reduce N oxides (NO_3^-, NO_2^-, NO, N_2O) when O_2 becomes limiting (Bremner, 1997). Chemodenitrification refers to the same reduction process, but is not carried by micro-organisms. However, chemodenitrification is only likely to be significant

in soils where NO_2^- tends to accumulate (Haynes and Sherlock, 1986). The pathway of denitrification is usually presented as follows:

$$\begin{array}{ccccccccc} & (+5) & & (+3) & & (+2) & (+1) & (0) \\ NO_3^- & \rightarrow & NO_2^- & \rightarrow & NO & \rightarrow & N_2O & \rightarrow & N_2 \end{array}$$

The key factors affecting denitrification in soils are the moisture content of the soil, availability of O_2, the amount of readily available C, pH, temperature and NO_3^- concentration (Bremner, 1997). As the moisture content determines the availability of O_2 in the soil, it is a major factor influencing the denitrification activity. Linn and Doran (1984) and Aulakh et al. (1992) reported critical values for water filled pore space of between 60% and 90% for significant denitrification to occur in differently textured soils. The availability of organic matter is also an important factor moderating both the rate and total extent of denitrifiaction, as the most abundant denitrifiers are heterotrophs, which require organic compounds as electron donors and as a source of cellular material (Haynes and Sherlock, 1986). The overall rate of denitrification has an optimum in the range of 7 to 8 (Van Cleemput and Patrick, 1974) and can be strongly inhibited at soil pH values below 6 (Muller et al., 1980). The critical temperature below which denitrifiaction is strongly reduced ranges from 5 to 10°C (Focht and Verstraete, 1977).

1.4.6. Dissimilatory nitrate reduction to ammonium

Dissimilatory nitrate reduction to ammonium (DNRA) can occur under heavily reduced conditions (Sorensen, 1978). DNRA may thus be in direct competition with denitrification, especially in anoxic water-logged sediments (Kelso et al., 1997). However, Fazzolari et al. (1998) demonstrated that DNRA activity may be less sensitive than denitrification to an inhibitory effect by O_2 and therefore may also occur in aerobic soils.

This process is mainly carried out by obligate and facultative anaerobic bacteria with a fermentative metabolism (Koike and Sorensen, 1988).

CHAPTER 2

Relationship between soil organic C degradability and the evolution of the $\delta^{13}C$ signature in profiles under permanent grassland

This chapter is compiled from:

Accoe, F., Boeckx, P., Van Cleemput, O., Hofman, G., Zhang, Y., hua Li, R. and Guanxiong, C., 2002. Evolution of the $\delta^{13}C$ signature related to total carbon contents and carbon decomposition rate constants in a soil profile under grassland. Rapid Communications in Mass Spectrometry 16, 2184-2189.

Accoe, F., Boeckx, P., Van Cleemput, O., Hofman, G., 2003. Relationship between soil organic C degradability and the evolution of the $\delta^{13}C$ signature in profiles under permanent grassland. Rapid Communications in Mass Spectrometry 17, 2591-2596.

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Content

2. Relationship between soil organic C degradability and the evolution of the $\delta^{13}C$ signature in profiles under permanent grassland

2.1. Introduction

The transformation of plant litter, entering the soil, into the soil organic matter (SOM) pool through gradual decomposition and stabilization is of increasing interest with regard to issues of CO_2 uptake and C sequestration in soils (Solomon et al., 1993; Smith et al., 2000). ^{13}C natural abundance analysis is now a widely used tool in the research on quality and turnover of SOM (Balesdent and Mariotti, 1996). The $^{13}C/^{12}C$ ratio or $\delta^{13}C$ value (‰) in SOM remains close to the $^{13}C/^{12}C$ ratio in the original vegetation, but isotopic fractionation during decomposition of the plant litter and SOM can produce significant changes in the $^{13}C/^{12}C$ ratio (Melillo et al., 1989; Wedin et al., 1995). The magnitude and direction of the change in the $^{13}C/^{12}C$ ratio may vary with time and the prevailing environmental conditions (O'Brien and Stout, 1978).

Several studies, investigating the evolution of the $\delta^{13}C$ signature of SOM in undisturbed soil profiles with a permanent C_3 vegetation, have shown that the $\delta^{13}C$ signature generally tends to increase with increasing sampling depth in well or moderately drained mineral soils (Becker-Heidmann and Scharpenseel, 1986; Nadelhoffer and Fry, 1988; Becker-Heidmann and Scharpenseel, 1989; Balesdent and Mariotti, 1996; Bird and Pousai, 1997; Bol et al., 1999; Krul et al., 2002). In this study we investigated the evolution of the $\delta^{13}C$ signature of SOM in three profiles (0-40 cm depth) under permanent grassland (C_3 vegetation) of varying texture (a loamy sand, a loamy and a clay loam soil). We also studied the potential C dynamics at different depth intervals in these profiles, and

investigated to what extent the potential C dynamics were correlated with the evolution of the $\delta^{13}C$ signature in these depth intervals.

2.2. Materials and Methods

2.2.1. Site description and soil sampling

Soil samples were collected in May 2002 from three permanent grassland soils of varying texture at three different locations in Belgium. The first grassland soil was a wet, poorly drained Plagganthrept with a loamy sand texture (5.9% clay, 8.2% silt), located at Wechelderzande (4°46'E, 51°15'N). This soil has a slowly permeable subsoil, giving anaerobic conditions during wetter periods of the year. The second grassland soil was a moderately drained Glossic Hapludalf with a loamy texture (9.7% clay, 42.4% silt), located at Melle (3°47'E, 50°59'N). The third grassland soil was a moderately drained Oxyaquic Udifluvent with a clay loam texture (26.9% clay, 45.3% silt), located at Watervliet (3°35'E, 51°17'N) (Soil Map of Belgium, 1965; USDA, 1999). The pH-H_2O values in the 0-10 cm layer of the loamy sand, loamy and clay loam soil were 5.9, 6.3 and 7.2, respectively.

In order to study the evolution of the $\delta^{13}C$ signature of the SOM with increasing depth in the upper 40 cm of the soil profiles, nine replicate soil cores covering the whole area of the investigated grassland were taken from the 0-30 cm and 30-40 cm depth interval with a steel auger (respective auger diameters were 3.5 cm and 2.5 cm). The cores were sectioned into 2-cm (loamy soil profile) or 2.5-cm depth intervals (loamy sand and clay loam soil profiles). The nine replicate core sections from each depth interval were composited into three replicate bulk samples (each consisting of three randomly chosen core sections), mixed, air dried and sieved on a 2 mm sieve in order to remove root material. The bulk samples from the loamy sand and loamy soil profiles were then ground

in a planetary ball mill (PM400, Retsch, Germany) for subsequent chemical and isotopic analysis. As the samples from the calcareous clay loam soil (Watervliet) contained considerable amounts of inorganic C (2.6, 4.8, 7.3 and 9.5 g kg^{-1} soil in the 0-10, 10-20, 20-30 and 30-40 cm depth interval, respectively), which in general has a higher $\delta^{13}C$ value than the SOM (Midwood and Boutton, 1998), the inorganic C in these samples had to be removed prior to grinding and subsequent isotopic analysis. Removal of inorganic C in these samples was performed by adding 100 ml of 1 M HCl to 10 g soil and shaking during 1 hour, in accordance to Midwood and Boutton (1998). Next, the samples were washed with demineralized water to remove excess Cl^-, centrifuged, dried at 50°C and ground for isotopic analysis.

In order to study the potential C dynamics of the SOM at 0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm depth intervals in the profiles in triplicate, three replicate bulk samples were composited for each depth interval, each consisting of 12 replicate soil cores covering the whole area of the investigated grassland. The soil cores were taken with a steel auger (3.5 cm diameter for the 0-30 cm depth intervals, 2.5 cm diameter for the 30-40 cm depth interval). The bulk samples were mixed, sieved on a 3.15 mm sieve and stored at 4°C until the start of the incubation experiment.

2.2.2. Total C and $\delta^{13}C$ analysis

Measurements of total C content and ^{13}C natural abundance in the soil samples from the 2 cm and 2.5 cm depth intervals were performed using an ANCA-SL elemental analyzer coupled to an Isotope Ratio Mass Spectrometer (20-20, PDZ Europa, UK). The measured $^{13}C/^{12}C$ ratios were expressed as $\delta^{13}C$ values (‰) relative to the VPDB standard:

$$\delta^{13}C\ (‰) = \left(\frac{{}^{13}C/{}^{12}C_{\text{sample}} - {}^{13}C/{}^{12}C_{\text{standard}}}{{}^{13}C/{}^{12}C_{\text{standard}}} \right) * 1000 \qquad (2.1)$$

The working standard for the measurements was flour with a $\delta^{13}C$ value of -27.01 ± 0.04‰ (certified by Iso Analytical, UK). The analyses were performed in duplicate. Measurements of total organic and inorganic C content in the soil samples from the 10 cm depth intervals were performed using a CNS analyzer (Vario Max CNS, Elementar, Germany).

2.2.3. Potential C mineralization dynamics

Incubation experiments were conducted in order to examine the potential C mineralization dynamics of soil samples from the 0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm depth intervals from the three profiles. Before the start of the incubation experiment all the soil samples were dried to the gravimetric water content corresponding with a water filled pore space (WFPS) of 60% at the bulk density measured in the field (Table 2.1), using the following equation (Linn and Doran, 1984):

$$WFPS = w * \frac{\rho_b}{\varepsilon} = w * \frac{\rho_b}{\left(\frac{\rho_p - \rho_b}{\rho_p} \right)} \qquad (2.2)$$

where WFPS is the water filled pore space (%), w is the gravimetric water content (%), ε is the total soil porosity (-), ρ_b is the bulk density (g cm^{-3}) and ρ_p is the particle density (assumed to be approximately 2.65 g cm^{-3}).

Table 2.1. Bulk densities measured in the 0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm depth layers of the loamy sand, loamy and clay loam soil profiles

Depth (cm)	Bulk density ($g\ cm^{-3}$)		
	Loamy sand soil	Loamy soil	Clay loam soil
0-10	1.19	1.32	1.11
10-20	1.22	1.43	1.24
20-30	1.41	1.51	1.27
30-40	1.51	1.53	1.35

Out of the three replicate bulk samples from each depth interval, an amount of soil equivalent to 150 g oven-dry weight was placed into a 4.6 cm diameter PVC tube, and compressed manually to the corresponding bulk density measured in the field (Table 2.1). The tubes were covered with pin-holed parafilm in order to prevent drying out of the soil samples, but still enabling gas exchange. The PVC tubes were placed in sealed 1200 cm^3 glass jars fitted with a rubber septum for gas sampling and incubated at 15°C during approximately 55 days.

The evolution of the CO_2-production in each jar was measured by analyzing a 1 cm^3 headspace sample for CO_2 using a gas chromatograph (GC-14B, Shimadzu, Japan) with an ECD detector and a packed column (PORAPACK Q, mesh size 80/100) after different time intervals (9 to 10 sampling events in total) during the incubation period. Following each sampling event, the glass jars were opened and parafilm was removed from the PVC tubes during 15 min. to re-establish ambient conditions. The C mineralization rates (mg C kg^{-1} soil d^{-1}) were calculated as the slope of the linear regression, fitted through the evolution of the cumulative CO_2-C production between day 12 and day 55 of the incubations.

2.3. Results and discussion

2.3.1. Evolution of the organic C content and $\delta^{13}C$ signature of SOM in the profiles

The evolution of the total organic C content and the $\delta^{13}C$ signature of SOM in the loamy sand, loamy and clay loam soil profiles down to a depth of 40 cm is shown in Fig. 2.1. Fresh plant material originating from the loamy sand, loamy and clay loam grassland soils at the time of soil sampling showed $\delta^{13}C$ values of, respectively, -30.2 ± 0.3‰, -29.7 ± 0.5‰ and -30.9 ± 0.6‰ (average values of three replicates). These $\delta^{13}C$ values were all lower than the $\delta^{13}C$ value in the corresponding surface layer, which indicates that plant litter serves as a continuous input of ^{13}C depleted material into the SOM pool.

The clay loam soil showed the largest C contents among the three soils investigated over the entire profile depth (Fig. 2.1 A, B and C). This may reflect a larger input of plant litter and/or a larger storage capacity of SOM, due to a higher physical protection against decomposition, in the clay loam soil in relation to the loamy sand and loamy soils. A higher physical protection of SOM in the clay loam soil may be explained by the higher clay plus silt content (72%) in relation to the loamy sand soil (14%) and the loamy soil (52%), as it is generally observed that the preservation of SOM in soils is positively correlated with the clay or silt plus clay content (Christensen, 1992; Hassink, 1997).

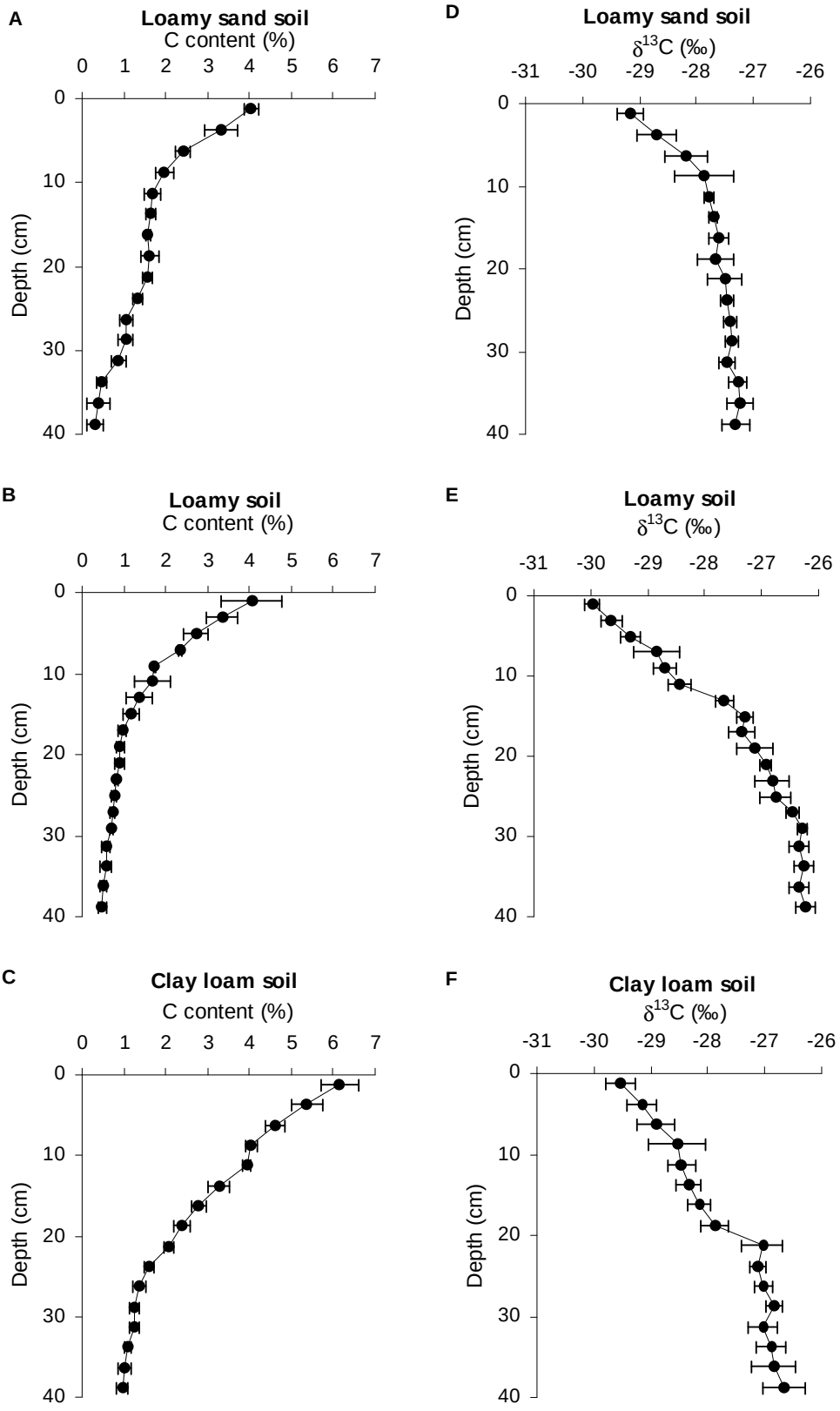


Fig. 2.1. Evolution of the total organic C content in the loamy sand (A), loamy (B) and clay loam soil profile (C), and evolution of the $\delta^{13}C$ value in the loamy sand (D), loamy (E) and clay loam soil profile (F) down to a depth of 40 cm (average values of three replicates, horizontal bars represent two standard deviations)

The organic C contents in the three profiles showed a strong decrease from the surface down to a certain depth, which varied from approximately 10 cm in the loamy sand soil profile to 30 cm in the clay loam profile, followed by a more gentle decrease down to 40 cm depth. In the three profiles, the decreasing C contents were accompanied by an increase in the $\delta^{13}C$ values of the SOM with increasing depth (Fig. 2.1 D, E and F). In the loamy and clay loam soil profiles, the $\delta^{13}C$ values showed a gradual increase of, respectively, 4‰ and 2.9‰ down to 40 cm depth in relation to the $\delta^{13}C$ value in the surface layer. In the loamy sand soil profile, the $\delta^{13}C$ values showed a strong increase of 1.3‰ down to a depth of approximately 10 cm. This strong increase at the surface of the profile was followed by a smaller, nearly linear increase of 0.6‰ down to 40 cm depth in the profile, which also coincided with a smaller decrease of the C contents. This resulted in a considerably smaller overall increase of the $\delta^{13}C$ value (1.9‰) in the loamy sand soil profile, in relation to the other profiles. This trend of increasing ^{13}C enrichment of the SOM with increasing depth in soil profiles has been observed in several other studies, investigating the evolution of the $\delta^{13}C$ signature of SOM in both well drained (Becker-Heidmann and Scharpenseel, 1986; Nadelhoffer and Fry, 1988; Becker-Heidmann and Scharpenseel, 1989; Balesdent and Mariotti, 1996; Bird and Pousai, 1997; Bol et al., 1999; Krul et al., 2002) and poorly drained (Becker-Heidmann and Scharpenseel, 1989; Bol et al., 1999), undisturbed soil profiles with a permanent C_3 vegetation.

This increase in ^{13}C enrichment of the SOM with increasing depth in the soil profiles can be partially explained by the fact that the natural abundance of atmospheric CO_2 has decreased with about 1‰ since pre-industrial times, due to the input of depleted CO_2 into the atmosphere from fossil C burning and deforestation (Keeling et al., 1984). Another possible mechanism explaining the observed trend of increasing $\delta^{13}C$ values is isotopic discrimination against ^{13}C during organic matter decomposition, combined with the higher degree of transformation of SOM with depth in the profile (Becker-Heidmann

and Scharpenseel, 1986; Nadelhoffer and Fry, 1988; Balesdent and Mariotti, 1996). In their metabolism, decomposing organisms would prefer ^{13}C depleted molecules for respiration, while ^{13}C enriched molecules tend to be utilised in the production of biomass and the end-products of the metabolism (Blair et al., 1985; Gleixner et al., 1993). As a result, SOM decay may lead to a progressive ^{13}C enrichment in the mixture of residual substrate and microbial products and metabolites (Balesdent and Mariotti, 1996). However, plant litter consists of isotopically distinct biochemical compounds which have different rates of decomposition (Melillo et al., 1989; Agren et al., 1996; Balesdent and Mariotti, 1996; Boutton, 1996). In particular, lignin is substantially depleted in ^{13}C relative to bulk plant tissue and decomposes at a significantly lower rate than the other biochemical fractions in the early stages of plant litter decomposition (Benner et al., 1987; Balesdent and Mariotti, 1996). As such, ^{13}C enrichment of SOM due to microbial respiration would be more or less balanced by the slower decay of ^{13}C depleted lignin in the early stages of plant litter decomposition (Balesdent and Mariotti, 1996). At more advanced stages of litter decay, lignin and other residual fractions would decompose at more similar rates (Berg et al., 1984; Nadelhoffer and Fry, 1988; Wedin et al., 1995) and microbial recycling of C would dominate (Balesdent and Mariotti, 1996), thus resulting in a gradual ^{13}C enrichment of the residual litter and the associated SOM pool in well or moderately drained, aerobic mineral soils. In chronically or permanently saturated soils however, with slow rates of SOM decomposition, $\delta^{13}C$ values can remain constant or even decrease with soil depth as a result of differential preservation of ^{13}C depleted, lignin-derived compounds or lipids (Stout et al., 1981; Benner et al., 1987; Nadelhoffer and Fry, 1988; Wedin et al., 1995). This might partially explain the smaller increase of the $\delta^{13}C$ values below 10 cm depth in the loamy sand soil profile, which is a poorly drained, chronically saturated soil. The smaller overall increase of the $\delta^{13}C$ value in the loamy sand soil profile might also be explained by a more intense translocation of young, ^{13}C depleted

material from the surface layer down the profile, in relation to the more fine-textured loamy and clay loam soil profiles.

The Rayleigh equation (Mariotti et al., 1981) was fitted to the observed C contents and corresponding $\delta^{13}C$ values of the SOM at the different depths in the three profiles (Fig. 2.2). The Rayleigh equation describes the gradual enrichment in ^{13}C of SOM as resulting from isotopic fractionation associated with C mineralization, where δ_o and C_o stand for the initial $\delta^{13}C$ signature and the initial C content, respectively, and where ε stands for the isotope enrichment factor:

$$\delta = \delta_o + \varepsilon \ln [C/C_o] \qquad (2.3)$$

δ_o and C_o were approximated by the $\delta^{13}C$ value and the C content in the surface layer of the profile (0-2 or 0-2.5 cm depth in the loamy or loamy sand and clay loam soils, respectively). In the loamy and clay loam soil profiles, the relation between the $\delta^{13}C$ values and corresponding C contents in the whole profile (0-40 cm depth) could be fitted ($R^2 = 0.97$, $p<0.001$) by the Rayleigh equation (Fig. 2.2). In the loamy sand soil profile however, this relation could only be fitted by the Rayleigh equation ($R^2 = 0.97$, $p<0.001$) in the upper 25 cm of the profile, as the $\delta^{13}C$ values below 25 cm depth in the profile remained nearly constant with still gradually decreasing C contents. The enrichment factor ε associated with the Rayleigh fit of the observed data in the loamy soil profile ($\varepsilon = -1.91 \pm 0.07$‰) was significantly larger (in absolute value, $p<0.05$) than the enrichment factors observed in the loamy sand soil profile ($\varepsilon = -1.64 \pm 0.09$‰) and clay loam soil profile ($\varepsilon = -1.57 \pm 0.06$‰). These values are in accordance with the enrichment factor associated with C mineralization ($\varepsilon = -1.71$‰) reported by Balesdent and Mariotti (1996). These results indicate that the evolution of the $\delta^{13}C$ signature of SOM in the investigated profiles (0-40 cm depth in the loamy and clay loam soil profiles, 0-25 cm depth in the loamy sand

soil profile) was largely determined by the isotopic fractionation associated with C mineralization. The divergence of the data from the Rayleigh approximation below 25 cm depth in the loamy sand soil profile suggests that in this profile other factors (accumulation or more intense translocation of ^{13}C depleted material) started to influence the evolution of the $\delta^{13}C$ signature.

As the observed ^{13}C-enrichment with increasing depth was correlated to the stage of decomposition of the SOM, we next investigated if there was a correlation between the rate of change of these $\delta^{13}C$ values and the potential C dynamics at different depth intervals in these profiles.

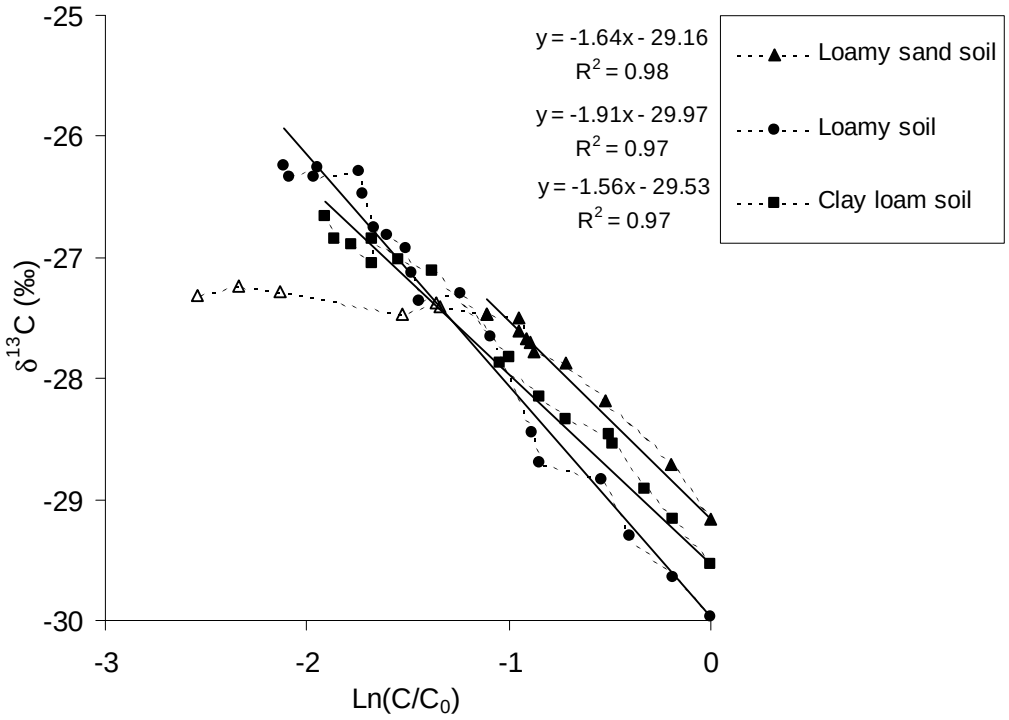


Fig. 2.2. Relationship between $\ln(C/C_0)$ and the $\delta^{13}C$ values in the upper 40 cm of the three soil profiles (dotted line), and fit by the Rayleigh equation (full line); in the loamy sand soil profile, only the data from the upper 25 cm in the profile were used for the fit (data from below 25 cm depth are indicated by empty triangles); C and C_0 stand for the C content in the different depth intervals and in the surface layer, respectively

2.3.2. Potential C dynamics and relationship with ^{13}C enrichment of the SOM

The measured C mineralization rates for the 0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm depth intervals from the three profiles are presented in Table 2.2, together with the total organic C contents and the C decomposition rate constants. The C decomposition rate constants (yr^{-1}), which are an indicator of the degradability of the SOM, were calculated by dividing the observed C mineralization rate by the corresponding C content in each depth interval.

Table 2.2. Organic C contents, C mineralization rates and C decomposition rate constants in the 0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm depth intervals from the three grassland profiles (standard deviations in brackets); values in the same column and from the same sampling depth that share the same letter are not significantly different ($\alpha = 0.05$)

Soil	Depth (cm)	C content (g kg^{-1} soil)	C mineralization rate (mg CO_2-C kg^{-1} soil d^{-1})	C decomposition rate constant (yr^{-1})
Loamy sand	0-10	30.3(0.3) ^{a}	4.63(0.16) ^{a}	0.0557(0.0019) ^{a}
	10-20	17.6(1.0) ^{a}	1.49(0.05) ^{a}	0.0308(0.0011) ^{a}
	20-30	13.0(0.5) ^{a}	0.71(0.03) ^{a}	0.0200(0.0007) ^{a}
	30-40	5.7(0.9) ^{a}	0.20(0.01) ^{a}	0.0130(0.0004) ^{a}
Loamy	0-10	28.8(0.2) ^{a}	5.19(0.04) ^{b}	0.0657(0.0005) ^{b}
	10-20	11.0(0.2) ^{b}	1.56(0.01) ^{a}	0.0519(0.0005) ^{b}
	20-30	7.8(0.1) ^{b}	0.80(0.03) ^{ab}	0.0372(0.0012) ^{b}
	30-40	5.7(0.1) ^{a}	0.56(0.04) ^{b}	0.0360(0.0028) ^{b}
Clay loam	0-10	54.1(0.2) ^{b}	6.47(0.42) ^{c}	0.0436(0.0028) ^{c}
	10-20	23.0(0.4) ^{a}	1.68(0.13) ^{a}	0.0267(0.0021) ^{a}
	20-30	13.3(0.1) ^{a}	1.00(0.07) ^{b}	0.0274(0.0020) ^{c}
	30-40	9.4(0.1) ^{b}	0.44(0.02) ^{b}	0.0170(0.0008) ^{c}

In the three profiles, the C contents and C mineralization rates decreased with increasing sampling depth. The observed C mineralization rates (m_C) showed a significant, positive correlation ($m_C = 0.14C_{tot} - 0.52$, $R^2 = 0.87$, $p<0.001$) with the total organic C contents (C_{tot}). In the upper 30 cm of the profiles, the largest C mineralization rates were observed in the clay loam soil profile, followed by the loamy and loamy sand soil profile. The C mineralization rates showed the largest relative difference among the three profiles in the surface layer. The C decomposition rate constants also decreased with increasing depth in the profiles (Table 2.2). This reflects that the stability of SOM against microbial degradation is considerably higher in the deeper layers of the soil profile in relation to the SOM in the surface layer, due to a more enhanced decomposition stage. In all the investigated layers (from 0-40 cm depth) from the loamy soil profile and in the upper layer from the loamy sand soil profile, the C decomposition rate constants were considerably larger than the corresponding values in the clay loam soil profile. This may be attributed to the higher silt and clay content in the clay loam soil, as SOM generally tends to be more stabilized and physically protected against microbial degradation due to the association with silt and clay particles (Tiessen and Stewart, 1983).

As we observed a varying ^{13}C enrichment of the SOM with increasing depth in the three profiles, we investigated if there was a correlation between the rate of change of these $\delta^{13}C$ values and the potential C dynamics in the different depth intervals. Therefore, we calculated the average change of the $\delta^{13}C$ value per depth increment in each 10 cm depth interval ($\Delta\delta^{13}C$ value, expressed in ‰ cm^{-1}), as the slope of a linear regression of the evolution of the $\delta^{13}C$ values (as illustrated in Fig. 2.3. for the $\Delta\delta^{13}C$ values in the loamy soil profile). The calculated $\Delta\delta^{13}C$ values for the three profiles, together with the goodness-of-fit (R^2) of the linear regressions and the standard errors on the $\Delta\delta^{13}C$ values are shown in Table 2.3.

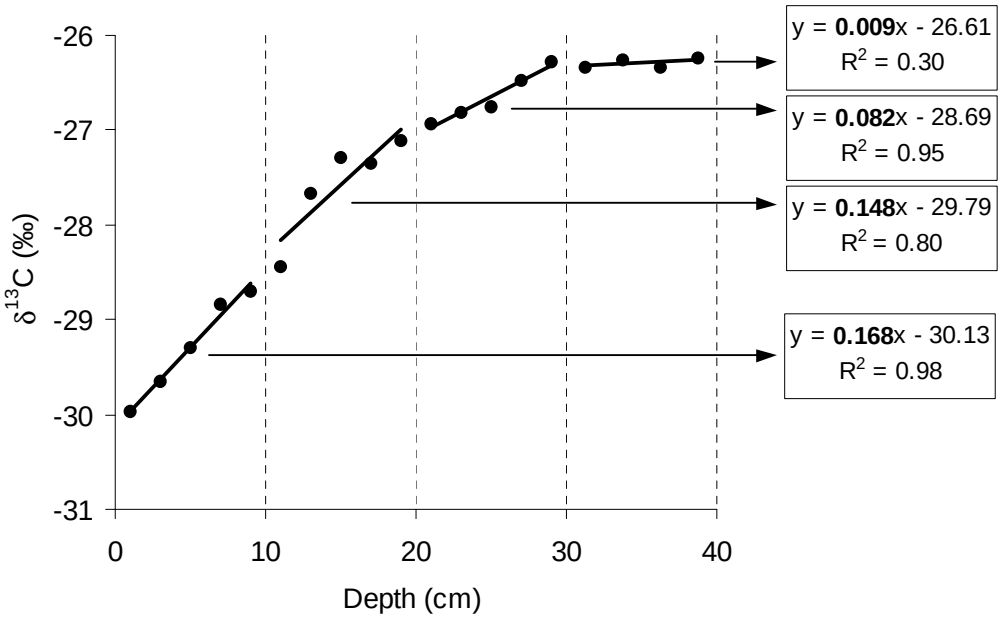


Fig. 2.3. Calculation of the $\Delta\delta^{13}C$ values in the 0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm depth intervals of the loamy soil profile through linear regression of the evolution of the $\delta^{13}C$ values ($\Delta\delta^{13}C$ values are indicated in bold in the linear regression equations)

Table 2.3. $\Delta\delta^{13}C$ values calculated by linear regression in the 0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm depth intervals from the three profiles, together with the goodness-of-fit (R^2) of the linear regressions (standard errors in brackets)

Soil	Depth (cm)	$\Delta\delta^{13}C$ value (‰ cm^{-1})	R^2 (-)
Sandy loam	0-10	0.175(0.012)	0.99
	10-20	0.018(0.011)	0.58
	20-30	0.018(0.002)	0.98
	30-40	0.014(0.011)	0.34
Loamy	0-10	0.168(0.013)	0.98
	10-20	0.148(0.043)	0.80
	20-30	0.082(0.011)	0.95
	30-40	0.009(0.010)	0.30
Clay loam	0-10	0.129(0.007)	0.99
	10-20	0.077(0.010)	0.97
	20-30	0.018(0.019)	0.31
	30-40	0.048(0.007)	0.97

The observed $\Delta\delta^{13}C$ values tended to decrease with increasing sampling depth in the three profiles (except for the $\Delta\delta^{13}C$ value in the 30-40 cm depth interval in the clay loam soil profile). In order to investigate the relationship between the $\Delta\delta^{13}C$ values and the potential C dynamics in the different depth intervals, in terms of the C mineralization rates (m_C) and the C decomposition rate constants (drc_C), these values were plotted versus the corresponding $\Delta\delta^{13}C$ values in Fig. 2.4. There was a significant, positive correlation ($drc_C = 0.22\Delta\delta^{13}C + 0.019$, $R^2=0.75$, $p<0.001$, $n=12$) between the C decomposition rate constants from the four sampling depths in the three profiles and the corresponding $\Delta\delta^{13}C$ values. A less significant, positive correlation ($m_C = 25.3\Delta\delta^{13}C + 0.12$, $R^2=0.59$, $p<0.005$, $n=12$) existed between the C mineralization rates from the four sampling depths in the three profiles and the corresponding $\Delta\delta^{13}C$ values. A stronger, positive correlation between the C decomposition rate constants and the $\Delta\delta^{13}C$ values was observed when only the data from the upper 30 cm in the profiles ($drc_C = 0.22\Delta\delta^{13}C + 0.019$, $R^2=0.86$, $p<0.001$, $n=9$) or from the upper 20 cm in the profiles ($drc_C = 0.21\Delta\delta^{13}C + 0.020$, $R^2=0.78$, $p<0.05$, $n=6$) were considered.

These results suggest that the $\Delta\delta^{13}C$ values in the surface layers (0-30 cm depth) of profiles under permanent grassland, and to a lesser extent the $\Delta\delta^{13}C$ values in the deeper soil layers (30-40 cm depth), may be interpreted as a direct indicator of the degradability of the SOM, in terms of the C decomposition rate constant. The observed relationship between the evolution of the ^{13}C enrichment and the decomposition rate constants of SOM in the investigated grassland profiles may be explained by the fact that its degradability is proportional to the labile fraction of the SOM. Fresh litter input, which generally has a more negative $\delta^{13}C$ signature than the SOM in the profile, serves as a continuous source of labile C, which is gradually incorporated into the soil profile. In this way, the evolution of the $\delta^{13}C$ signature might also be interpreted as a decreasing fraction of relatively 'young', labile C in the SOM with increasing depth in the profile. The soil type may thus

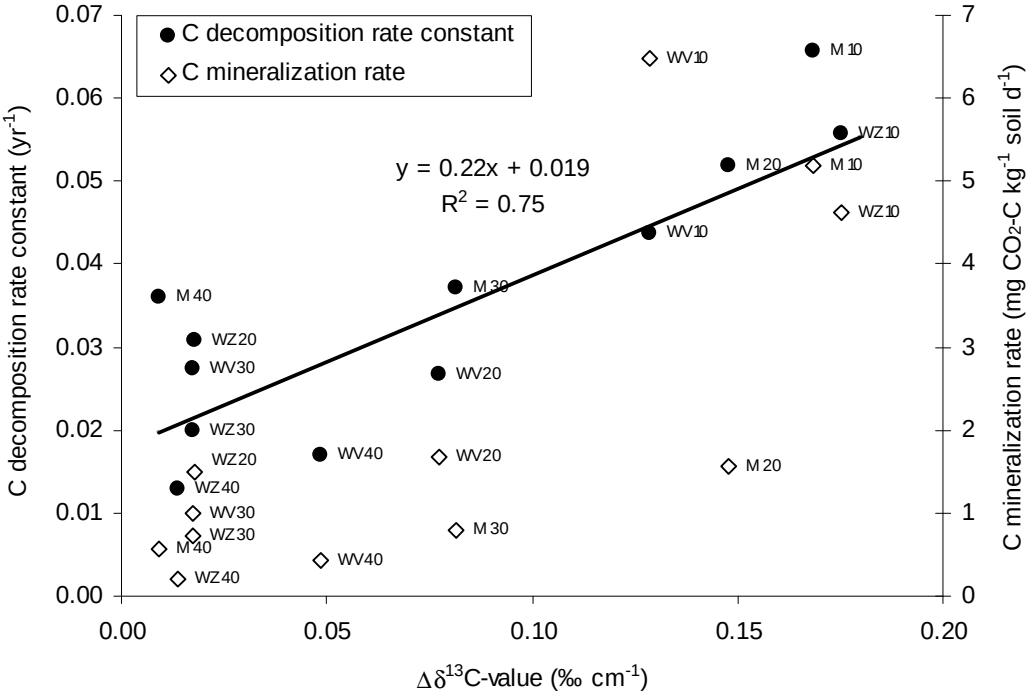


Fig. 2.4. Relationship between the C decomposition rate constants and the corresponding $\Delta\delta^{13}C$ values (linear regression indicated by a full line), and relation between the potential C mineralization rates and the corresponding $\Delta\delta^{13}C$ values in the 0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm depth intervals of the three soil profiles; the soils and depth intervals from which the data points are originating are indicated by the letters WZ (Wechelderzande, loamy sand soil), M (Melle, loamy soil) or WV (Watervliet, clay loam soil), followed by the numbers 10 (0-10 cm), 20 (10-20 cm), 30 (20-30 cm) or 40 (30-40 cm depth)

also have an influence on the evolution of the $\delta^{13}C$ signature with increasing depth, in terms of the rate at which solid and soluble compounds, originating from the surface layer, are translocated into the soil profile (Becker-Heidmann and Scharpenseel, 1989).

As the $\Delta\delta^{13}C$ values tend to be inversely related to the stability of the SOM and are more easily accessible, whereas incubation experiments to determine potential C mineralization rates from soil samples are generally time-consuming and laborious, the $\Delta\delta^{13}C$ values might serve as a practical tool for getting a rapid indication of soil C stability in the surface layers (0-30 cm depth) of profiles under permanent grassland.

2.4. Conclusions

The ^{13}C enrichment of SOM which we observed with increasing depth in the three profiles under permanent grassland is consistent with the findings of several other studies. The significant fit by the Rayleigh equation of the $\delta^{13}C$ profiles reflects that the observed ^{13}C enrichment with increasing depth is mainly driven by isotopic fractionation associated with C mineralization along with the decomposition process. The evolution of the $\delta^{13}C$ signature in the loamy sand soil profile, which diverged from the Rayleigh approximation below 25 cm depth, suggests that other factors, like differential preservation or accumulation of ^{13}C depleted material, may also influence the $\delta^{13}C$ evolution in poorly drained, chronically wet soil profiles.

The strong, significant correlation which we observed between the C decomposition rate constants and the corresponding $\Delta\delta^{13}C$ values in the soil layers down to 30 cm depth in the investigated profiles, suggests that these $\Delta\delta^{13}C$ values may be interpreted as a direct indicator of the stability of the SOM in these layers. In this way, the $\Delta\delta^{13}C$ values might serve as a practical tool for getting a rapid indication of soil C stability in the surface layers (0-30 cm depth) of profiles under permanent grassland.

However, further research is needed to investigate the influence of soil type, in terms of drainage capacity and the translocation of material from the surface layer into the profile, and grassland age, in terms of SOM content, on the evolution of the $\delta^{13}C$ signature in profiles under permanent grassland. The isotope enrichment factor ε relates the variation of the quantity of C to the variation of its ^{13}C enrichment, which in turn tended to be related to the quality of the C in the investigated soil profiles. Thus we suggest that more extended research in other permanent grassland ecosystems might elucidate whether this enrichment factor ε is also related to the C dynamics in profiles under permanent grassland.

CHAPTER 3

Characterization of soil organic matter fractions from grassland and cultivated soils via C content, C/N ratio and $\delta^{13}C$ signature

This chapter is compiled from:

Accoe, F., Boeckx, P., Van Cleemput, O., Hofman, G., Hui, X., Bin, H. and Guanxiong, C., 2002. Characterization of soil organic matter fractions from grassland and cultivated soils via C content and $\delta^{13}C$ signature. Rapid Communications in Mass Spectrometry 16, 2157-2164.

Content

3. Characterization of soil organic matter fractions from grassland and cultivated soils via C content, C/N ratio and $\delta^{13}C$ signature

3.1. Introduction

^{13}C natural abundance analysis and physical fractionation are widely used tools in the research on quality and turnover of soil organic matter (SOM). The ^{13}C natural abundance or $\delta^{13}C$ value (‰) of SOM is mainly determined by the $\delta^{13}C$ value of the plant litter from which it is derived. Plants with the C_3 photosynthetic pathway (Calvin pathway) discriminate more against $^{13}CO_2$ during photosynthesis than C_4 plants (Hatch and Slack pathway) (Smith and Epstein, 1971). As a result, C_3 plants have $\delta^{13}C$ values ranging from approximately -32‰ to -22‰, while C_4 plants have $\delta^{13}C$ values ranging from approximately -17‰ to -9‰ (Boutton, 1996). Thus, the $\delta^{13}C$ values reported for C_3 and C_4 plants differ on average by 14‰. This large difference in $\delta^{13}C$ values enables us to study the turnover of whole soil C and different SOM fractions in soils with a former C_3 vegetation converted to a C_4 vegetation (or vice-versa) (Balesdent et al., 1987; Puget et al., 1995; Ryan et al., 1995).

After incorporation into the SOM pool, the isotopic signature of plant litter may be slightly altered as the decomposition process proceeds (O'Brien and Stout, 1978; Melillo et al., 1989; Wedin et al., 1995). One possible source of alteration of the isotopic signature may be the different decomposition rates of isotopically distinct biochemical components of plant litter (Melillo et al., 1989; Agren et al., 1996; Boutton, 1996). In particular, lignin is substantially depleted in ^{13}C relative to bulk plant tissue and decomposes at a significantly lower rate than the other biochemical fractions (Benner et al., 1987). Although several studies have shown that the relative proportion of lignin in plant tissue

increases as decomposition proceeds, this differential preservation does not appear to induce lower $\delta^{13}C$ values in the residual litter or the associated SOM pool in well-drained mineral soils (Nadelhoffer and Fry, 1988; Melillo et al., 1989; Wedin et al., 1995; Boutton, 1996). Shifts in the isotopic signature can also be induced by isotopic discrimination associated with microbial decomposition of SOM. In their metabolism, decomposing organisms would prefer ^{13}C-depleted molecules for respiration while ^{13}C-enriched molecules tend to be utilised in the production of biomass and the end-products of metabolism (Blair et al., 1985; Gleixner et al., 1993), which may induce a ^{13}C enrichment in the residual SOM.

Physical fractionation of SOM is based on the concept that SOM fractions (1) with different density (density fractionation) or (2) associated with mineral particles of different size (size fractionation) vary in structure and turnover, due to different degrees of organo-mineral complexation (Christensen, 1992). The light fraction of SOM, which mainly consists of partially decomposed plant residues, serves as a readily decomposable substrate for the soil microbial biomass and is a short-term reservoir of plant nutrients (Gregorich et al., 1994). SOM associated with clay and silt particles (<50 μm according to USDA classification) generally shows a greater stability against microbial degradation than SOM in larger size fractions (Tiessen and Stewart, 1983). Davidson et al. (1987) suggested that water soluble organic C (WSOC) is a favourable substrate for the soil microbial biomass, and it has been reported that a large portion of the WSOC is readily decomposable (Zsolnay and Steindl, 1991).

In this study, the variation in ^{13}C enrichment due to isotopic fractionation associated with SOM decomposition was investigated among five size and density fractions, WSOC and microbial biomass C (MBC) from the upper layer of a continuous grassland soil (C_3 vegetation). The distribution and incorporation of newly introduced C into these SOM fractions was investigated by $\delta^{13}C$ analysis of the same fractions

originating from a C_3-humus soil which was converted (since 19 years) to continuous maize cultivation (C_4 vegetation) and a rotation of maize cultivation with grassland.

3.2. Materials and methods

3.2.1. Site description and soil sampling

Soil samples were taken in November 2000 from the experimental agricultural station of the Ghent University located at Melle in Belgium (3°47'E, 50°59'N). The soil is a moderately drained Glossic Hapludalf with a degraded argillic horizon and a loam texture (9.7% clay, 42.4% silt) (Soil Map of Belgium, 1965; USDA, 1999). The site was arable land with C_3 crops prior to the establishment of experimental fields with different management treatments in 1966. The management treatments studied here were continuous grassland (CG, since 1966), continuous maize cropping (CM, since 1981) and a rotation of three years maize cropping followed by three years grassland (R, since 1981). From 1966 to 1981, the CM and R soils were cultivated with C_3 crops instead of maize (continuously or in rotation with three years grassland, respectively). At the time of soil sampling, the R soil was in the second year of maize cropping. Every time before sowing of the maize, the soil was worked with a rotary cultivator to a depth of about 20 cm. The general soil characteristics of the 0-20 cm layer from the CG, CM and R soil are shown in Table 3.1.

In the sampling procedure we intended to obtain composite soil samples representative of each management treatment. Based on the results and sampling procedures from other studies (Tiessen and Stewart, 1983; Van Kessel et al., 1994; Shang and Tiessen, 2000), investigating the variability of total C and $\delta^{13}C$ analysis in cultivated and non-cultivated soils, we composited and mixed 24 subsamples from each field. The 24 subsamples were taken from the upper 20 cm in each field (as this was the working depth

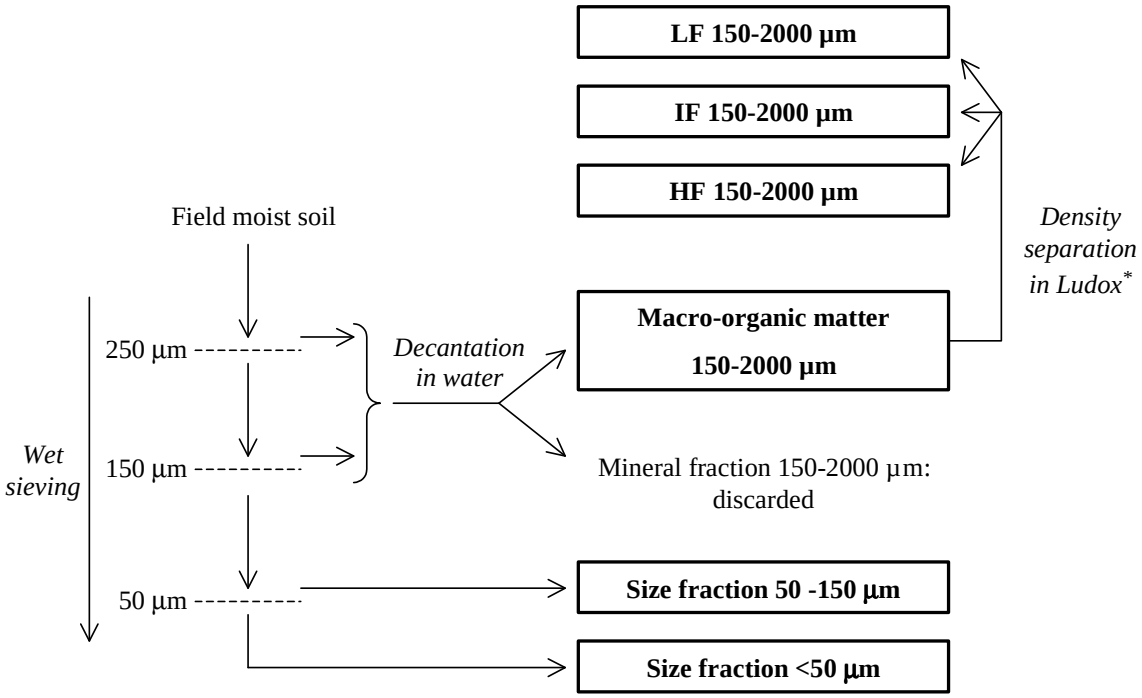
in the cultivated soils) with a steel auger (3.5 cm diameter). Fresh soil samples were sieved to pass a 2 mm screen and stored at 4°C until fractionation or chemical analysis.

Table 3.1. General soil characteristics of the 0-20 cm layer from the continuous grassland (CG), rotation (R) and continuous maize (CM) soil (standard errors in brackets)

Soil	C content	N content	pH-H_2O	Bulk density
	(g kg^{-1} soil)		(-)	(g cm^{-3})
CG	22.5(0.5)	1.75(0.04)	5.4	1.38(0.07)
R	14.9(0.3)	1.40(0.01)	6.4	1.43(0.05)
CM	8.3(0.2)	0.86(0.05)	6.6	1.49(0.06)

3.2.2. Size and density fractionation of soil organic matter

The SOM was separated into five size and density fractions: the light (LF 150-2000 µm; density <1.13 g cm^{-3}), intermediate (IF 150-2000 µm; 1.13< density <1.37 g cm^{-3}) and heavy density fraction (HF 150-2000 µm; density >1.37 g cm^{-3}) of the macro-organic matter (150-2000 µm), the size fraction 50-150 µm and the size fraction <50 µm (Fig. 3.1). Density fractionation of the macro-organic matter was performed by the method of Meijboom et al. (1995). Field-moist, mixed soil samples of 500 g were wet sieved over two sieves with tap water (top sieve mesh size 250 µm, bottom sieve mesh size 150 µm). The soil was pushed through the top sieve until the water passing the sieve became clear, in order to destroy all macro-aggregates >250 µm. Next, the two size fractions retained on the sieves were washed into a bucket and swirled with a jet of water to separate the organic matter from the mineral fraction by decantation. Swirling and decanting were repeated until no more floating organic matter appeared.



*Ludox : silica suspensions with densities 1.13 and 1.37 g cm^{-3}

Fig. 3.1. Schematic overview of the procedure for size and density fractionation of SOM; LF 150-2000 µm = light density fraction (d < 1.13 g cm^{-3}), IF 150-2000 µm = intermediate density fraction (1.13 g cm^{-3}< d <1.37 g cm^{-3}), HF 150-2000 µm = heavy density fraction (density >1.37 g cm^{-3})

The remaining mineral fraction was discarded. The obtained organic matter from both size fractions was combined to be further separated into a light (LF 150-2000 µm), intermediate (IF 150-2000 µm) and heavy density fraction (HF 150-2000 µm) by subsequent submersion in colloidal silica suspensions (Ludox, Dupont) with a density of 1.37 g cm^{-3} and 1.13 g cm^{-3}. The obtained density fractions were washed with demineralized water and dried at 50°C during 48 hours.

The size fractions 50-150 µm and <50 µm were obtained by wetsieving of field-moist, mixed soil samples of 100 g over three sieves with tap water (top sieve mesh size 250 µm, middle sieve mesh size 150 µm, bottom sieve mesh size 50 µm). The soil was also pushed through the top sieve in order to destroy all macro-aggregates >250 µm. The suspension passing the bottom sieve was collected and left about 48 hours at 4°C to settle. After settling, the clear solution was removed and the soil size fraction <50 µm was collected. The fraction retained on the 50 µm sieve and the fraction <50 µm were also dried at 50°C during 48 hours.

The size and density fractionation was replicated three times for each composite soil sample and fraction yields were compared among replicate fractionations to verify the accuracy of the fractionation procedure. The three replicates of each fraction were combined for grinding with a planetary ball mill (PM400, RETSCH, Germany) and subsequent chemical and isotopic analysis.

3.2.3. MBC and WSOC

MBC was determined by the fumigation-extraction method (Voroney et al., 1993). Soluble C in fumigated and non-fumigated soil samples was extracted with demineralized water, by shaking on a mechanical shaker for one hour and filtering the soil suspensions through Whatman No. 5 filter paper. Demineralized water instead of K_2SO_4 was used for

the extractions in order to enable accurate $\delta^{13}C$ analysis, which may be complicated by the large amount of S in the K_2SO_4 extracts. Gregorich et al. (2000) found that the flush of C induced by fumigation and extracted by either 125mM K_2SO_4 or demineralized water was similar and the authors suggested that in both cases the organic C extracted was probably derived from the same fraction of SOM. The amount of MBC was then calculated according to the following formula:

$$\text{MBC (g C kg}^{-1}\text{ soil)} = (C_f - C_{nf})/k_{EC} \quad (3.1)$$

where C_f and C_{nf} were the amounts of C extracted from the fumigated and non-fumigated samples, and k_{EC} = 0.35 (representing the extraction efficiency of MBC) according to Sparling et al. (1990). WSOC was also extracted with demineralized water by shaking for one hour, centrifuging at 4000 rpm during 10 min. and filtering through Whatman No. 5 filter paper. The fumigation-extraction procedure and extraction of WSOC was replicated three times for each composite soil sample. All extracts were frozen till further analysis. Soluble organic C content in the water extracts was determined with a total organic C analyzer (TOC-5000, Shimadzu, Japan). The water extracts were then freeze-dried (HETO FD 3, Ankersmit, Germany) in order to increase their C contents considerably to enable $\delta^{13}C$ analysis of WSOC and MBC.

3.2.4. Stable C isotope analysis

Measurements of ^{13}C natural abundance in soils and SOM fractions were performed using an ANCA-SL elemental analyzer coupled to an Isotope Ratio Mass

Spectrometer (20-20, PDZ Europa, UK). The measured $^{13}C/^{12}C$ ratios are expressed as $\delta^{13}C$ values (‰) relative to the VPDB standard:

$$\delta^{13}C\ (‰) = \left(\frac{{}^{13}C/{}^{12}C_{\text{sample}} - {}^{13}C/{}^{12}C_{\text{standard}}}{{}^{13}C/{}^{12}C_{\text{standard}}} \right) * 1000 \qquad (3.2)$$

The working standard for the measurements was flour with a $\delta^{13}C$ value of -27.01 ± 0.04‰ (certified by Iso Analytical, UK).

The proportion (*f*) of C_4-derived C in the SOM fractions from the R and CM soils was calculated using the following equation (Balesdent et al., 1987):

$$f = \frac{(\delta_S - \delta_{ref,C3})}{(\delta_{ref,C4} - \delta_{ref,C3})} \qquad (3.3)$$

where δ_S is the $\delta^{13}C$ value of a given SOM fraction isolated from the soil converted to C_4 vegetation, and $\delta_{ref,C3}$ or $\delta_{ref,C4}$ are the $\delta^{13}C$ values of the same SOM fraction from a reference soil under a C_3 or C_4 vegetation, respectively. The CG soil can be considered as the C_3 reference soil. Since there was no reference soil under C_4 vegetation, the difference ($\delta_{ref,C4}$ - $\delta_{ref,C3}$) was estimated as (δ_{maize} - δ_{grass}) or the difference between the $\delta^{13}C$ values of maize residues (-11.60 ± 0.07‰) and grass residues (-30.26 ± 0.04‰) (Balesdent and Mariotti, 1996). Hereby it is assumed that the shift in isotopic composition of plant tissue during decomposition and integration into the SOM pool is equal for grass and maize residues.

The $\delta^{13}C$ value of MBC was estimated as the $\delta^{13}C$ value of the C extracted from the fumigated sample in excess of that extracted from the non-fumigated sample, as follows:

$$\delta^{13}C_{MBC} = \frac{\left(\delta^{13}C_f C_f - \delta^{13}C_{nf} C_{nf}\right)}{\left(C_f - C_{nf}\right)} \quad (3.4)$$

where C_f and C_{nf} were the amounts of C extracted from the fumigated and non-fumigated samples and $\delta^{13}C_f$ and $\delta^{13}C_{nf}$ were the $\delta^{13}C$ values of the (freeze-dried) fumigated and non-fumigated extracts, respectively.

3.2.5. Total C and N analysis

Total C and N analysis of whole soil and the size fractions 50-150 μm and <50 μm was performed using the Isotope Ratio Mass Spectrometer (20-20, PDZ Europa, UK). Because of the large C and N contents, total C and N analysis of the macro-organic matter fractions was performed using a CN analyser (NC 2100 SOIL, CE Instruments, Italy).

3.3. Results and discussion

3.3.1. Whole soil C

The CG soil showed the largest total C content in the upper 20 cm (Table 3.1). In the R soil and the CM soil, the total C contents were respectively 34% and 63% lower than in the CG soil. These lower total C contents may be attributed to a lower input of root-derived organic matter in comparison to the CG soil and the disruption of soil aggregation and higher SOM turnover as a consequence of tillage in cultivated soils (Christensen, 1992; Puget et al., 1995).

3.3.2. Density fractionation of macro-organic matter

The LF 150-2000 µm fraction consisted mainly of recognisable plant material in an early stage of decomposition and showed the largest C content (Table 3.2). The HF 150-2000 µm fraction contained more humified, darker and amorphous organic matter and showed the smallest C content among the three density fractions. The decreasing C contents in the IF and HF 150-2000 µm fractions indicate an increasing ash content and an increasing association of the organic matter with soil minerals in these heavier fractions.

The total amount of macro-organic matter obtained after density fractionation was largest in the CG soil (12.9 g fraction kg^{-1} soil), followed by the R (5.1 g fraction kg^{-1} soil) and the CM soil (2.2 g fraction kg^{-1} soil) (Table 3.2). In the CG soil, the amount of HF 150-2000 µm fraction (71% of the macro-organic matter) was considerably larger than the amounts of IF 150-2000 µm fraction (23%) and LF 150-2000 µm fraction (6%). The difference in macro-organic matter content in the R and CM soil in relation to the CG soil

Table 3.2. Amounts and C contents of the size and density fractions (macro-organic matter = size fraction 150-2000 µm; LF 150-2000 µm = light density fraction, d < 1.13 g cm^{-3}; IF 150-2000 µm = intermediate density fraction, 1.13 < d <1.37 g cm^{-3}; HF 150-2000 µm = heavy density fraction, d >1.37 g cm^{-3}; size fraction 50-150 µm; size fraction <50 µm), and distribution of C among all the SOM fractions considered (size and density fractions; WSOC = water soluble organic C; MBC = microbial biomass C) from the 0-20 cm layer in the continuous grassland (CG), rotation (R) and continuous maize (CM) soil (standard deviations in brackets)

	Weight (g kg^{-1} soil)			C content (g kg^{-1} fraction)			Distribution of total C (g kg^{-1} soil)		
	CG	R	CM	CG	R	CM	CG	R	CM
Whole soil	1000	1000	1000	22.5(0.5)	14.9(0.3)	8.3(0.2)	22.5(0.5)	14.9(0.3)	8.3(0.2)
Size and density fractions									
Macro-organic matter	12.91(0.21)	5.06(0.19)	2.19(0.20)	227(6)	247(12)	270(8)	2.93(0.09)	1.25(0.08)	0.59(0.06)
LF 150-2000 µm	0.79(0.11)	0.83(0.11)	0.52(0.07)	361(4)	335(6)	321(5)	0.28(0.04)	0.28(0.04)	0.17(0.02)
IF 150-2000 µm	3.00(0.31)	1.64(0.23)	0.77(0.05)	329(3)	290(9)	301(6)	0.99(0.11)	0.48(0.07)	0.23(0.02)
HF 150-2000 µm	9.13(0.06)	2.58(0.29)	0.90(0.18)	182(4)	191(4)	214(3)	1.66(0.04)	0.49(0.06)	0.19(0.04)
50-150 µm	366(8)	349(18)	342(11)	13.9(0.6)	8.8(0.1)	2.7(0.1)	5.1(0.2)	3.1(0.2)	0.9(0.1)
<50 µm	477(12)	497(23)	484(19)	25.4(0.8)	18.3(0.5)	14.7(0.1)	12.1(0.5)	9.1(0.5)	7.1(0.3)
WSOC							0.026(0.003)	0.016(0.006)	0.020(0.003)
MBC							0.58(0.03)	0.45(0.02)	0.21(0.02)

was most pronounced in the amounts of HF 150-2000 µm fraction and IF 150-2000 µm fraction. Compared with the CG soil, the amount of LF 150-2000 µm fraction differred to a much lesser extent in the CM and was the same in the R soil.

The increasing amounts of C stored from the LF 150-2000 µm fraction towards the HF 150-2000 µm fraction in the CG soil (Table 3.2) reflect an accumulation and stabilization of C into the IF and HF 150-2000 µm fractions, resulting from an undisturbed transfer of partially decomposed plant material in the LF 150-2000 µm fraction towards more humified material in the HF 150-2000 µm fraction, along with the decomposition process. However, this trend of increasing C contents in the IF and HF 150-2000 µm fractions was not present in the R and CM soil. This may indicate that tillage in the R and CM soil disturbed the transfer process and accumulation of soil C into the IF and HF 150-2000 µm fractions.

3.3.3. Distribution of total C among size and density fractions

The distribution of whole soil C among the five size and density fractions in the CG, R and CM soil, expressed in C contents (g C kg^{-1} soil) and as the proportion (%) of the total amount of C recovered in the five fractions, is shown in Table 3.2 and Fig. 3.2, respectively. In the three management treatments, the largest amount of soil C was stored in the size fraction <50 µm, followed by the size fraction 50-150 µm and the macro-organic matter. The amount of C stored in the macro-organic matter decreased from 2.93 g C kg^{-1} soil in the CG soil to 0.59 g C kg^{-1} soil in the CM soil (Table 3.2). This trend was also reflected in the proportion of total C present in the macro-organic matter, which decreased in the order CG (14.6%) > R (9.3%) > CM (6.8%) (Fig. 3.2). With decreasing total C contents (in the order CG>R>CM), the C enrichment ratio (ratio of g C kg^{-1} fraction to g C kg^{-1} whole soil) of the size fraction <50 µm increased from 1.1 in the CG

soil to 1.8 in the CM soil, while the C enrichment ratio in the size fraction 50-150 µm decreased from 0.6 (CG soil) to 0.3 (CM soil). Consequently, the proportion of total C present in the size fraction <50 µm increased in the order CG (60.2%) < R (67.9%) < CM (82.3%), while the proportion in the size fraction 50-150 µm decreased in the order CG (25.2%) > R (22.8%) > CM (10.8%) (Fig. 3.2).

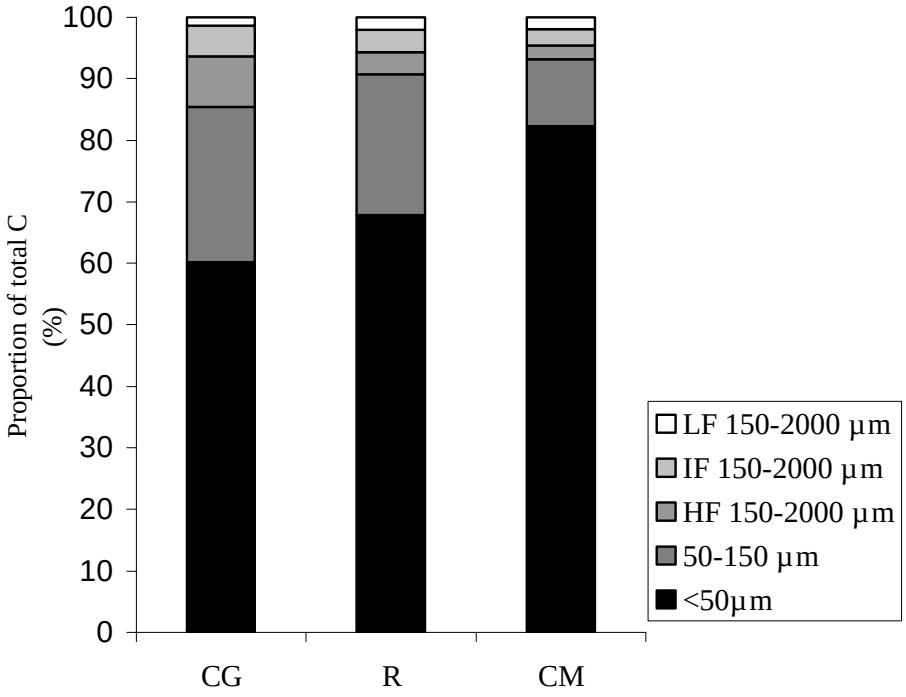


Fig. 3.2. Proportions of total C recovered in the size and density fractions from the 0-20 cm layer in the continuous grassland (CG), rotation (R) and continuous maize (CM) soil

These results show that the relative contribution to the total C content of C stored in the macro-organic matter and in the size fraction 50-150 µm decreased with decreasing total C contents, while the relative contribution of C associated with the clay- and silt-sized fraction (<50 µm) increased. This indicates that C in the clay- and silt-sized fraction was less affected by soil disruption due to tillage, than C in the macro-organic matter and in the size fraction 50-150 µm. This may be attributed to the greater stability and physical protection against microbial degradation of the clay and silt associated organic matter, in relation to the organic matter in larger size fractions (Tiessen and Stewart, 1983).

3.3.4. C/N ratios of the size and density fractions

The C/N ratios of the size and density fractions in the CG, R and CM soils are shown in Table 3.3. In the three soils investigated, the C/N ratios tended to decrease in the order LF 150-2000 µm fraction > IF and HF 150-2000 µm fractions > size fraction 50-150 µm > size fraction <50 µm. The C/N ratios observed in the LF, IF and HF 150-2000 µm fractions are in the same range as the values reported by Hassink (1995) (16.1-35.9, 15.9-26.0 and 11.1-20.9, respectively) and Meijboom et al. (1995) (18-24, 15-21 and 13-16, respectively). The generally higher C/N ratio observed in the LF 150-2000 µm fraction in relation to the IF and HF 150-2000 µm fractions, reflects that the LF 150-2000 µm fraction consists of organic matter in a less decomposed state. The decrease in C/N ratios from the macro-organic matter fractions towards the <50 µm fraction which we observed is consistent with the findings of several other studies (e.g. Tiessen and Stewart, 1983; Catroux and Schnitzer, 1987; Christensen, 1992), and indicates an increasing degree of humification from the coarser to the finer particle-size fractions of SOM. In all the size and density fractions, except in the size fraction <50 µm, the C/N ratios tended to increase in the order CG<R<CM. This might be explained by a higher C/N ratio in the maize residues in relation to the C/N ratio in the grass residues.

Table 3.3. C/N ratios of the size and density fractions from the 0-20 cm layer in the continuous grassland (CG), rotation (R) and continuous maize (CM) soil

SOM fraction	Soil		
	CG	R	CM
Macro-organic matter	16.4	17.4	23.8
LF 150-2000 µm	20.1	21.1	29.2
IF 150-2000 µm	16.0	17.0	20.6
HF 150-2000 µm	16.1	16.1	24.5
50-150 µm	12.6	14.4	18.8
<50 µm	11.3	10.2	8.6

3.3.5. Amounts of WSOC and MBC

The amounts of WSOC and MBC (expressed in g C kg^{-1} soil) in the three management treatments are shown in Table 3.2. The amounts of WSOC corresponded with approximately 0.1% of the total C content in the CG and R soils, and with approximately 0.2% of the total C content in the CM soil. In the three management treatments, the amount of MBC corresponded with approximately 3% of the total C content. The WSOC content in the CG plot was significantly larger ($p<0.05$) than the WSOC contents in both the R and CM soil. This is in accordance with results of Gregorich et al. (2000) who found substantially lower WSOC contents under continuous maize than under grass in different soil types. The WSOC contents of the R and the CM soil, however, did not differ significantly. The largest amount of MBC was also found in the CG soil. MBC amounts decreased in the same order as the total C contents in the R and the CM soil, which is in agreement with the results of Liang et al. (1998), who found a positive correlation between the amounts of MBC and whole soil C in soils under continuous corn.

3.3.6. $\delta^{13}C$ analysis of SOM fractions from the CG soil

The $\delta^{13}C$ values of whole soil C and the different SOM fractions from the three management treatments are presented in Table 3.4. The differences between the $\delta^{13}C$ values of all the SOM fractions and the $\delta^{13}C$ value of whole soil C in every management treatment are shown in Fig. 3.3 (negative or positive values indicate, respectively, depletion or enrichment in ^{13}C compared to whole soil C). As the input of organic matter into soil occurs through decomposition of dead plant material at the surface, the isotopic signature of the SOM is mainly determined by the isotopic signature of the plant material. $\delta^{13}C$ values of different tissues from the same plant, however, may show a variation of 1-3‰ (Wedin et al, 1995). The measured $\delta^{13}C$ values of below-ground and above-ground grass tissue were respectively -30.78 ± 0.05‰ and -29.74 ± 0.04‰, indicating that the above-ground tissue was more enriched in ^{13}C. Since the relative proportion by the different plant parts to the general litter input was not known, the $\delta^{13}C$ value of the input was assumed to be the average of the above and below-ground $\delta^{13}C$ values, namely -30.26 ± 0.04‰.

Among the size and density fractions, the LF 150-2000 µm fraction had the lowest $\delta^{13}C$ value, which was closest to the average $\delta^{13}C$ value of the grass residues and deviated most from the $\delta^{13}C$ value of whole soil C (Fig. 3.3). The $\delta^{13}C$ values of the IF 150-2000 µm fraction and the HF 150-2000 µm fraction were respectively 0.4‰ and 1.5‰ higher than in the LF 150-2000 µm fraction, which means that there was an increasing enrichment in ^{13}C with increasing density in the macro-organic matter. This enrichment in ^{13}C may be attributed to isotopic fractionation associated with microbial respiration during the decomposition process of dead plant material in soils (Balesdent et al., 1987; Melillo et al., 1989; Boutton, 1996). This trend of increasing ^{13}C enrichment from the LF towards

Table 3.4. $\delta^{13}C$ values of the SOM fractions from the 0-20 cm layer in the continuous grassland (CG), rotation (R) and continuous maize (CM) soil, and proportions of C_4-derived C in the R and CM soil (standard errors in brackets)

	$\delta^{13}C$ (‰)			C_4-derived C (%)	
	CG	R	CM	R	CM
Whole soil	-28.8(0.3)	-26.8(0.1)	-22.7(0.2)	11	33
Size and density fractions					
Macro-organic matter	-29.0(0.1)	-26.0(0.1)	-19.3(0.1)	16	52
LF 150-2000 µm	-30.0(0.2)	-21.2(0.2)	-15.8(0.1)	47	77
IF 150-2000 µm	-29.6(0.2)	-27.4(0.1)	-19.0(0.2)	11	57
HF 150-2000 µm	-28.5(0.1)	-27.3(0.3)	-22.8(0.1)	6	31
50-150 µm	-29.0(0.1)	-27.1(0.1)	-21.0(0.3)	10	43
<50 µm	-28.6(0.1)	-27.1(0.1)	-22.6(0.1)	8	33
WSOC	-29.5(0.3)	-27.2(0.4)	-24.5(0.5)	13	27
MBC	-27.9(0.7)	-23.8(0.3)	-21.3(0.5)	23	36

the HF 150-2000 µm fraction, and the higher C/N ratio in the LF 150-2000 µm fraction (Table 3.3) reflects that the LF 150-2000 µm fraction consisted mainly of organic matter in an early stage of decomposition while the IF and HF 150-2000 µm fractions contained relatively more humified material. The size fraction 50-150 µm had the same $\delta^{13}C$ value as the macro-organic matter, while the size fraction <50 µm showed the highest $\delta^{13}C$ value among the three size fractions (Table 3.4). This higher ^{13}C enrichment, together with the smaller C/N ratio (Table 3.3), reflects that the clay and silt associated organic matter was relatively more decomposed and transformed by microbial pocesses than the organic matter in the larger size fractions.

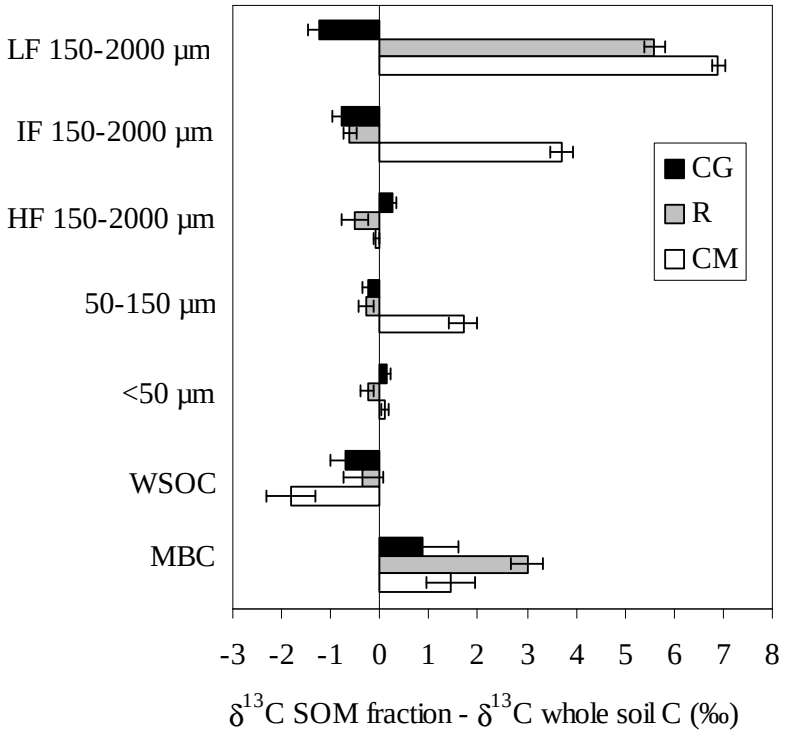


Fig. 3.3. Difference between the $\delta^{13}C$ values of the SOM fractions and whole soil C from the 0-20 cm layer in the continuous grassland (CG), rotation (R) and continuous maize (CM) soil (horizontal bars represent standard errors)

3.3.7. $\delta^{13}C$ analysis of SOM fractions from the R and CM soil

At the time of establishment of the R and CM management treatments, the SOM had a C_3 isotopic signature. Due to the incorporation of maize residues with a much higher $\delta^{13}C$ value of -11.60 ± 0.07‰, the $\delta^{13}C$ values of the SOM in the R and the CM soil shifted gradually towards higher values. When considering the CG soil as a reference soil, the $\delta^{13}C$ values of whole soil C in the R and the CM soil increased with respectively 2‰ and 6.1‰ (Table 3.4). Based on these $\delta^{13}C$ values, we calculated that respectively 11% and 33% of the total C contents in the R and CM soil were maize-derived C. Thus, the total amount of C_4-derived C incorporated after 19 years of maize cultivation in the CM

soil, and 10 years (in total) of maize cultivation in the R soil amounted to, respectively, 2.74 ± 0.07 and 1.57 ± 0.1 g C_4-C kg^{-1} soil. This equals a net input rate of 429 ± 11 kg C_4-C ha^{-1} per year in the upper 20 cm of the CM soil (taking into account a bulk density of 1.49 g cm^{-3}) and a net input rate of 450 ± 8 kg C_4-C ha^{-1} per year in the upper 20 cm of the R soil (taking into account a bulk density of 1.43 g cm^{-3}). The smaller increase of the $\delta^{13}C$ value of whole soil C in the R soil mainly reflects the lower total input of maize residues during the last 19 years in relation to the CM soil. However, when we consider the smaller proportion of maize-derived C together with the larger total C content in the R soil, we may conclude that there was a considerable contribution to the total C content of grass-derived C, due to the incorporation of grass residues before every switch to three years of maize cultivation.

Both in the R and CM soil, the influence of C_4-C incorporation on the $\delta^{13}C$ values was most pronounced in the LF 150-2000 µm fraction (Table 3.4). In the R soil, the $\delta^{13}C$ values of the other size and density fractions showed a much smaller positive shift relative to the fractions in the CG soil. In the CM soil, however, the $\delta^{13}C$ values of all the SOM fractions showed a considerable positive shift relative to the fractions from the CG soil. The proportion of relatively 'young', C_4-derived C was largest in the LF 150-2000 µm fraction, and decreased considerably with increasing density among the macro-organic matter fractions. Comparing the three size fractions, the proportions of C_4-derived C declined from the macro-organic matter towards the smallest size fraction. These results reflect a decreasing turnover rate of SOM with increasing density among the macro-organic matter fractions, and with decreasing fraction size. The decreasing proportions of C_4-derived C in smaller size fractions also reflect a slow transfer of newly introduced and partially decomposed plant material from the macro-organic matter fraction to the smallest size fraction during the decomposition process.

3.3.8. Stable C isotope analysis of WSOC and MBC

WSOC in the CG soil had the most negative $\delta^{13}C$ value (Table 3.4). In the R and CM soil, the $\delta^{13}C$ values were respectively 2.3 and 5‰ higher, which reflects an increasing proportion of maize-derived C. In the three management treatments the $\delta^{13}C$ values of WSOC showed a negative shift in relation to whole soil C, which varied between 0.4 and 1.8‰ (Fig. 3.3). MBC showed the same trend of increasing $\delta^{13}C$ values among the three management treatments. However, the contribution of maize-derived C in both R and CM soil was larger in the MBC (23 and 36%, respectively) than in the WSOC (13 and 27%, respectively), which is in accordance with the results of Ryan et al. (1995) and Gregorich et al. (2000). The average $\delta^{13}C$ values of MBC in all the management treatments were less negative than the value of whole soil C, indicating an enrichment in ^{13}C (Fig. 3.3). This enrichment ranged from 0.9‰ in the CG to 3‰ in the R soil.

Several authors found that MBC was enriched in ^{13}C relative to the bulk SOM in grassland soils (Gregorich et al., 2000; Santruckova et al., 2000) and soils with maize cultivation (Ryan et al., 1995; Gregorich et al., 2000). This enrichment may be attributed to isotopic discrimination associated with microbial metabolism, during which decomposing organisms would preferentially use isotopically lighter molecules for respiration (Blair et al., 1985; Gleixner et al., 1993), and isotopically heavier molecules for biomass synthesis (Gleixner et al., 1993; Santruckova et al., 2000). However, it is also possible that the enrichment in ^{13}C of MBC relative to whole soil C in the R and CM soil can be mainly attributed to the larger proportion of C_4-derived C in MBC relative to whole soil C in both management treatments (Table 3.4).

3.4. Conclusions

Analysis of the distribution of soil C among size fractions in the CG, R and CM soils showed that the relative contribution to the total C content in the macro-organic matter and in the size fraction 50-150 μm diminished with decreasing total C contents, while the relative contribution of C associated with the clay- and silt-sized fraction <50 μm increased. This reflects a greater stability against microbial degradation of the clay and silt associated organic C. The shifts in the $\delta^{13}C$ values together with the decrease in C/N ratios, which were observed in the size and density fractions from the CG, R and CM soils, reflected an increasing degree of microbial degradation and a decreasing turnover rate (1) with increasing density among the macro-organic matter fractions, and (2) with decreasing particle size among the size fractions considered.

CHAPTER 4

Gross N transformation rates and net N mineralization rates related to the C and N contents of soil organic matter fractions in grassland soils of different age

This chapter is compiled from:

Accoe, F., Boeckx, P., Busschaert, J., Hofman, G., Van Cleemput, O. Gross N transformation rates and net N mineralization rates related to the C and N contents of soil organic matter fractions in grassland soils of different age. (submitted to Soil Biology and Biochemistry).

Content

4. Gross N transformation rates and net N mineralization rates related to the C and N contents of soil organic matter fractions in grassland soils of different age

4.1. Introduction

Soil organic matter (SOM) plays a major role in soil quality as it improves the physical, chemical and biological properties of the soil. When arable land, which has been under long-term cultivation, is converted to permanent grassland, the SOM content gradually tends to increase due to greater organic material inputs, combined with a slower rate of SOM decomposition due to the absence of annual cultivation (Whitehead, 1995a; Haynes and Beare, 1996). This increase in SOM content is a slow process and therefore changes in the quantity and quality of the total SOM pool are usually difficult to detect in the short-term after conversion of arable land to grassland (Hassink et al., 1997; Haynes, 1999). SOM is heterogeneous and is composed of a series of pools ranging from active to passive (Schimel et al., 1985). Size and density fractionation techniques are often used for physically dividing SOM into pools which differ in composition and biological functioning (Christensen, 1992). Size fractionation is based on the observation that SOM in the sand-sized fraction (>50 µm) is generally more labile than SOM in the clay- and silt-sized fractions (Tiessen and Stewart, 1983). Density fractionation is based on the observation that during humification parts of SOM become more associated with the mineral fraction and thus occur in organomineral complexes of higher density (Barrios et al., 1996). Therefore, size and density fractionation may enable us to identify both labile, active fractions of SOM, which may respond much faster to management changes than the total SOM content, and passive fractions, which are more related to long-term SOM dynamics (Janzen et al., 1992; Barrios et al., 1996).

The net N mineralization from plant residues and SOM in grassland soils results from the balance between gross N mineralization and immobilization by the soil microbial biomass. The quality and quantity of soil organic C has been suggested to be a major factor affecting N dynamics in soils (Hart et al., 1994). Changes in SOM contents can influence the N dynamics in soils, because of the importance of available C for microbial immobilization of N (Compton and Boone, 2002) and denitrification (Whitehead, 1995c). Schimel (1986) for example found that N immobilization was lower in cropland than in native grassland, suggesting that microbial activity in the cropland was limited by C substrate availability. Barret and Burke (2000) suggested that soils with high SOM content and high C/N ratios may immobilize more N than soils with less SOM because of a limitation of reduced C substrate for microbial metabolism. Organic substrates with high C/N ratios often support microbial communities that are N-limited and generally exhibit higher rates of N immobilization, presumably because these micro-organisms require additional N to metabolize material with a high C content relative to the N content (Sollins et al., 1984; Janssen, 1996).

The first objective of this study was to investigate the accumulation of SOM and eventual shifts in the distribution of organic C and N among five size and density fractions of SOM after conversion of long-term arable land to permanent grassland. The second objective was to investigate the influence of the total SOM content and the distribution of C and N among the size and density fractions on (1) the gross N transformation rates (mineralization, nitrification and immobilization), determined by means of the ^{15}N-isotope dilution technique, and (2) on the long-term net N mineralization and immobilization rates. All experiments were carried out on three sandy loam grassland soils of 6, 14 and approximately 50 years old, respectively.

4.2. Materials and Methods

4.2.1. Site description and soil sampling

Soil samples were collected from three adjacent permanent grassland soils of different age, located at Deerlijk (50°49' N, 3°23' E), Belgium, during August and September 2002. These soils had been converted from continuous arable cropping (during at least 20 years) to permanent grassland (mainly *Lolium perenne*) since respectively 6, 14 and approximately 50 years at the time of sampling. The 6 years old grassland (6.8% clay, 17.3% silt) and the 14 years old grassland (8.8% clay, 31.5% silt) were moderately dry sandy loam soils, whereas the 50 years old grassland (5.9% clay, 21.3% silt) was a moderately wet sandy loam soil. The 6, 14 and 50 years old grassland soil will be further referred to as the D6, D14 and D50 soil, respectively. Total C and N content, bulk density and pH-H_2O in the 0-10 and 10-20 cm soil layers of the three soils are shown in Table 4.1. The grasslands were grazed and cut one to two times per year, and received an annual input of 230 kg organic N ha^{-1} as cattle manure and 120 kg mineral N ha^{-1}.

From each grassland soil, three replicate bulk samples from the 0-10 and 10-20 cm soil layers were composited, each consisting of 20 replicate soil cores covering the entire area of the investigated grassland. The soil cores were taken with a steel auger (3.5 cm diameter). The replicate bulk samples were homogenized and sieved on a 2 mm sieve to remove root material. Part of the fresh bulk samples was stored in plastic bags at 4°C until the start of the ^{15}N isotope dilution experiments, the rest was air-dried and stored until size and density fractionation or the start of the long-term incubation experiments. The size and density fractionations and incubation experiments were all performed in triplicate, using subsamples of the three replicate bulk samples from each plot and soil layer.

Table 4.1. Total C and N content, bulk density and pH-H_2O in the 0-10 and 10-20 cm layers of the D6, D14 and D50 soil (mean values of three replicates, standard deviations in brackets); values followed by the same letter in the same layer are not significantly different (P<0.05); values followed by * in the 0-10 cm layer are significantly different from the corresponding values in the 10-20 cm layer

Soil	Depth	C content	N content	Bulk density	pH-H_2O
	(cm)	(g kg^{-1} soil)		(g cm^{-3})	(-)
D6	0-10	20.9 (0.8)a*	1.80 (0.07)a*	1.29 (0.06)	5.8
	10-20	13.2 (0.4)a	1.11 (0.04)a	1.35 (0.05)	6.1
D14	0-10	22.9 (1.0)a*	2.06 (0.06)a*	1.24 (0.10)	5.9
	10-20	11.0 (0.8)a	0.97 (0.08)a	1.34 (0.06)	6.0
D50	0-10	45.8 (2.8)b*	3.75 (0.22)b*	1.17 (0.06)	6.0
	10-20	25.3 (0.2)b	1.74 (0.01)b	1.15 (0.03)	6.1

4.2.2. Size and density fractionation of soil organic matter

The soil organic matter was separated into five fractions: the light (density <1.13 g cm^{-3}), intermediate (1.13 g cm^{-3}< density <1.37 g cm^{-3}) and heavy density fraction (density >1.37 g cm^{-3}) of particulate macro-organic matter (150-2000 µm), the size fraction 50-150 µm and the size fraction <50 µm.

The three density fractions of the macro-organic matter were obtained following a slightly modified version of the method developed by Meijboom et al. (1995). Instead of using field-moist soil samples, 250 g of air dried soil from each replicate bulk sample was rewetted with 750 ml of water in a 2000 ml glass beaker and mildly dispersed by shaking on an orbital shaker at 175 rev. $min.^{-1}$ during one hour. By means of this pretreatment, most of the macro-aggregates (>250 µm) were broken down in order to release the particulate organic matter before the start of the wet-sieving procedure. The soil

suspension was then wet-sieved over two stacked sieves (top sieve mesh size 250 µm, bottom sieve mesh size 150 µm) by means of a wet sieving machine (AS200 Control g, Retsch). Any intact macro-aggregates (>250 µm) remaining on the top sieve were gently pushed through, to ensure a complete breakdown of the macro-aggregates. The two size fractions retained on the sieves were washed into a bucket and swirled with a jet of water to separate the macro-organic matter from the mineral fraction by decantation. The macro-organic matter was then further separated into a light (LF 150-2000 µm), intermediate (IF 150-2000 µm) and heavy density fraction (HF 150-2000 µm) by subsequent submersion in colloidal silica suspensions (Ludox, Dupont) with a density of 1.37 g cm^{-3} and 1.13 g cm^{-3}. The obtained density fractions were washed with demineralized water, dried at 50°C during 48 hours and ground with a planetary ball mill (PM400, Retsch) for total C and N analysis. As there was always a certain amount of heavy density particulate organic matter which couldn't be separated from the mineral fraction by decantation, this mineral fraction was also analyzed for its total C and N content. The C and N contents reported for the HF 150-2000 µm density fraction in the following sections are the sum of the C and N contents in the mineral fraction 150-2000 µm and the heavy density fraction, obtained after density separation.

For the separation of the size fractions 50-150 µm and <50 µm, 50 g of air dried soil from each replicate bulk sample was rewetted with 150 ml of water in a 500 ml glass beaker and dispersed in the same way as described before. The soil suspension was then wet-sieved over three stacked sieves on the sieving machine (top sieve mesh size 250 µm, middle sieve mesh size 150 µm, bottom sieve mesh size 50 µm). The suspension passing the bottom sieve was collected and centrifuged (Labofuge GL, Heraeus Sepatech) at 3000 rev. $min.^{-1}$ during 5 min. in order to obtain the size fraction <50 µm. The obtained size fractions 50-150 µm and <50 µm were also dried at 50°C during 48 hours. As micro-aggregates (<250 µm) are stable to slaking by rapid-wetting and wet-sieving (Tisdall and Oades, 1982), it could be assumed that the size fraction 50-150 µm consisted of a mixture

of water-stable micro-aggregates >50µm, particulate organic matter and sand, and that the <50 µm fraction mainly consisted of water-stable micro-aggregates and to a lesser extent of primary mineral particles.

4.2.3. Incubations

We conducted two sets of laboratory incubations in order to study the N dynamics in the three grassland soils. Fully-mirrored ^{15}N isotope dilution experiments (7-day incubations) were conducted to determine the short-term potential gross N transformation rates. 70-day incubations have been carried out to determine the long-term potential net N mineralization rates.

For the ^{15}N isotope dilution experiments, the fresh soil samples were shortly dried to obtain a gravimetric water content (GWC) corresponding to a water filled pore space of 50% at the bulk density measured in the field (Table 4.1), minus the amount of ^{15}N-labelling solution which would be added to the soils at the beginning of the experiment (corresponding with 6.7% GWC). The soils were pre-incubated during 7 days at 15°C. At the start of the incubation experiment, in total 18 disposable jars per soil layer and grassland soil were filled with an amount of soil equivalent to 60 g oven-dry weight. In order to determine the potential gross N mineralization (ammonification) and NH_4^+-immobilization rates, 4 ml of a ^{15}N-enriched (10.23 atom%) $^{15}NH_4{}^{14}NO_3$-solution, equivalent to 30 mg N kg^{-1} soil, was added by means of a disposable syringe to 9 of the jars. In order to determine the potential gross nitrification and NO_3^--immobilization rates, 4 ml of a ^{15}N-enriched (10.4 atom%) $^{14}NH_4{}^{15}NO_3$-solution of the same concentration was added to the 9 other jars. After label addition, the soils were thoroughly mixed in order to ensure a homogeneous label distribution, the bulk densities were adjusted to the values measured in the field (resulting in a water filled pore space of 50%), covered with pin-holed parafilm to enable gas exchange and incubated at 15°C. After 1, 3 and 7 days of

incubation, three replicate $^{15}NH_4{}^{14}NO_3$- and $^{14}NH_4{}^{15}NO_3$-labelled samples were extracted with 180 ml of 2M KCl (60 min. shaking) for NH_4^+-, NO_3^-- and ^{15}N-analysis.

For the 70-day incubations, the air-dried soil samples were rewetted to a gravimetric water content corresponding with a water filled pore space of 50% at the bulk density measured in the field (Table 4.1) and pre-incubated during 7 days at 15°C. At the start of the incubation experiment, 18 disposable jars per soil layer and grassland soil were filled with an amount of soil equivalent to 60 g oven-dry weight, which was adjusted to the bulk density observed in the field, thus resulting in a water filled pore space of 50%. The jars were covered with pin-holed parafilm, incubated at 15°C and after 7, 14, 28, 42, 56 and 70 days of incubation, three replicate samples were extracted with 180 ml of 2M KCl (60 min. shaking) for NH_4^+- and NO_3^--analysis. The potential net N mineralization rates (net production rate of NH_4^+- and NO_3^--N) were calculated by means of a linear regression of the evolution of the total mineral N content at the different sampling dates from day 7 till day 70 of the incubation experiment.

4.2.4. Chemical analysis

Analyses of the total C and N content in the soil samples and the different SOM fractions was performed using a CNS analyzer (Vario Max CNS, Elementar, Germany). The NH_4^+ and NO_3^- concentrations in the KCl extracts were determined colorimetrically by means of a continuous flow analyzer (Skalar, The Netherlands). Isotope ratio analysis of the NH_4^+- and NO_3^--pool was performed after chemical conversion to N_2O. NH_4^+ was converted quantitatively to N_2O using NaOBr according to a protocol adapted from Hauck (1982) and Saghir et al. (1993). NO_3^- was converted quantitatively to N_2O according to Stevens and Laughlin (1994). Isotope ratio analysis of the produced N_2O was carried out using a trace gas preparation unit (ANCA-TGII, PDZ Europa, UK) coupled to an Isotope Ratio Mass Spectrometer (20-20, PDZ Europa, UK).

4.2.5. Calculation of the gross N transformation rates

The potential gross N mineralization and gross NH_4^+ consumption rates were calculated from the $^{15}NH_4$-pool dilution in the $^{15}NH_4{}^{14}NO_3$-labelled samples between day 1, 3 and 7 following the equations of Kirkham and Bartholomew (1954):

$$m = \frac{\left[NH_4^+\right]_0 - \left[NH_4^+\right]_t}{t} \times \frac{\log\left(APE_0 / APE_t\right)}{\log\left(\left[NH_4^+\right]_0 / \left[NH_4^+\right]_t\right)} \quad (4.1)$$

$$c_A = m - \frac{\left[NH_4^+\right]_t - \left[NH_4^+\right]_0}{t} \quad (4.2)$$

where m = gross N mineralization rate (mg N kg^{-1} d^{-1}); c_A = NH_4^+ consumption rate (mg N kg^{-1} d^{-1}); t = time (days); APE_0 = atom% ^{15}N in excess of the NH_4^+-pool at time 0; APE_t = atom% ^{15}N in excess of the NH_4^+-pool at time t; $[NH_4^+]_0$ = total NH_4^+ concentration (mg N kg^{-1}) at time 0; $[NH_4^+]_t$ = total NH_4^+ concentration (mg N kg^{-1}) at time t. The potential gross nitrification (n) and gross NO_3^- consumption rates (c_N) were calculated from the $^{15}NO_3$-pool dilution in the $^{14}NH_4{}^{15}NO_3$-labelled samples between day 1, 3 and 7 following the same equations, by substituting the NO_3^- concentrations and atom% ^{15}N in excess of the NO_3^--pool into equations (4.1) and (4.2). The potential gross NH_4^+ immobilization rate was then calculated by subtracting the gross nitrification rate from the gross NH_4^+ consumption rate, in the assumption that NH_4^+ consumption through volatilization was zero. In the assumption that NO_3^- consumption through denitrification was negligible at a water filled pore space of 50% (Linn and Doran, 1984), the gross NO_3^- immobilization rate was equivalent to the gross NO_3^- consumption rate.

4.2.6. Statistical analysis

All statistical analyses of the data were performed using SPSS (version 11.0.1). Statistical analysis of the differences in the N transformation rates and differences in C and N contents between plots or SOM fractions within depth was conducted by analysis of variance (one-way ANOVA). Pairwise comparisons of means was conducted using Tukey's honestly significant difference test with a significance level of $\alpha = 0.05$. Stepwise multiple linear regression analysis (backward procedure, significance level for the F value was 0.1 for removal from the model) was used to study the relations between the gross and net N transformation rates and the SOM contents.

4.3. Results

4.3.1. Total organic C and N contents

At 0-10 cm depth, both total C and N contents showed an increase with increasing age of the investigated grasslands (Table 4.1). Total C and N contents in the D14 and D50 soil were respectively about 1.1 and 2.1 times larger than in the D6 soil. At 10-20 cm depth, no trend of increasing C and N contents with increasing age was observed, but the C and N contents in the D50 soil were significantly ($P<0.05$) larger than in the D6 and D14 soils. In the three grassland soils, total C and N contents at 0-10 cm depth were significantly larger (1.6 to 2.2 times) than at 10-20 cm depth.

4.3.2. C and N contents in the size and density fractions of soil organic matter

The C and N contents and the proportions of total C and N recovered in the different size and density fractions from the D6, D14 and D50 soils are shown in Tables 4.2 and 4.3, respectively. In both investigated soil layers, the largest C and N contents were found in the size fraction <50 µm. The proportions of total C and N stored in the size fraction <50 µm ranged from 44.2 to 56.9% in the 0-10 cm soil layers, and from 62.4 to 78.4% in the 10-20 cm soil layers. The C and N contents and the proportions of total C and N stored in the SOM fractions generally decreased in the order <50 µm > 50-150 µm > HF 150-2000 µm > IF 150-2000 µm > LF 150-2000 µm. In the three soils investigated, the proportions of total C and N stored in the size fraction <50 µm were considerably larger in the 10-20 cm soil layer in relation to the 0-10 cm layer, whereas the proportions of total C and N in all the SOM fractions >50 µm were smaller.

In the 0-10 cm soil layer, the C and N contents in the <50 µm, 50-150 µm and HF 150-2000 µm fractions increased in the order D6<D14<D50, whereas the C and N contents in the LF 150-2000 µm and IF 150-2000 µm fractions remained nearly constant or even decreased. The increase in C and N contents was, however, only significant ($P<0.05$) for the HF 150-2000 µm fraction. If we compare the C and N contents of the SOM fractions from the D50 soil with the corresponding SOM fractions from the D6 soil, the largest relative increase in C and N contents occurred in the HF 150-2000 µm fraction (C and N contents respectively 5.1 and 6.1 times larger), followed by the 50-150 µm fraction (C and N contents respectively 2.7 and 2.6 times larger) and the <50 µm fraction (C and N contents respectively 2 and 1.8 times larger). This was also reflected in the proportions of total C and N stored in the HF 150-2000 µm fraction and the 50-150 µm fraction, which were significantly larger in the D50 soil than in the D6 and D14 soils,

Table 4.2. C contents and the proportions of total C in the different size and density fractions from the 0-10 and 10-20 cm layers of the D6, D14 and D50 soil (mean values of three replicates, standard deviations in brackets); values followed by the same lowercase letter within a SOM fraction or depth and among grassland soils are not significantly different (P<0.05); values followed by the same uppercase letter within a grassland soil or depth and among SOM fractions are not significantly different; values followed by * in the 0-10 cm layer are significantly different from the corresponding values in the 10-20 cm layer

		C content ($g\ kg^{-1}$ soil)			Proportion of total C (%)		
Depth (cm)	SOM fraction	Soil D6	Soil D14	Soil D50	Soil D6	Soil D14	Soil D50
0-10	LF 150-2000 µm	0.32 (0.08) aA*	0.54 (0.11) bA*	0.17 (0.05) aA	1.7	2.5	0.4
	IF 150-2000 µm	2.08 (0.27) aB*	1.18 (0.12) bA*	2.25 (0.18) aB*	10.8	5.5	5.0
	HF 150-2000 µm	1.63 (0.13) aB*	3.27 (0.27) bB*	8.35 (0.58) cC*	8.5	15.1	18.6
	50-150 µm	5.40 (0.45) aC*	6.10 (1.49) aC*	14.33 (0.19) bD*	28.2	28.2	31.9
	<50 µm	9.74 (0.77) aD*	10.54 (0.35) aD*	19.87 (0.46) bE*	50.8	48.7	44.2
10-20	LF 150-2000 µm	0.14 (0.04) aA	0.07 (0.02) bA	0.08 (0.01) aA	1.1	0.6	0.4
	IF 150-2000 µm	0.32 (0.01) aA	0.17 (0.03) bA	0.34 (0.05) aB	2.6	1.6	1.4
	HF 150-2000 µm	1.07 (0.11) aB	0.66 (0.08) bB	1.69 (0.15) cC	8.8	6.2	7.1
	50-150 µm	2.34 (0.12) aC	2.27 (0.08) aC	6.38 (0.14) bD	19.3	21.3	26.8
	<50 µm	8.26 (0.09) aD	7.48 (0.86) aD	15.32 (0.55) bE	68.1	70.3	64.3

Table 4.3. N contents and the proportions of total N in the different size and density fractions from the 0-10 and 10-20 cm layers of the D6, D14 and D50 soil (mean values of three replicates, standard deviations in brackets); values followed by the same lowercase letter within a SOM fraction or depth and among grassland soils are not significantly different ($P<0.05$); values followed by the same uppercase letter within a grassland soil or depth and among SOM fractions are not significantly different; values followed by * in the 0-10 cm layer are significantly different from the corresponding values in the 10-20 cm layer

		N content ($g\ kg^{-1}$ soil)			Proportion of total N (%)		
Depth (cm)	SOM fraction	Soil D6	Soil D14	Soil D50	Soil D6	Soil D14	Soil D50
0-10	LF 150-2000 µm	0.018(0.004)aA*	0.030(0.006)bA*	0.011(0.002)aA*	1.1	1.6	0.3
	IF 150-2000 µm	0.131(0.017)aB*	0.074(0.010)bA*	0.166(0.017)aB*	8.0	3.9	4.6
	HF 150-2000 µm	0.114(0.025)aAB*	0.255(0.023)bB*	0.690(0.045)cC*	7.0	13.6	19.1
	50-150 µm	0.438(0.050)aC*	0.488(0.140)aC*	1.124(0.038)bD*	26.9	26.0	31.1
	<50 µm	0.926(0.071)aD*	1.032(0.031)aD*	1.629(0.037)bE*	56.9	54.9	45.0
10-20	LF 150-2000 µm	0.008(0.002)aA	0.003(0.001)bcA	0.005(0.001)acA	0.8	0.4	0.3
	IF 150-2000 µm	0.019(0.001)aA	0.008(0.002)bA	0.019(0.019)aA	1.9	0.8	1.2
	HF 150-2000 µm	0.067(0.003)aB	0.043(0.011)aB	0.127(0.020)bB	6.7	4.6	8.1
	50-150 µm	0.161(0.013)aC	0.144(0.036)aC	0.433(0.011)bC	16.0	15.3	27.8
	<50 µm	0.750(0.015)aD	0.744(0.102)aD	0.976(0.041)bD	74.7	78.9	62.6

whereas the proportions of total C and N stored in the <50 µm fraction were significantly smaller. In the 10-20 cm soil layer, the C and N contents in the LF 150-2000 µm and IF 150-2000 µm fractions also remained nearly constant or decreased with increasing age of the investigated grasslands. In contrast with the 0-10 cm soil layer, no trend of increasing C nor N contents was observed in the <50 µm, 50-150 µm and HF 150-2000 µm fractions in the order of D6<D14<D50. However, the C and N contents in these fractions were still significantly larger in the D50 soil than in the D6 and D14 soils.

Table 4.4. C/N ratios of the different size and density fractions from the 0-10 and 10-20 cm layers of the D6, D14 and D50 soil (mean values of three replicates, standard deviations in brackets)

		C/N ratio (-)		
Depth (cm)	SOM fraction	Soil D6	D14	D50
0-10	LF 150-2000 µm	18.1 (0.1)	17.8 (0.8)	15.6 (2.3)
	IF 150-2000 µm	15.9 (0.2)	16.0 (0.5)	13.6 (0.3)
	HF 150-2000 µm	14.4 (1.1)	12.8 (0.4)	12.1 (0.2)
	50-150 µm	12.4 (0.4)	12.6 (0.5)	12.8 (0.3)
	<50 µm	10.5 (0.1)	10.2 (0.1)	12.2 (0.1)
10-20	LF 150-2000 µm	18.1 (0.4)	19.7 (0.1)	18.4 (1.0)
	IF 150-2000 µm	17.1 (0.8)	22.1 (3.4)	17.8 (0.9)
	HF 150-2000 µm	15.9 (1.3)	15.7 (2.3)	13.5 (0.9)
	50-150 µm	14.6 (1.0)	15.8 (0.7)	14.7 (0.1)
	<50 µm	11.0 (0.1)	10.1 (0.2)	15.7 (0.1)

In both layers of the D6 and D14 soil, the C/N ratios of the SOM fractions decreased in the order LF 150-2000 µm > IF 150-2000 µm > HF 150-2000 µm > 50-150 µm > <50 µm (Table 4.4). In the D50 soil, the C/N ratios of the SOM fractions also decreased in the order LF 150-2000 µm > IF 150-2000 µm > HF 150-2000 µm, but no

further trend of decreasing C/N ratios was observed in the 50-150 µm and <50 µm fractions. The C/N ratios of the SOM fractions were generally lower in the 0-10 cm than in the 10-20 cm layer.

4.3.3. Gross N transformation rates and net N mineralization rates

The size and ^{15}N-enrichment of the NH_4^+- and NO_3^-- pools at day 1, 3 and 7 after the ^{15}N-label additions, are shown in Table 4.5 and Table 4.6, respectively. In both investigated layers, the NH_4^+- contents decreased as a function of time, except in the 10-20 cm layer of the D50 soil, where the NH_4^+- contents increased slightly from day 1 to 3, followed by a strong decrease between day 3 and 7. In both layers of the D14 and D50 soil, the NO_3^--contents increased as a function of time, whereas in the D6 soil the NO_3^--contents remained nearly constant during the entire incubation period.

The gross N transformation rates, which have been calculated for the intervals day 1-day 3 and day 3-day 7, together with the weighted average transformation rates for the interval day 1-day 7, are shown in Table 4.7. The average (day 1-day 7) gross N mineralization and gross nitrification rates tended to increase with increasing SOM contents, in the order D6<D14<D50 in both soil layers investigated (Table 4.7). The average (day 1-day 7) potential gross N mineralization rates in the 0-10 cm layer were approximately 2 times (D6 and D14 soils) to 3 times larger (D50 soil) than in the 10-20 cm layer. These gross mineralization rates correspond with a gross mineralization of 643, 982 and 1876 kg N ha^{-1} y^{-1} in the upper 20 cm of the D6, D14 and D50 soil, respectively. The average potential gross nitrification rates in the 0-10 cm layer were, respectively, 1.8, 1.6 and 1.5 times larger than in the 10-20 cm layer.

Table 4.5. Size of the NH_4^+- and NO_3^--pool at day 1, 3 and 7 after addition of the NH_4NO_3-solution in the 0-10 cm and 10-20 cm layers of the D6, D14 and D50 soil (mean values of three replicates, standard deviations in brackets)

Soil	Depth (cm)	NH_4^+ (mg N kg^{-1} soil)			NO_3^- (mg N kg^{-1} soil)		
		d_1	d_3	d_7	d_1	d_3	d_7
D6	0-10	11.8(1.7)	5.1(0.7)	1.8(0.9)	25.1(3.6)	22.3(2.2)	24.2(3.8)
	10-20	11.2(1.5)	6.9(0.7)	3.3(0.9)	14.2(2.3)	13.2(0.5)	14.3(0.6)
D14	0-10	12.8(0.9)	9.7(1.3)	4.4(1.8)	22.6(2.2)	28.5(3.4)	36.2(3.9)
	10-20	12.8(1.7)	11.7(0.8)	6.5(0.3)	14.0(0.6)	18.5(2.4)	24.9(2.2)
D50	0-10	18.7(3.8)	9.2(1.5)	2.3(0.1)	67.9(9.0)	74.5(2.6)	86.6(3.4)
	10-20	30.3(1.3)	32.5(2.3)	9.6(0.7)	35.9(4.3)	43.5(1.8)	45.3(2.4)

Table 4.6. ^{15}N-enrichment of the NH_4^+-pool at day 1, 3 and 7 after addition of the $^{15}NH_4{}^{14}NO_3$-solution, and ^{15}N-enrichment of the NO_3^--pool at day 1, 3 and 7 after addition of the $^{14}NH_4{}^{15}NO_3$-solution in the 0-10 cm and 10-20 cm layers of the D6, D14 and D50 soil (mean values of three replicates, standard deviations in brackets)

Soil	Depth (cm)	NH_4^+ (atom% ^{15}N in excess)			NO_3^- (atom% ^{15}N in excess)		
		d_1	d_3	d_7	d_1	d_3	d_7
D6	0-10	6.8(0.3)	4.9(0.5)	2.4(0.4)	4.9(0.6)	4.2(0.6)	3.5(0.4)
	10-20	7.2(0.4)	6.6(0.4)	4.6(0.2)	7.1(0.3)	6.2(0.3)	5.2(0.1)
D14	0-10	7.9(0.2)	6.3(0.3)	2.8(0.9)	4.9(0.2)	4.3(0.1)	3.6(0.1)
	10-20	8.7(0.3)	7.8(0.4)	5.8(0.2)	6.7(0.2)	6.0(0.1)	5.0(0.1)
D50	0-10	3.5(0.2)	2.3(0.1)	0.5(0.1)	2.7(0.1)	2.4(0.1)	2.0(0.1)
	10-20	3.9(0.3)	3.7(0.2)	2.9(0.2)	3.5(0.1)	3.2(0.1)	2.5(0.2)

Table 4.7. Gross N mineralization, nitrification, NH_4^+ and NO_3^- immobilization rates calculated for the intervals day 1-day 3 and day 3-day 7 of the ^{15}N isotope dilution experiments, weighted average values of the gross rates for the interval day 1-day 7 (mean values of three replicates, standard deviations in brackets), net N mineralization rates measured between day 7 and day 70 of the long-term incubation experiments and gross N immobilization rates calculated by difference between the gross N mineralization rates and long-term net N mineralization rates in the 0-10 cm and 10-20 cm layers of the D6, D14 and D50 soil (mean values of three replicates, standard deviations in brackets); values at the same depth followed by the same letter are not significantly different; values followed by * in the 0-10 cm layer are significantly different from the corresponding values in the 10-20 cm layer

	N transformation rates (mg N kg^{-1} soil d^{-1})					
	D6		D14		D50	
	0-10	10-20	0-10	10-20	0-10	10-20
Gross N mineralization *(^{15}N isotope dilution)*						
d_1-d_3	1.44 (0.33)	0.42 (0.61)	1.29 (0.32)	0.65 (0.13)	3.12 (0.16)	0.56 (0.23)
d_3-d_7	0.62 (0.28)	0.46 (0.14)	1.49 (0.16)	0.71 (0.10)	3.39 (0.31)	1.37 (0.16)
d_1-d_7	0.89 (0.20)a*	0.45 (0.16)a	1.42 (0.20)b*	0.69 (0.02)a	3.30 (0.18)c*	1.10 (0.18)b
Gross nitrification *(^{15}N isotope dilution)*						
d_1-d_3	1.94 (0.62)	0.92 (0.13)	1.76 (0.35)	0.94 (0.08)	5.13 (1.18)	2.29 (0.07)
d_3-d_7	1.09 (0.17)	0.68 (0.14)	1.68 (0.15)	1.11 (0.07)	3.73 (0.08)	3.02 (0.86)
d_1-d_7	1.37 (0.09)a*	0.76 (0.14)a	1.70 (0.21)a*	1.05 (0.02)a	4.20 (0.35)b*	2.78 (0.56)b
Gross NH_4^+ immobilization *(^{15}N isotope dilution)*						
d_1-d_3	2.81 (0.70)	1.65 (0.94)	1.07 (0.47)	0.25 (0.52)	2.73 (1.51)	-2.82 (1.58)
d_3-d_7	0.36 (0.28)	0.68 (0.09)	1.15 (0.33)	0.90 (0.30)	1.39 (0.60)	4.08 (1.34)
d_1-d_7	1.17 (0.40)a	1.00 (0.32)a	1.12 (0.38)a	0.71 (0.28)a	1.83 (0.41)a	2.72 (0.89)b
Gross NO_3^- immobilization *(^{15}N isotope dilution)*						
d_1-d_3	3.36 (2.01)	1.41 (1.56)	-1.22 (1.50)	-1.31 (1.38)	1.83 (4.81)	-1.56 (2.95)
d_3-d_7	0.61 (0.78)	0.39 (0.15)	-0.25 (1.70)	-0.50 (1.17)	0.71 (1.35)	2.58 (1.14)
d_1-d_7	1.57 (0.27)a	0.78 (0.44)ab	0.42 (0.59)a	0.19 (0.33)a	1.58 (1.65)a	1.84 (0.96)b
Net N mineralization *(long- term incubation)*						
	0.32 (0.08)a*	0.09 (0.03)a	0.33 (0.03)a*	0.12 (0.07)a	0.58 (0.10)a*	0.06 (0.03)a
Gross N immobilization *(calculated by difference, long-term incubation)*						
	0.57 (0.13)a	0.36 (0.15)a	1.09 (0.21)a*	0.58 (0.09)a	2.72 (0.38)b*	1.04 (0.21)b

Calculation of the gross NH_4^+ and NO_3^- immobilization rates using the formulas (4.1) and (4.2) resulted in negative values in some cases (NO_3^- immobilization rates in the D14 soil and NH_4^+ and NO_3^- immobilization rates between day 1 and 3 in the 10-20 cm layer of the D50 soil) (Table 4.7). However, in nearly all cases these negative values were not significantly different from zero. Thus, when negative values were obtained for one of the replicate immobilization rates between day 1-day 3 or day 3-day 7, these could be set to zero for the calculation of the weighted average immobilization rates between day 1 and 7. Negative values for gross immobilization rates, calculated with the same formulas, have often been reported in the literature (Watson and Mills, 1998; Watson et al., 2000; Wang et al., 2001; Verchot et al., 2002). Müller et al. (2004) suggested that these negative rates would be related to fast immobilization and subsequent remineralization of the added ^{15}N-enriched NH_4^+- or NO_3^--pool, which cannot be accounted for by means of the analytical solution for gross rate calculations.

In both layers of the D6 soil and the 10-20 cm layer of the D50 soil, the sum of the average (day 1-day 7) gross NH_4^+ and NO_3^- immobilization rates (gross consumption of mineral N) was larger than the average (day 1-day 7) gross mineralization rates (gross production of mineral N), which was reflected by the decrease in total mineral N contents between day 1 and day 7 (Table 4.5). This indicates that net N-immobilization occurred during the incubation period in these soils. In the other soil layers investigated, the average gross mineralization rates were larger (D14 soil) or nearly equal (0-10 cm layer of the D50 soil) in relation to the sum of the average gross NH_4^+ and NO_3^- immobilization rates, which was reflected by an increase in the total mineral N contents between day 1 and day 7 (Table 4.5). This indicates that net N mineralization occurred in these soil layers during the incubation.

In the 0-10 cm layer, the largest long-term net mineralization rate was observed in the D50 soil, which was 1.8 times larger than in the D6 and D14 soils. In the 10-20 cm

layer however, the D50 soil showed the smallest net mineralization rate among the three soils. The measured net mineralization rates correspond with a net mineralization of 195, 208 and 274 kg N ha^{-1} y^{-1} in the upper 20 cm of the D6, D14 and D50 soil, respectively.

We also estimated the gross N immobilization rates (NH_4^+ plus NO_3^- immobilization) during the long-term incubation experiments by subtracting the long-term net N mineralization rates from the average (day 1-day 7) gross N mineralization rates, which were determined by the ^{15}N-isotope dilution technique. This technique for calculating gross N immobilization rates is called the difference method (according to Hart et al., 1994). The gross immobilization rates calculated by this method will be referred to as gross immobilization *(difference)*, in order to distinguish them from the rates calculated by the ^{15}N-isotope dilution method, which will be referred to as gross immobilization *(^{15}N dilution)*. The gross N immobilization rates *(difference)* ranged from 0.57, 1.09 to 2.72 mg N kg^{-1} soil d^{-1} in the 0-10 cm layer, and from 0.36, 0.58 to 1.04 mg N kg^{-1} soil d^{-1} in the 10-20 cm layer of the D6, D14 and D50 soils, respectively (Table 4.7). These rates correspond with a gross N immobilization *(difference)* of, respectively, 444, 776 and 1605 kg N ha^{-1} y^{-1} in the upper 20 cm of the D6, D14 and D50 soil.

In the further discussion of the results, the gross N transformation rates mentioned in the text will refer to the weighted average gross N transformation rates calculated between day 1 and day 7.

4.3.4. Relations between gross N transformation rates, net N mineralization rates and SOM contents

The Pearson correlation coefficients between the N transformation rates (gross N mineralization, gross nitrification, gross immobilization *(difference*) and long-term net N mineralization rates, all expressed in mg N kg^{-1} soil d^{-1}) and the C contents, N contents

(expressed in mg C or N kg^{-1} soil) and C/N ratios of whole soil and the individual SOM fractions are shown in Table 4.8. The linear regression models corresponding with the largest Pearson correlation coefficients for each of the N transformation rates are included in Table 4.9. As in some cases negative values were obtained for the gross NH_4^+ and NO_3^- immobilization rates (*^{15}N dilution*) (Table 4.7), the correlation between these rates and the SOM contents was not investigated.

The gross mineralization rates showed a strong, positive correlation with the total C and N contents (Table 4.8, r=0.948 and r=0.963, respectively; Table 4.9, model 1). Considering the C and N contents in the individual SOM fractions, the gross N mineralization rates showed the strongest correlation with the C and N content in the HF 150-2000 µm fraction (Table 4.8, r=0.976; Table 4.9, model 2), followed by the N contents in the <50 µm fraction (Table 4.8, r=0.973) and the C and N contents in the 50-150 µm fraction (Table 4.8, r=0.957 and r=0.953). The gross nitrification rates showed a stronger correlation with the total C contents (Table 4.8, r=0.939; Table 4.9, model 4) than with the total N contents (Table 4.8, r=0.885) , and were best correlated with the C and N contents in the <50 µm fraction (Table 4.8, r=0.965 and r=0.898, respectively; Table 4.9, model 5) among the individual SOM fractions. The gross immobilization rates *(difference)* also showed strong, positive correlations with the total C and N contents (Table 4.8, r=0.933 and r=0.930; Table 4.9, models 7 and 8). Considering the individual SOM fractions, the gross immobilization rates *(difference)* were best correlated with the C and N contents in the HF 150-2000 µm fraction (Table 4.8, r=0.952 and r=0.954; Table 4.9, models 9 and 10) and the N contents in the <50 µm fraction (Table 4.8, r=0.954; Table 4.9, model 11). The long-term net N mineralization rates showed much weaker, positive correlations with the total C and N contents (Table 4.8, r=0.748 and r=0.828, respectively; Table 4.9, model 12) than the gross N mineralization rates, and showed the strongest correlation with the N contents in the IF 150-2000 µm fraction (Table 4.8, r=0.848; Table 4.9, model 13). None of the N transformation rates considered were

significantly correlated with the whole soil C/N ratio, and only very weak correlations were found with the C/N ratios of the SOM fractions.

Table 4.8. Pearson correlation coefficients and significance of the correlations between the N transformation rates (weighted average gross N mineralization and nitrification for the interval day 1-day 7, gross N immobilization *(difference)* and long-term net N mineralization rates) and the C contents, N contents and C/N ratios of whole soil, LF 150-2000 µm, IF 150-2000 µm, HF 150-2000 µm, 50-150 µm and <50 µm fractions

	Gross mineralization *(d_1-d_7)*	Gross nitrification *(d_1-d_7)*	Gross immobilization *(difference)*	Net mineralization *(long-term incubation)*
C_{tot}	0.948***	0.939***	0.933***	0.748***
C_{LF}	0.073	-0.096	0.004	0.337
C_{IF}	0.669***	0.504*	0.588*	0.818***
C_{HF}	0.976***	0.846***	0.952***	0.801***
$C_{50\text{-}150\ \mu m}$	0.957***	0.917***	0.940***	0.762***
$C_{<50\ \mu m}$	0.870***	0.965***	0.891***	0.542*
N_{tot}	0.963***	0.885***	0.930***	0.828***
N_{LF}	0.125	-0.051	0.053	0.383
N_{IF}	0.740***	0.571*	0.663**	0.848***
N_{HF}	0.976***	0.851***	0.954***	0.795***
$N_{50\text{-}150\ \mu m}$	0.953***	0.882***	0.927***	0.793***
$N_{<50\ \mu m}$	0.973***	0.898***	0.954***	0.782***
C/N_{tot}	0.062	0.436	0.150	-0.316
C/N_{LF}	-0.709***	-0.649**	-0.670**	-0.673**
C/N_{IF}	-0.614**	-0.516*	-0.580*	-0.583*
C/N_{HF}	-0.590**	-0.648**	-0.573*	-0.496*
$C/N_{50\text{-}150\ \mu m}$	-0.431	-0.312	-0.376	-0.541*
$C/N_{<50\ \mu m}$	0.184	0.551*	0.268	-0.212

*, **, *** : r-values significant at p<0.05, 0.01 and 0.001 respectively

Table 4.9. Linear and multiple linear regression models for the variation of the N transformation rates (weighted average gross N mineralization and nitrification for the interval day 1-day 7, gross N immobilization *(difference)* and long-term net N mineralization rates), as influenced by (1) total C contents, total N contents and whole soil C/N ratio, and (2) C contents, N contents and C/N ratios of the LF 150-2000 µm, IF 150-2000 µm, HF 150-2000 µm, 50-150 µm and <50 µm fractions

		Unstandardized regression coefficient	Standardized regression coefficient
Gross N mineralization rate *(d_1-d_7)*			
Model 1 (R^2=0.93, p<0.001)	Constant	-0.59426**	
	N_{tot}	0.00100***	
Model 2 (R^2=0.95, p<0.001)	Constant	0.41165***	
	N_{HF}	0.00416***	
Model 3 (R^2=0.97, p<0.001)	Constant	-2.27128**	
	$N_{50\text{-}150\ \mu m}$	0.00124*	0.431
	$N_{<50\ \mu m}$	0.00215**	0.679
	$C/N_{50\text{-}150\ \mu m}$	0.09757**	0.188
	$C/N_{<50\ \mu m}$	-0.04500*	-0.093
Gross nitrification rate *(d_1-d_7)*			
Model 4 (R^2=0.88, p<0.001)	Constant	-0.32716	
	C_{tot}	0.00010***	
Model 5 (R^2=0.93, p<0.001)	Constant	-1.15886***	
	$C_{<50\ \mu m}$	0.00026***	
Model 6 (R^2=0.92, p<0.001)	Constant	-2.90391**	
	C_{tot}	0.00009***	0.885
	C/N_{tot}	0.22298*	0.214
Gross N immobilization rate *(difference)*			
Model 7 (R^2=0.88, p<0.001)	Constant	-0.47965*	
	C_{tot}	0.00007***	
Model 8 (R^2=0.87, p<0.001)	Constant	-0.50431**	
	N_{tot}	0.00082***	
Model 9 (R^2=0.91, p<0.001)	Constant	0.24657**	
	C_{HF}	0.00029***	
Model 10 (R^2=0.91, p<0.001)	Constant	0.31393***	
	N_{HF}	0.00346***	
Model 11 (R^2=0.91, p<0.001)	Constant	-1.52868***	
	$N_{<50\ \mu m}$	0.00256***	
Net N mineralization rate *(long-term incubation)*			
Model 12 (R^2=0.69, p<0.001)	Constant	-0.08995	
	N_{tot}	0.00018***	
Model 13 (R^2=0.72, p<0.001)	Constant	0.05916	
	N_{IF}	0.00274***	
Model 14 (R^2=0.83, p<0.001)	Constant	0.69438**	
	N_{tot}	0.00018***	0.858
	C/N_{tot}	-0.06560**	-0.384
Model 15 (R^2=0.84, p<0.001)	Constant	0.34105*	
	N_{tot}	0.00020***	0.909
	$C/N_{<50\ \mu m}$	-0.03993**	-0.397

Via multiple linear regression analysis we further investigated whether (1) a combination of the N contents in the individual SOM fractions, (2) a combination of the total N or C contents and the whole soil C/N ratios, or (3) a combination of the N or C contents in the individual SOM fractions and their C/N ratios could explain a larger proportion of the variability of gross and net N transformation rates, than the total SOM contents or the SOM contents in the individual fractions. As the C contents were highly correlated with the N contents in the whole soil and the individual SOM fractions (Pearson correlation coefficients ranging from 0.91 to 1), the C and N contents were only entered separately for the multiple linear regression analysis.

For all rates considered, no combination of the N contents in the individual SOM fractions could be calculated that accounted for a larger proportion of the variability of these rates than the total C or N contents or any single SOM fraction. For the gross N mineralization rates, multiple linear regression analysis resulted in one significant model (Table 4.9, model 3), which included the N contents and C/N ratios of the 50-150 µm and <50 µm fractions. This model explained 97% of the variability of the gross N mineralization rates. For the gross nitrification rates, one significant model was calculated (Table 4.9, model 6). In model 6, the whole soil C/N ratio explained only an additional 4% of the variability of gross nitrification rates compared to the linear regression model with the total C contents only (Table 4.9, model 4). Multiple linear regression analysis of the gross N immobilization rates *(difference)* resulted in the linear regression models including total C and N contents, the C and N contents in the HF 150-2000 µm fraction and the N content in the <50 µm fraction, as discussed before (Table 4.9, models 7-11). For the net N mineralization rates two significant models were calculated (Table 4.9, models 14 and 15). Model 14 included a negative correlation with the total C/N ratios, and explained 83% of the variability of the net mineralization rates. 84% of the variability of the net mineralization rates was accounted for by model 15, which included a negative correlation with the C/N ratio of the <50 µm fraction.

In order to investigate whether the long-term potential net N mineralization rates were related to the short-term potential gross N mineralization rates, the long-term net N mineralization rates in the six soil layers investigated were plotted versus the corresponding gross N mineralization rates in Figure 4.1. The relation between net and gross N mineralization rates could be significantly fitted by a logarithmic equation (net m = 0.24Ln(gross m) + 0.23, R^2=0.69, $p<0.05$). When the outlier (corresponding with the mineralization rates in the 10-20 cm layer of the D50 soil) was removed from the dataset, a much better fit was obtained (net m = 0.25Ln(gross m) + 0.27, R^2=0.92, $p<0.01$).

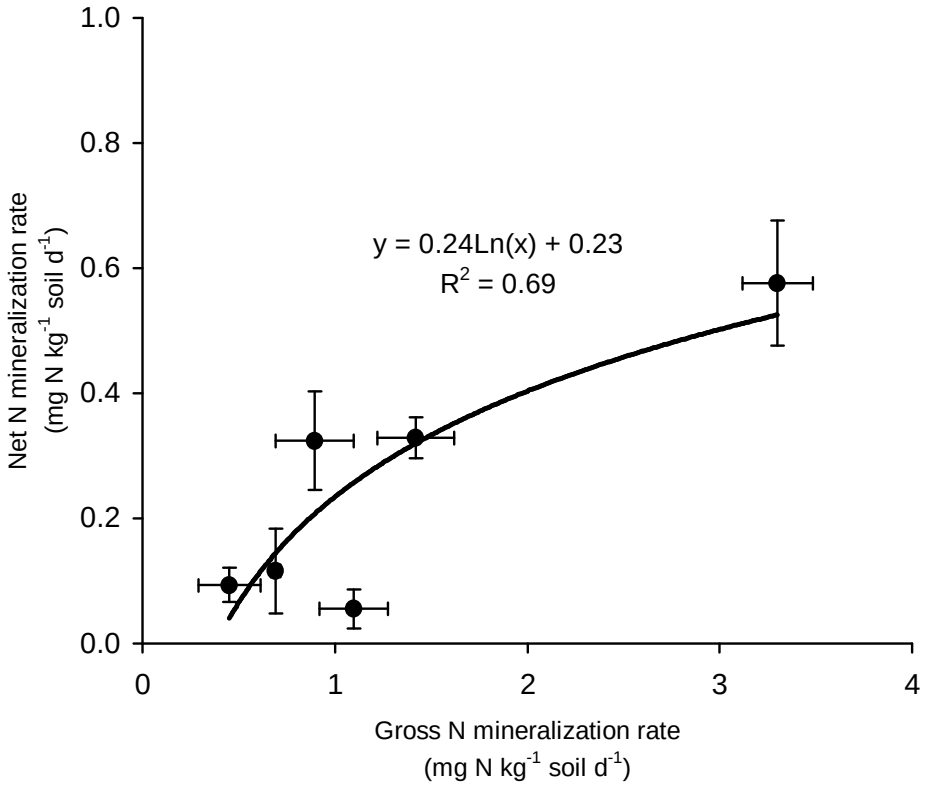


Fig. 4.1. Plot of the net N mineralization rates versus the gross N mineralization rates observed in the 0-10 cm and 10-20 cm layers of the D6, D14 and D50 soil, and fit of the relation by a logarithmic regression equation

4.4. Discussion

4.4.1. Total organic C and N contents

Accumulation of SOM when long-term arable land is converted to permanent grassland can be attributed to greater organic matter inputs under grassland through dead plant material (mainly roots), combined with a slower rate of soil organic matter decomposition due to absence of annual cultivation (Whitehead, 1995a; Haynes and Beare, 1996). Taking account of the bulk densities measured in the soils (Table 4.1), the total amounts of C and N stored in the 0-10 cm layer of the D6, D14 and D50 soil were 27, 28, and 54 t C ha^{-1}, and 2.3, 2.6 and 4.4 t N ha^{-1}, respectively (Least significant difference at $P<0.05$ = 8 t C ha^{-1} and 0.6 t N ha^{-1}, respectively). Thus, the difference in C and N storage in the 0-10 cm layer of the D6 and D14 soils was not yet significant.

In the assumption that the initial SOM contents, before conversion to permanent grassland, and the average annual input of organic material in the three soils were comparable, these results reflect that SOM accumulation after conversion of arable land to permanent grassland is a slow process, which tends to be detectable in the total C and N contents only in the long-term after conversion (D50 soil). In the 10-20 cm layer, the total amounts of C and N stored were respectively 18, 15 and 29 t C ha^{-1}, and 1.5, 1.3 and 2.0 t N ha^{-1} (Least significant difference at $P<0.05$ = 3 t C ha^{-1} and 0.3 t N ha^{-1}, respectively). The smaller increase in C and N storage in the 10-20 cm layer in relation to the 0-10 cm layer reflects that in temperate grassland systems, SOM accumulation largely tends to occur in the surface layer (Loiseau and Soussana, 1999).

4.4.2. C and N contents in the size and density fractions of soil organic matter

The contribution of the macro-organic matter fractions to the total organic C and N contents in the 0-10 cm layers ranged from 0.3 to 2.5% for the LF 150-2000 µm fraction, from 3.9 to 10.8% for the IF 150-2000 µm fraction and from 7.0 to 19.1% for the HF 150-2000 µm fraction (Tables 4.2 and 4.3). These values correspond well with the results of Hassink (1995), who reported ranges of 0.5 to 2.3% for the LF 150-2000 µm fraction, 1.3 to 8.4% for the IF 150-2000 µm fraction and 5.8 to 23.1% for the HF 150-2000 µm fraction from the 0-10 cm layer of grassland soils (> 8 years old) with a loamy texture. However, the contributions of the macro-organic matter to the total organic N content in this study were considerably larger than the values reported by Warren and Whitehead (1988), who found that 0.55 to 6.3% of total organic N was stored in the SOM fraction >200 µm in the 0-15 cm layer of grassland soils (> 20 years old). This difference may be partially explained by the smaller sampling depth (0-10 cm depth) and minimal particle size of the macro-organic matter (>150 µm) which were considered in our study.

In the 10-20 cm layer, the contribution to the total organic C and N contents of the LF 150-2000 µm fraction, which ranged from 0.4 to 1.1%, was generally slightly smaller than in the 0-10 cm layer. The contributions of the IF and HF 150-2000 µm fractions, however, which respectively ranged from 0.8 to 2.6% and from 4.5 to 8.8%, were considerably smaller than in the 0-10 cm layer and this was also observed by Hassink (1995).

The C/N ratios observed in the LF, IF and HF 150-2000 µm fractions from the 0-10 cm layers were generally slightly smaller than the values reported by Hassink (1995) (20, 18 and 14, respectively), Meijboom et al. (1995) (18-24, 15-21 and 13-16, respectively) and Warren and Whitehead (1988) (16.4-27.3 for the macro-organic matter

fraction >200 µm). This may be explained by the fact that these studies considered the macro-organic matter fraction with a size up to 8 mm (Hassink, 1995; Meijboom et al., 1995) or 6 mm (Warren and Whitehead; 1988), thus containing a larger amount of grass root material, which generally has a C/N ratio ranging from 25 to 45 (Whitehead, 1970).

The decrease of the C/N ratios in the order LF>IF>HF reflects that the LF 150-2000 µm fraction consisted mainly of partially decomposed plant residues, whereas the IF 150-2000 µm and HF 150-2000 µm fractions consisted of more humified, organo-mineral complexed SOM (Meijboom et al., 1995). The decrease in C/N ratios from the macro-organic matter fractions towards the <50 µm fraction which we observed (except in the 10-20 cm layer of the D50 soil) is consistent with the findings of several other studies (e.g. Tiessen and Stewart, 1983; Catroux and Schnitzer, 1987; Christensen, 1992) and is indicative of an increasing degree of humification from the coarser to the finer particle-size fractions of SOM.

In the 0-10 cm layer, total C and N contents and the amounts of C and N stored in the HF 150-2000 µm, 50-150 µm and <50 µm fractions tended to increase in the order D6<D14<D50, whereas this trend was not observed in the LF 150-2000 µm and IF 150-2000 µm fractions. Though there was no significant ($P<0.05$) difference detectable yet between total C and N contents nor C and N contents in the 50-150 µm and <50 µm fraction in the D6 and D14 soil, C and N contents in the HF 150-2000 µm fraction were already significantly larger (about 2 times) in the D14 soil. This indicates that in the short-term after conversion of arable land to permanent grassland, SOM derived from dead plant material initially tends to accumulate in the HF 150-2000 µm fraction. This suggests that the HF 150-2000 µm fraction could serve as a good and relatively easily detectable indicator of early SOM accumulation, or early changes in SOM content in general, induced by the conversion of cultivation to permanent grassland. The fact that no SOM accumulation was observed in the LF or IF 150-2000 µm fractions shows that these two

fractions can be considered to be SOM pools with a very short turnover time, which is consistent with the conclusions of Römkens et al. (1999). These results also indicate that the transfer of C and N from the macro-organic matter fractions towards the more humified 50-150 µm and <50 µm SOM fractions is a slow process, and that SOM accumulation in the 50-150 µm and <50 µm fraction is only detectable in the long-term after conversion of arable land to permanent grassland.

4.4.3. Gross N transformation rates and net N mineralization rates

The gross N mineralization rates observed in the 0-10 cm layer in our study (ranging from 0.89 to 3.30 mg N kg^{-1} soil d^{-1}) were comparable with the rates reported by Jamieson et al. (1999) (0.36 to 2.36 mg N kg^{-1} soil d^{-1}) and Murphy et al. (1999) (1.3 to 3.3 mg N kg^{-1} soil d^{-1}), but lower than the rates reported by Davidson et al. (1990) and Corre et al. (2002) (4.9 to 8.2 mg N kg^{-1} soil d^{-1}). The gross nitrification rates (ranging from 1.37 to 4.20 mg N kg^{-1} soil d^{-1} in the 0-10 cm layer) were comparable with the range observed by Watson et al. (2000) (1.89 to 3.71 mg N kg^{-1} soil d^{-1}), but higher than the rates observed by Davidson et al. (1990) (0.59 to 0.81 mg N kg^{-1} soil d^{-1}) and Corre et al. (2002) (0.3 to 2.8 mg N kg^{-1} soil d^{-1}). The net N mineralization rates in the 0-10 cm layer of the investigated grassland soils (0.32-0.58 mg N kg^{-1} soil d^{-1}) were in the same range as reported by Hassink (1994) (0.38-0.89 mg N kg^{-1} soil d^{-1}) for ungrazed grassland soils with comparable SOM contents.

When the gross N transformation rates in soils are estimated by means of ^{15}N isotope dilution experiments, the gross NH_4^+ consumption rates (NH_4^+ immobilization and nitrification) and gross NO_3^- consumption rates (NO_3^- immobilization) may be stimulated (priming effect) and thus overestimated, as the substrates for these processes (NH_4^+ and NO_3^-, respectively) are added to the soils (Davidson et al., 1991). Therefore, the calculated gross NH_4^+ and NO_3^- consumption rates may not reflect the N transformation processes as

they occur in the field, but rather represent the *potential* NH_4^+ immobilization, NO_3^- immobilization and nitrification activity in these soils (Watson et al., 2000). As for the calculation of the gross N mineralization rates only the product pool of this process (NH_4^+), and not the substrate pool is added in the ^{15}N-isotope dilution experiments, the thus obtained gross N mineralization rates can be assumed to be unaffected by the $^{15}NH_4$ addition (Davidson et al., 1991). In this study, the gross NH_4^+ consumption rates (d_1-d_7) were always larger than the gross mineralization rates (d_1-d_7), which obviously is not sustainable (Table 4.7). In the long-term incubation experiments, net mineralization was observed in all layers investigated. In the ^{15}N-isotope dilution experiments, however, net mineralization was only observed in the D14 soil and the 0-10 cm layer of the D50 soil, whereas in the other layers net immobilization occurred. The total gross N immobilization rates (*^{15}N dilution*) (sum of the NH_4^+ and NO_3^- immobilization rates) in the D6, D14 and D50 soils ranged from 2.74, 1.54 to 3.41 mg N kg^{-1} soil d^{-1} in the 0-10 cm layer, and from 1.78, 0.90 to 4.56 mg N kg^{-1} soil d^{-1} in the 10-20 cm layer (Table 4.7). The gross N immobilization rates (*^{15}N dilution*) were thus 1.3 to 5 times larger than the gross N immobilization rates *(difference)*, which is in contrast with the results of Hart et al. (1994), who found a very close agreement between the two methods for calculating gross N immobilization in a forest soil. These observations may suggest that NH_4^+ and NO_3^- consumption were stimulated and thus overestimated in our ^{15}N-isotope dilution experiments. As the gross N mineralization rates do not tend to be influenced by ^{15}N-addition in the isotope dilution experiments, we assume that the gross N immobilization rates *(difference)* are more representative for the gross N immobilization rates as they occur in the field than the gross N immobilization rates (*^{15}N dilution*).

4.4.4. Relations between gross N transformation rates, net N mineralization rates and SOM contents

The very strong correlation, which was observed between the gross N mineralization rates and the total N contents (Table 4.8) reflects that gross N mineralization is largely determined (93%) by the total N availability. Multiple linear regression analysis showed that up to 97% of the variability of the gross N mineralization rates could be accounted for by the N contents in the 50-150 µm and <50 µm fractions, together with their C/N ratios (Table 4.9, model 3). The standardized regression coefficient for the $N_{<50\mu m}$ fraction was approximately 1.5 times larger than for the $N_{50\text{-}150\mu m}$ fraction, which indicates a larger relative importance of the $N_{<50\mu m}$ fraction in the regression model (Table 4.9, model 3). Monaghan and Barraclough (1997) found that the contribution of macro-organic matter N (> 200 µm, d < 1 g cm^{-3}) to gross N mineralization in grassland soils was relatively small (only 2.3 to 3.4%) and they suggested that most of the N mineralized in grassland soils is derived from SOM associated with mineral particles, which is in accordance with our results.

Considering the N contents in the individual SOM fractions, the net N mineralization rates in our study were strongly correlated with the IF 150-2000 µm fraction and not significantly correlated with the LF 150-2000 µm fraction (Table 4.8). This is in contrast with the results of Hassink (1995) who found that the LF 150-2000 µm fraction showed the strongest correlation among the macro-organic matter fractions with the net N mineralization rates in the top 25 cm of grassland soils. The net N mineralization rates showed a much weaker correlation with the total N contents than the gross N mineralization rates (Table 4.8). This reflects that net N mineralization results from the balance between gross mineralization and immobilization, which is also controlled by other factors than total N availability, like C content (Table 4.9, models 7 and 9) or C/N ratio of the SOM (Table 4.9, models 14 and 15). Several studies suggest that N

immobilization and the balance between mineralization and immobilization is influenced by the available C content (Woodmansee and Duncan, 1980; Hart et al., 1993; Whitehead, 1995a; Barret and Burke, 2000) and the C/N ratio of the SOM (van Veen et al., 1984; Whitehead, 1995a; Janssen, 1996). In our study, the gross N immobilization rates *(difference)* showed a strong, positive correlation with the total C contents and especially with the C contents in the HF 150-2000 µm fraction. This is in accordance with the results of Barret and Burke (2000), who also found a significant positive, but weaker correlation (R^2=0.58) between the gross N immobilization rates and total C contents in five grassland soils.

The relation between net N mineralization and gross N mineralization rates could be well described by means of a logarithmic equation (Fig. 4.1). As net N mineralization equals gross N mineralization minus gross N immobilization *(difference)*, this logarithmic relationship indicates that the ratio of net to gross N mineralization (or the ratio of gross immobilization *(difference)* to gross mineralization) tended to decrease with increasing gross N mineralization rates in the investigated grassland soils. The ratio of the net to gross mineralization rates ranged from 0.36, 0.23 to 0.18 in the 0-10 cm layers, and from 0.20, 0.17 to 0.05 in the 10-20 cm layers of the D6, D14 and D50 soils, respectively. These results shows that the ratio of gross immobilization to gross mineralization tended to increase, with increasing SOM contents in both layers of the investigated grasslands. This trend has also been observed in forest soils by Hart et al. (1994) and reflects that the microbial demand for N (immobilization) tended to increase with increasing C availability and with increasing age of the investigated grassland soils.

In our study no significant correlations were found between the gross N immobilization rates and the whole soil C/N ratios (Table 4.8). For the net N mineralization rates, however, we did find significant, negative correlations with the whole soil C/N ratios and the C/N ratios of the <50 µm fraction via multiple linear

regression analysis (Table 4.9, models 14 and 15). Whole soil C/N ratios and the C/N ratios of the <50 µm fraction accounted for respectively 14 and 15% of the variability of the net N mineralization rates, in addition to the variability explained by the total N contents. In this way, the very small net N mineralization rate which was observed in the 10-20 cm layer of the D50 soil in relation to the D6 and D14 soils, may be partially explained by the relatively high C/N ratio in the <50 µm fraction in the D50 soil (Table 4.4).

4.5. Conclusions

The total C and N contents mainly tended to increase in the 0-10 cm layer with increasing age of the investigated grassland soils. Significant differences in total SOM storage were, however, only detectable in the long-term (D50 soil) after conversion of arable land to permanent grassland. In the assumption that the initial SOM contents, before conversion to permanent grassland, and the average annual input of organic material in the soils were comparable, these results indicate that stabilization of SOM and thus sequestration of C upon conversion of arable land to grassland is a slow process. The largest relative increase in C and N contents occurred in the HF 150-2000 µm fraction, followed by the 50-150 µm and <50 µm fractions. Our results suggest that the HF 150-2000 µm fraction could serve as a good indicator of early SOM accumulation, induced by the conversion of cultivation to permanent grassland. We didn't observe any clear trends in C and N contents in the LF 150-2000 µm fraction, and the C and N contents in the LF 150-2000 µm fraction were not significantly correlated with the gross N transformation rates nor with the net N mineralization rates in the investigated grassland soils. Therefore we suggest that the LF 150-2000 µm fraction might be considered together with the IF 150-2000 µm fraction in future studies, as the combined LF+IF 150-2000 µm fraction (density <1.37 g cm^{-3}).

The gross N mineralization, nitrification, and immobilization rates *(difference)* in the investigated grassland soils showed strong, positive correlations with the total C and N contents. Our results indicate that gross N mineralization is largely determined by the total N availability, whereas net N mineralization is also controlled by other factors (C content and C/N ratio of the SOM), as it results from the balance between gross mineralization and immobilization. The relation between long-term net mineralization rates and gross mineralization rates could be fitted by means of a logarithmic equation, which reflects that

the ratio of gross immobilization *(difference)* to gross mineralization tended to increase with increasing SOM contents. Since microbial demand for N (immobilization) tended to increase with increasing SOM content in the investigated grassland soils, this indicates that potential N retention in soils through immobilization tends to be limited by the available C content.

CHAPTER 5

Estimation of gross N transformation rates and potential N retention after addition of ^{15}N-labelled NH_4NO_3 to permanent grassland soils

This chapter is compiled from:

Accoe, F., Boeckx, P., Videla, X., Hofman, G., Van Cleemput, O. Estimation of gross N transformation rates and potential N retention after addition of ^{15}N-labelled NH_4NO_3 to permanent grassland soils (submitted to Soil Science Society of America Journal).

Content

5. Estimation of gross N transformation rates and potential N retention after addition of ^{15}N-labelled NH_4NO_3 to permanent grassland soils

5.1. Introduction

The flux of N through mineralization-immobilization turnover (MIT) in grassland soils is a major determinant for the N supply for plant uptake and for N loss processes (Ledgard et al., 1998). The balance between N mineralization and immobilization is at an undefined equilibrium which tends to vary with time and soil properties (Barraclough and Jarvis, 1989). Several field studies with ^{15}N-labelled mineral fertilizer have shown that significant amounts of labelled fertilizer can be retained in the soil organic N pool, as a result of immobilization by the microbial biomass (Bristow et al., 1987; Hart et al., 1993; Whitehead, 1995b). The extent to which inorganic N is immobilized is related to the supply of readily available C, as this regulates the microbial activity (Okereke and Meints, 1985). Some of the immobilized fertilizer N in the soil organic matter (SOM) is subsequently remineralized, though this is believed to occur relatively slowly (Whitehead, 1995b).

The gross N transformation rates (mineralization, nitrification, NH_4^+ and NO_3^- immobilization) which occur in soils can only be estimated by application of the ^{15}N isotope pool dilution methodology in single or paired ^{15}N-labelling experiments (Barraclough, 1991). In paired ^{15}N-labelling experiments, ^{15}N-labelled NH_4^+ and unlabelled NO_3^- is added to the soil in one experiment, while unlabelled NH_4^+ and ^{15}N-labelled NO_3^- is added in a parallel experiment (Barraclough, 1991). The ^{15}N isotope pool dilution methodology is based on the principle that after enrichment of an N pool (NH_4^+ or NO_3^-) with ^{15}N, an influx of non-enriched N into this pool, via mineralization or

nitrification, lowers the ^{15}N-abundance (dilution) whereas an efflux, via NH_4^+ immobilization and nitrification or via NO_3^- immobilization and denitrification, does not. Thus, the decrease in ^{15}N abundance of the enriched pool is a measure for the gross production of the enriched N compound. In addition to this approach, the increase in ^{15}N-abundance of other, non-enriched pools can be used to quantify the gross transformation rates of these pools (Wessel and Tietema, 1992). Because several N fluxes can simultaneously dilute or enrich the ^{15}N abundance of a pool, these fluxes can only be accurately estimated using numerical techniques (Mary et al., 1998).

The aim of this study was to investigate the evolution of the gross N transformation rates and the potential N retention after mineral fertilizer application in three grassland soils of varying texture. Differently ^{15}N-labelled NH_4NO_3 (at a rate of 100 mg N kg^{-1} soil) was added to the soils in paired experiments. These soils were incubated during 30 days in the laboratory. Size and ^{15}N-enrichment of the NH_4^+, NO_3^-, and soil organic N pools were measured at 0, 1, 3, 7, 14 and 30 days after NH_4NO_3-application. The C mineralization rates were also monitored during the incubation experiments. The experimental data were simulated with the numerical simulation model FLUAZ (Mary et al., 1998) in order to estimate the gross N transformation rates.

5.2. Materials and methods

5.2.1. Site description and soil sampling

Soil samples were collected in September 2002 from three permanent grassland soils of varying texture at three different locations in Belgium. The first grassland soil was a wet, poorly drained Plagganthrept with a loamy sand texture, located at Wechelderzande (4°46’E, 51°15’N). The second grassland soil was a moderately drained Glossic Hapludalf with a loamy texture, located at Melle (3°47’E, 50°59’N). The third grassland soil was a

moderately drained Oxyaquic Udifluvent with a clay loam texture, located at Watervliet (3°35'E, 51°17'N). The sand, silt and clay content of the soil samples were determined by particle-size analysis following the pipette method of Robinson-Köhn (De Leenheer, 1966; Gee and Bauder, 1986). The soils were classified according to USDA (1999). The general soil characteristics of the 0-10 cm layer of the three soils are shown in Table 5.1. The three soils had comparable C/N ratios, ranging from 11.0 (Watervliet) to 11.3 (Wechelderzande). From each grassland soil, 20 replicate soil cores covering the whole area of the investigated grassland were taken from the 0-10 cm layer with a steel auger (3.5 cm diameter). The soil cores were bulked and stored in plastic bags at 4°C until the start of the ^{15}N-isotope dilution experiments.

Table 5.1. General soil characteristics of the 0-10 cm layer in the Wechelderzande, Melle and Watervliet grassland soils

Location	C content (%)	N content (%)	pH-H_2O (-)	Silt content (%)	Clay content (%)	Bulk density (g cm^{-3})
Wechelderzande	3.03	0.268	5.9	8.2	5.9	1.19
Melle	2.88	0.261	6.3	42.4	9.7	1.32
Watervliet	5.41	0.493	7.2	45.3	26.9	1.11

5.2.2. Incubations

For each of the three soils, a fully-mirrored ^{15}N-isotope dilution experiment has been conducted in the laboratory, in order to study the gross N transformation rates during 30 days after addition of differently ^{15}N-labelled NH_4NO_3. Before the start of the ^{15}N isotope dilution experiments, the fresh soil samples were homogenized and sieved on a 3.15 mm sieve to remove root material and shortly air-dried to obtain the gravimetric water content corresponding with a water filled pore space of 50% at the bulk density

measured in the field (Table 5.1), minus the amount of ^{15}N-labelling solution which would be added to the soils at the beginning of the experiment (corresponding with 6% gravimetric moisture content). The soils were pre-incubated during 7 days at 15°C. After the pre-incubation period, half the amount of the soil was labelled with a ^{15}N-enriched (10.23 atom%) $^{15}NH_4{}^{14}NO_3$-solution, equivalent to an addition of 50 mg NH_4^+-N and 50 mg NO_3^--N kg^{-1} soil. The other half of the soil was labelled with a ^{15}N-enriched (10.4 atom%) $^{14}NH_4{}^{15}NO_3$-solution at the same dosis. After label addition, the soils were thoroughly mixed in order to ensure a homogeneous label distribution. From both the $^{15}NH_4{}^{14}NO_3$- and $^{14}NH_4{}^{15}NO_3$-labelled bulk samples of each of the three grasslands soils, 15 disposable jars were filled with an amount of soil equivalent to 50 g oven-dry weight. The bulk densities of the soil samples were adjusted to the values measured in the field (resulting in a water filled pore space of 50%), covered with pin-holed parafilm to enable gas exchange and incubated at 15°C. After 1, 3, 7, 14 and 30 days of incubation, 3 replicate $^{15}NH_4{}^{14}NO_3$- and $^{14}NH_4{}^{15}NO_3$-labelled incubations were removed and extracted with 250 ml of 2M KCl (60 min. shaking). After shaking, the soil suspensions were centrifuged (Heraeus Sepatech, Labofuge GL) at 3000 rev. $min.^{-1}$ during 5 min. and the clear supernatans was immediately frozen for later NH_4^+-, NO_3^-- and ^{15}N-analysis. In order to remove any residual inorganic ^{15}N from the soil samples, the extraction was repeated twice by shaking during 30 min. with 150 ml 2M KCl followed by centrifugation. The extracted soil samples were then quickly dried at 50°C during 48 hours and ground with a planetary ball mill (PM400, Retsch, Germany) for ^{15}N-analysis of the organic N, in order to study the ^{15}N-immobilization. This extraction procedure was also carried out just before and 15 min. after the $^{15}NH_4{}^{14}NO_3$- and $^{14}NH_4{}^{15}NO_3$-label additions (initial and day 0 extraction).

From the $^{15}NH_4{}^{14}NO_3$-labelled bulk samples three additional disposable jars per grassland soil were filled with an amount of soil equivalent to 150 g oven-dry weight, in order to follow the evolution of the CO_2-production during the incubation. The bulk

densities of these soil samples were also adjusted in order to obtain a water filled pore space of 50% and covered with pin-holed parafilm. These samples were placed in sealed 1200 cm^3 glass jars fitted with a rubber septum for gas sampling and incubated at 15°C for 30 days. The evolution of the CO_2-production in each jar was measured by analyzing a 1 cm^3 headspace sample for CO_2 using a gas chromatograph (GC-14B, Shimadzu, Japan) with an ECD detector and a packed column (PORAPACK Q, mesh size 80/100) after 1, 3, 7, 14, 23 and 30 days of incubation. Following each sampling event, the glass jars were opened and parafilm was removed from the samples during 15 min. to re-establish ambient conditions.

5.2.3. Chemical analysis

Analyses of the total C and N contents in the soil samples were performed using a CNS analyzer (Vario Max CNS, Elementar, Germany). The NH_4^+- and NO_3^--concentrations in the KCl extracts were determined colorimetrically by means of a continuous flow analyzer (Skalar, The Netherlands). Isotope ratio analysis of the NH_4^+- and NO_3^--pool was performed after chemical conversion to N_2O. NH_4^+ was converted to N_2O using NaOBr according to a protocol adapted from Hauck (1982) and Saghir et al. (1993). The samples with a NH_4^+-concentration too low for conversion to N_2O were spiked by an addition of 700 µl of an $(NH_4)_2SO_4$-solution at natural abundance, with a concentration of 10.7 mmol N l^{-1}, to 45 ml of the samples. NO_3^- was converted to N_2O according to Stevens and Laughlin (1994). Isotope ratio analysis of the produced N_2O was carried out using an ANCA-TGII trace gas preparation unit (PDZ Europa, UK) coupled to a Continuous Flow Isotope Ratio Mass Spectrometer (20-20, PDZ Europa, UK). ^{15}N-analysis of the soil samples was performed using an ANCA-SL elemental analyzer coupled to a Continuous Flow Isotope Ratio Mass Spectrometer (20-20, PDZ Europa, UK).

5.2.4. Calculation of the N fluxes

The N fluxes during the incubation experiments were estimated numerically using the FLUAZ model developed by Mary et al. (1998). The eight N fluxes which can be taken into account in the FLUAZ model are (Fig. 5.1): mineralization (= ammonification, m), immobilization of NH_4^+ (ia) and NO_3^- (in), remineralization or release of previously immobilized N (r), humification (h), nitrification (n), volatilization (v) and denitrification (d). The calculations in this model are based on the isotopic dilution and isotopic enrichment principles (Monaghan and Barraclough, 1995). In order to use the model, measurements of the size and atom% ^{15}N in excess of the NH_4^+-, NO_3^-- and total soil organic N pool from either a single or a paired labelling experiment are needed as input data. The biomass N pool which is simulated in the model is that part of the biomass which is actively growing and accounts for the immobilization and remineralization of added mineral N (Mary et al., 1998).

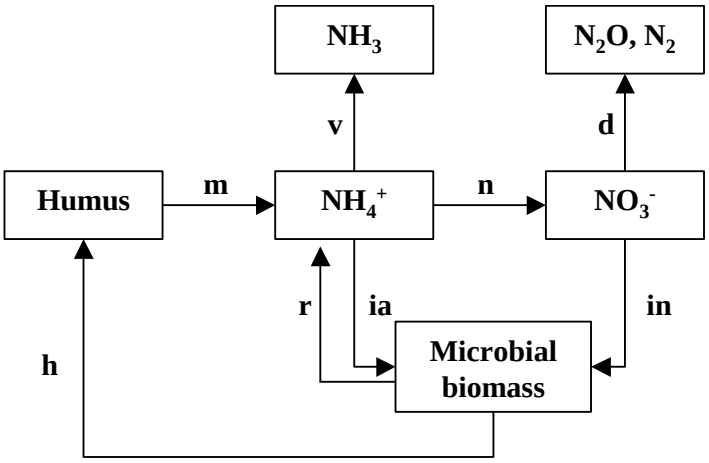


Fig. 5.1. Compartment model of the N pools and fluxes considered in FLUAZ; m = mineralization (= ammonification), n = nitrification, v = volatilization, d = denitrification, ia = immobilization of NH_4^+, in = immobilization of NO_3^-, r = remineralization, h = humification

The FLUAZ model combines a numerical integration method (Runge-Kutta algorithm) for the differential equations, describing the N and ^{15}N fluxes between the four N pools (NH_4^+-N, NO_3^--N, humus and biomass N; Fig. 5.1), with a non-linear fitting method (Haus-Marquardt algorithm) for the calculation (optimization) of the different N fluxes in the model. The optimal fit of the experimental data was calculated by minimizing the MWE (mean weighted error) criterion, which is a function of the difference between simulated and measured variables and the experimental variance of the measured variables. In this way, the measured variables with the largest experimental variability have the lowest weight in the optimization procedure (Mary et al., 1998). Further details on the FLUAZ model can be found in Mary et al. (1998).

In this study, the FLUAZ model was used to estimate the gross N mineralization rate, the gross nitrification rate, the gross NH_4^+ and NO_3^- immobilization rate, the remineralization rate and denitrification rate within the five time intervals (day 0-1, 1-3, 3-7, 7-14 and 14-30) considered during the incubation of the three soils. The humification and volatilization rates were assumed to be zero. The gross N mineralization, immobilization, remineralization and denitrification rates were allowed to follow zero order kinetics, whereas the gross nitrification rate was allowed to follow first order kinetics, in accordance with Mary et al. (1998).

5.3. Results

5.3.1. Size and atom% ^{15}N in excess of the NH_4^+- and NO_3^--pool

The initial NH_4^+-content in the loamy sand (Wechelderzande), loamy (Melle) and clay loam soil (Watervliet), just before addition of the labelled NH_4NO_3-solutions, was respectively 42.4, 1.6 and 1.5 mg N kg^{-1} soil. The initial NO_3^--content in the three soils was respectively 28.6, 39.3 and 57.6 mg N kg^{-1} soil. This indicates that, during the pre-incubation period, a considerable accumulation of NH_4^+-N had occurred in the loamy sand soil, which was not the case in the loamy and clay loam soils. The loamy and clay loam soils, however, showed a relatively larger accumulation of NO_3^--N than the loamy sand soil during the pre-incubation period. The evolution of the NH_4^+- and NO_3^--contents in the three soils during the incubation (between 15 min. (day 0) and 30 days after addition of the ^{15}N-labelled NH_4NO_3-solutions) is shown in Fig. 5.2.

In the loamy sand soil (Fig. 5.2 A), the NH_4^+-content increased between day 0 (96.9 mg N kg^{-1} soil) and day 3 (107.2 mg N kg^{-1} soil), followed by a linear decrease between day 3 and day 30 (10.6 mg N kg^{-1} soil). The NO_3^--content showed a steady increase between day 0 (70.1 mg N kg^{-1} soil) and day 30 (177.3 mg N kg^{-1} soil). However, the mean net nitrification rate between day 0 and day 14 (5.2 mg N kg^{-1} soil d^{-1}) was 2.5 times larger than the mean net nitrification rate between day 14 and day 30 (2.1 mg N kg^{-1} soil d^{-1}). The total mineral N content showed a strong increase between day 0 and day 3 (corresponding with a mean net mineralization rate of 9.3 mg N kg^{-1} soil d^{-1}), followed by a smaller increase between day 3 and 14 (corresponding with a mean net mineralization rate of 1.3 mg N kg^{-1} soil d^{-1}), and a decrease between day 14 and 30 (corresponding with a mean net immobilization rate of -1.4 mg N kg^{-1} soil d^{-1}).

The NH_4^+-content in the loamy soil (Fig. 5.2 B) decreased linearly between day 0 (46.5 mg N kg^{-1} soil) and day 14 (3.9 mg N kg^{-1} soil), and remained nearly constant afterwards. The mean net nitrification rate between day 0 and 14 (3.6 mg N kg^{-1} soil d^{-1}) was 3.4 times larger than the mean net nitrification rate between day 14 and 30 (1.1 mg N kg^{-1} soil d^{-1}). In contrast with the loamy sand soil, the total mineral N content showed a continuous increase during the whole incubation period, corresponding with a mean net mineralization rate of 0.8 mg N kg^{-1} soil d^{-1}.

In the clay loam soil (Fig. 5.2 C), the NH_4^+-content showed a very fast decrease during the first 3 days after the NH_4NO_3-addition (from 47.3 to 1.8 mg N kg^{-1} soil) and remained nearly constant after day 3. The fast decrease of the NH_4^+-content coincided with a very fast increase of the NO_3^--content between day 0 (106.6 mg N kg^{-1} soil) and day 3 (186.2 mg N kg^{-1} soil). The NO_3^--content showed a much smaller increase between day 3 and day 14, and started to decrease again after day 14. Like in the two other soils, the mean net nitrification rates decreased in function of time and ranged from 27.4 mg N kg^{-1} soil d^{-1} (day 0-day 3), 2.8 mg N kg^{-1} soil d^{-1} (day 3-day 14) to -0.97 mg N kg^{-1} soil d^{-1} (day 14-day 30). During the first day after the NH_4NO_3-addition, the total mineral N contents decreased slightly, followed by a strong increase between day 1 and 3 (corresponding with a mean net mineralization rate of 32.4 mg N kg^{-1} soil d^{-1}). As the NH_4^+-contents remained nearly constant after day 3, the mean net N mineralization rates equalled the mean net nitrification rates between day 3 and 30.

The evolution of the atom% ^{15}N in excess of the NH_4^+-pool and the NO_3^--pool in the three soils between 15 min. (day 0) and 30 days after addition of the $^{15}NH_4{}^{14}NO_3$ and $^{14}NH_4{}^{15}NO_3$-solutions are shown in Fig. 5.3 and 5.4, respectively.

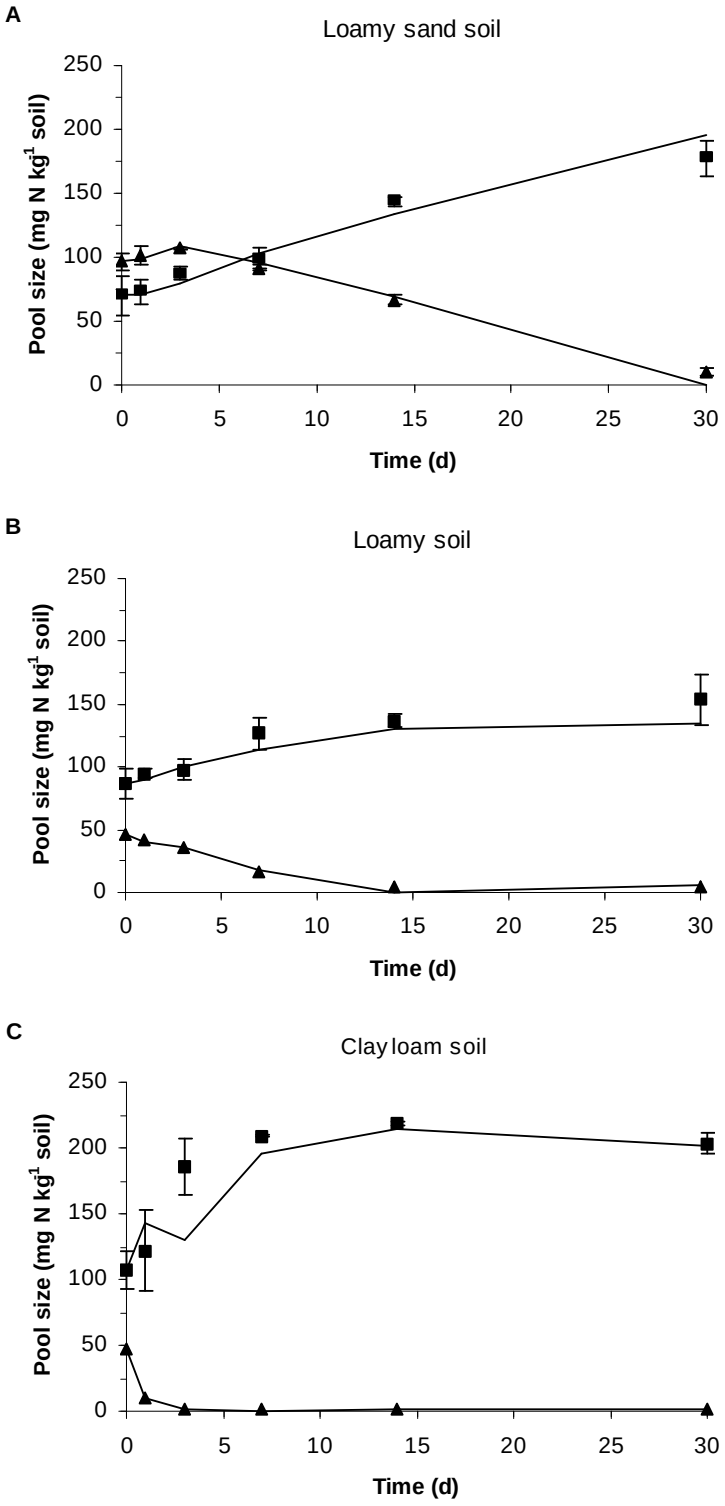


Fig. 5.2. Evolution of the NH_4^+- and NO_3^--contents (indicated by triangles and squares, respectively) in the loamy sand (A), loamy (B) and clay loam (C) soil between 15 min. (day 0) and 30 days after NH_4NO_3 addition; vertical bars represent two standard deviations; the evolution of the simulated values by the FLUAZ-model is indicated by the continuous lines

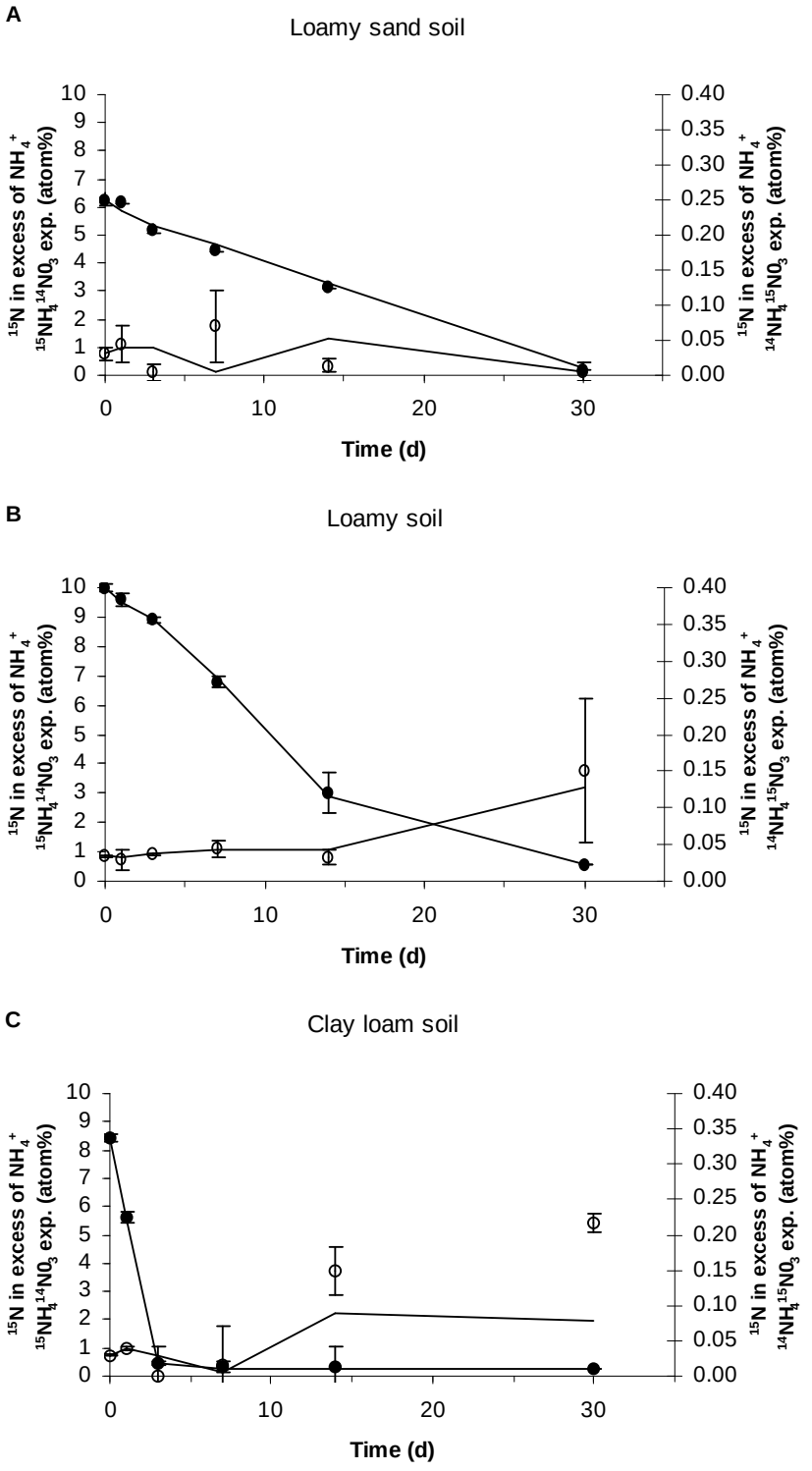


Fig. 5.3. Evolution of the atom% ^{15}N in excess of the NH_4^+-pool in the loamy sand (A), loamy (B) and clay loam soil (C) between 15 min. (day 0) and 30 days after addition of $^{15}NH_4{}^{14}NO_3$ and $^{14}NH_4{}^{15}NO_3$ (indicated by full and empty bullets, respectively); vertical bars represent two standard deviations; the evolution of the simulated values by the FLUAZ-model is indicated by the continuous line

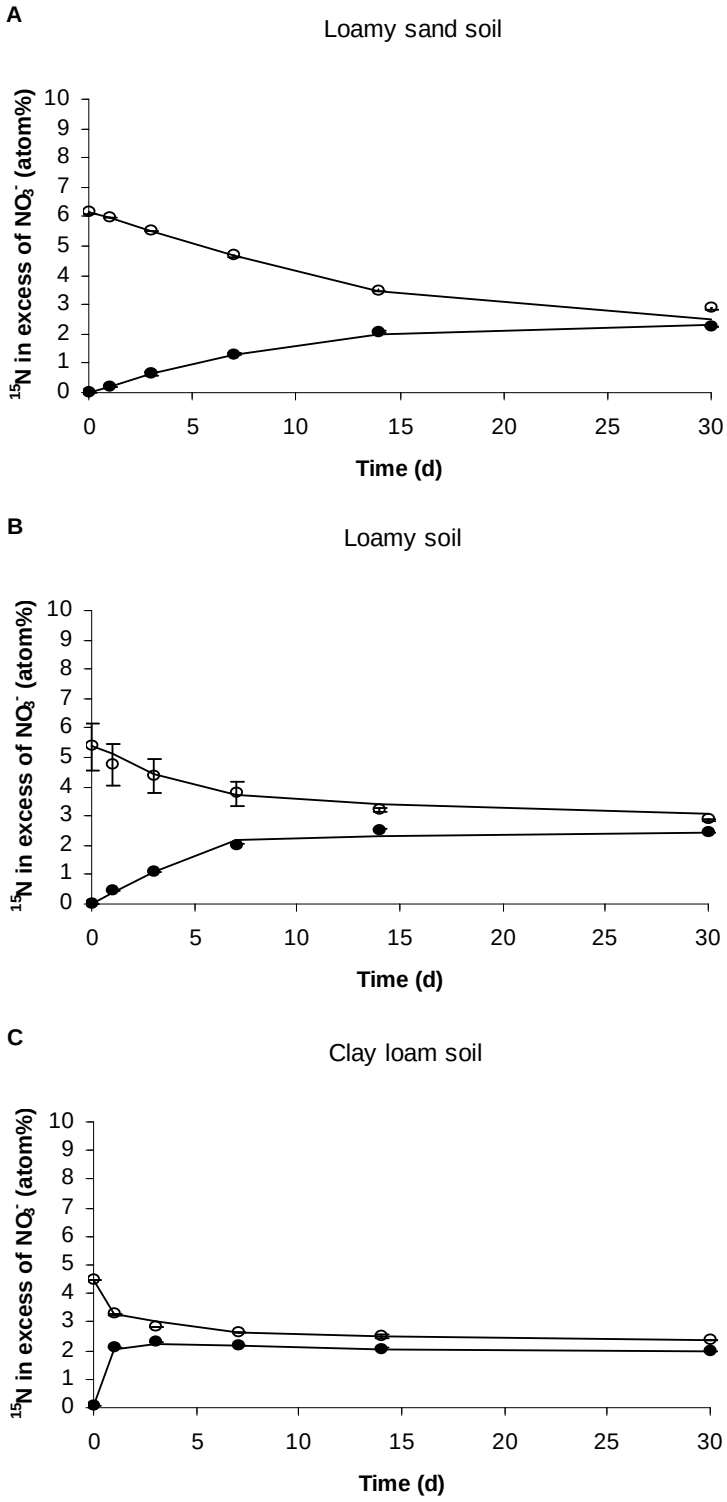


Fig. 5.4. Evolution of the atom% ^{15}N in excess of the NO_3^--pool in the loamy sand (A), loamy (B) and clay loam soil (C) between 15 min. (day 0) and 30 days after addition of $^{15}NH_4{}^{14}NO_3$ and $^{14}NH_4{}^{15}NO_3$ (indicated by full and empty bullets, respectively); vertical bars represent two standard deviations; the evolution of the simulated values by the FLUAZ-model is indicated by the continuous lines

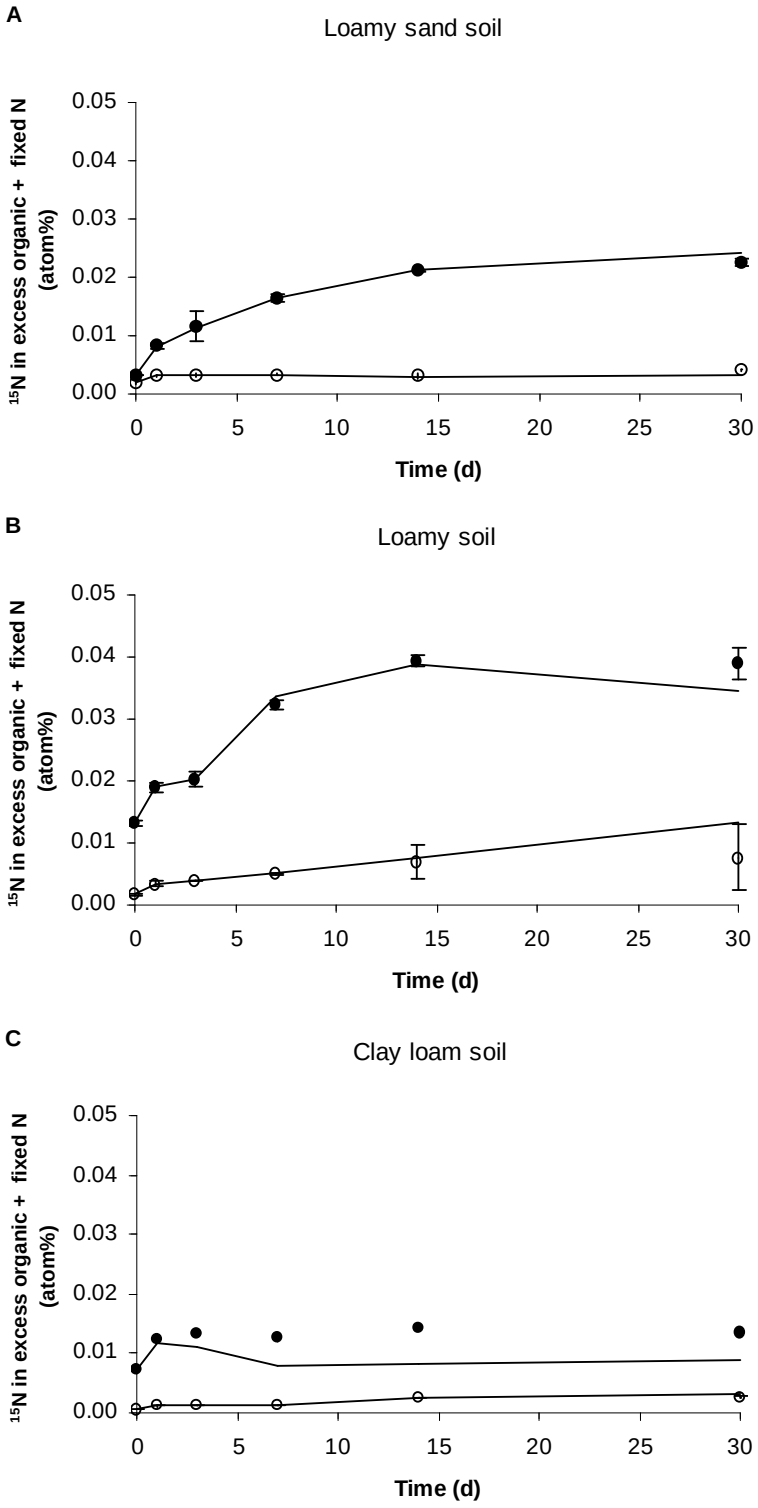


Fig. 5.5. Evolution of the atom% ^{15}N in excess of the soil organic and fixed N pool in the loamy sand (A), loamy (B) and clay loam soil (C) between 15 min. (day 0) and 30 days after addition of $^{15}NH_4{}^{14}NO_3$ and $^{14}NH_4{}^{15}NO_3$ (indicated by full and empty bullets, respectively); vertical bars represent two standard deviations; the evolution of the simulated values by the FLUAZ-model is indicated by the continuous lines

$^{15}NH_4{}^{14}NO_3$-addition

In the loamy sand and loamy soil, the atom% ^{15}N in excess of the NH_4^+-pool after $^{15}NH_4{}^{14}NO_3$-addition (Fig. 5.3 A and B) showed a gradual decrease during the whole incubation period from 6.3 (day 0) to 0.2 atom% in excess (day 30) and from 10.0 (day 0) to 0.6 atom% in excess (day 30), respectively. This indicates that there was a continuous input of NH_4^+-N at natural abundance into the ^{15}N-labelled NH_4^+-pool, resulting from mineralization (ammonification) of soil organic N. The lower initial atom% ^{15}N in excess of the NH_4^+-pool in the loamy sand soil in relation to the loamy soil can be attributed to the larger initial NH_4^+-content in the loamy sand soil. The atom% ^{15}N in excess of the NO_3^--pool in the loamy sand and loamy soil (Fig. 5.4 A and B) showed a logarithmic increase as the incubation proceeded, which indicates that ^{15}N-enriched NO_3^--N, resulting from nitrification, continuously entered the NO_3^--pool.

In the clay loam soil, the rapid decrease of the NH_4^+-content between day 0 and day 3 (Fig. 3.2 C) coincided with a very fast decrease of the atom% ^{15}N in excess of the NH_4^+-pool from 8.4 (day 0) to 0.45 atom% in excess (day 3), followed by a small but continuous decrease down to 0.25 atom% in excess at day 30 (Fig. 5.3 C). During the first day after the $^{15}NH_4{}^{14}NO_3$-addition, the atom% ^{15}N in excess of the NO_3^--pool (Fig. 5.4 C) increased fastly from 0.08 (day 0) to 2.1 atom% in excess (day 1), followed by a slower increase up to 2.3 atom% in excess at day 3. The observation that the atom% ^{15}N in excess of the NO_3^--pool at day 0 (15 min. after addition of the $^{15}NH_4{}^{14}NO_3$-solution) was already larger than zero, and the very fast increase of the atom% ^{15}N in excess between day 0 and 1 indicate that the added ^{15}N-labelled NH_4^+-N was very rapidly converted to NO_3^--N through nitrification. After day 3, the atom% ^{15}N in excess of the NO_3^--pool decreased linearly down to 1.9 atom% in excess at day 30. This ^{15}N-dilution of the NO_3^--pool after day 3 indicates that from then on there was a continuous input of NO_3^--N with a lower ^{15}N content through nitrification.

$^{14}NH_4{}^{15}NO_3$-addition

In the loamy sand and loamy soil, the atom% ^{15}N in excess of the NO_3^--pool (Fig. 5.4 A and B) showed a gradual decrease during the whole incubation period from 6.1 (day 0) to 2.9 atom% in excess (day 30) and from 5.4 (day 0) to 2.9 atom% in excess (day 30), respectively. In the clay loam soil, the atom% ^{15}N in excess of the NO_3^--pool (Fig. 5.4 C) decreased strongly between day 0 and 3 from 4.5 (day 0) to 2.8 atom% in excess (day 3), followed by a very slow decrease down to 2.3 atom% in excess (day 30). The observed dilution of the ^{15}N-labelled NO_3^--pools can be attributed to nitrification of NH_4^+-N at natural abundance. In the loamy and the clay loam soil, an increase in the atom% ^{15}N in excess of the NH_4^+-pool was observed between day 14 (0.03 atom% in excess) and 30 (0.15 atom% in excess), and between day 7 (0.01 atom% in excess) and 30 (0.22 atom% in excess), respectively (Fig. 5.3 B and C). However, this increase was only significant ($p<0.05$) in the clay loam soil. This ^{15}N-enrichment in the NH_4^+-pool when $^{14}NH_4{}^{15}NO_3$ was applied could be explained by the remineralization of previously immobilized ^{15}N, by a direct conversion of NO_3^- to NH_4^+ (via dissimilatory reduction) or by a combination of both processes occurring simultaneously in these soils.

5.3.2. ^{15}N-recovery in the soil organic and fixed N pool

The atom% ^{15}N in excess of the non-labelled loamy sand, loamy and clay loam soil was 0.0019, 0.0020 and 0.0026 atom%, respectively. The evolution of the atom% ^{15}N in excess, expressed as the difference between the atom% ^{15}N in excess of the labelled and non-labelled soils, and the amount of ^{15}N recovered in the soil organic and fixed N pool in the three soils between 15 min. (day 0) and 30 days after addition of the $^{15}NH_4{}^{14}NO_3$ and $^{14}NH_4{}^{15}NO_3$-solutions are shown in Fig. 5.5 and Table 5.2, respectively.

Table 5.2. Amounts of ^{15}N recovered (expressed in µg ^{15}N kg^{-1} soil) in the soil organic and fixed N pool at 15 min. (day 0), 1, 3, 7, 14 and 30 days after addition of $^{15}NH_4{}^{14}NO_3$ and $^{14}NH_4{}^{15}NO_3$ in the loamy sand, loamy and clay loam soil

Time (d)	$^{15}NH_4{}^{14}NO_3$-addition Soil			$^{14}NH_4{}^{15}NO_3$-addition Soil		
	Loamy sand	Loamy	Clay loam	Loamy sand	Loamy	Clay loam
0	84	357	353	50	47	27
1	215	508	602	82	94	58
3	300	543	649	86	106	61
7	429	866	617	87	135	63
14	552	1059	701	82	191	130
30	590	1043	664	106	206	131

$^{15}NH_4{}^{14}NO_3$-addition

The atom% ^{15}N in excess of the soil organic and fixed N pool in the loamy sand and the loamy soil showed a significant increase from day 0 till day 14 and remained nearly constant during the rest of the incubation period (Fig. 5.5 A and B). In the clay loam soil however, the atom% ^{15}N in excess only significantly increased between day 0 and day 1, and remained nearly constant during the rest of the incubation period (Fig. 5.5 C). The amount of $^{15}NH_4^+$-N recovered in the soil organic and fixed N pool immediately (15 min.) after $^{15}NH_4{}^{14}NO_3$-addition (Table 5.2) was smallest in the loamy sand soil (84 µg ^{15}N kg^{-1} soil) and approximately 4 times larger in the clay loam (353 µg ^{15}N kg^{-1} soil) and loamy soil (357 µg ^{15}N kg^{-1} soil). This represented 1.7 and 7.2% of the total amount of $^{15}NH_4^+$-N added in the loamy sand soil and the loamy and clay loam soils, respectively. This indicates that a rapid biotic or abiotic immobilization of the added NH_4^+-N occurred immediately after label addition and that this rapid process was most pronounced in the loamy and the clay loam soils. The total amount of $^{15}NH_4^+$-N recovered in the soil organic and fixed N pool at the end of the incubation was largest in the loamy soil (1043 µg ^{15}N

kg^{-1} soil), followed by the clay loam (664 µg ^{15}N kg^{-1} soil) and the loamy sand soil (590 µg ^{15}N kg^{-1} soil) (Table 5.2), which represented respectively 21, 13 and 12% of the total amount of $^{15}NH_4^+$-N added.

$^{14}NH_4{}^{15}NO_3$-addition

The amounts of $^{15}NO_3^-$-N recovered immediately after $^{14}NH_4{}^{15}NO_3$-addition ranged from 27 µg ^{15}N kg^{-1} soil in the clay loam soil to approximately 50 µg ^{15}N kg^{-1} soil in the loamy and loamy sand soil (Table 5.2). At the end of the incubation, the largest amount of $^{15}NO_3^-$-N was recovered in the loamy soil (206 µg ^{15}N kg^{-1} soil), followed by the clay loam (131 µg ^{15}N kg^{-1} soil) and the loamy sand soil (106 µg ^{15}N kg^{-1} soil). These amounts corresponded with respectively 4.1, 2.6 and 2.1% of the total amount of $^{15}NO_3^-$-N added. The amounts of $^{15}NO_3^-$-N recovered 30 days after $^{15}NH_4{}^{14}NO_3$-addition were thus on average approximately 5 times larger than the amounts recovered 30 days after $^{14}NH_4{}^{15}NO_3$-addition.

5.3.3. C mineralization rates

As C mineralization rates are a sensitive indicator of the microbial activity in soils, also the CO_2-production was measured during the incubation experiments. The average C mineralization rates which were observed during the five time intervals considered are shown in Table 5.3. In the loamy sand and loamy soils, the observed C mineralization rates were largest during the first day of incubation, and tended to decrease gradually as the incubation proceeded. In the clay loam soil, the same trend was observed as in the two other soils, but the C mineralization rates remained relatively large during the first three days of the incubation.

Table 5.3. Average C mineralization rates (expressed in mg C kg^{-1} soil d^{-1}) observed during the five time intervals considered in the loamy sand, loamy and clay loam soil (average values of three replicate measurements, standard deviations in brackets)

Interval	Soil		
	Loamy sand	Loamy	Clay loam
d_0-d_1	13.1(0.8)	9.9(0.1)	21.6(2.0)
d_1-d_3	6.7(0.5)	6.9(0.8)	23.9(0.7)
d_3-d_7	6.0(0.7)	5.8(0.4)	11.6(0.4)
d_7-d_{14}	4.2(0.6)	4.4(0.5)	5.6(0.3)
d_{14}-d_{30}	2.9(0.4)	5.1(0.7)	5.0(0.5)

5.3.4. Simulation of the data by FLUAZ

The simulated values of the NH_4^+- and NO_3^--contents, the atom% ^{15}N in excess of NH_4^+ and NO_3^-, and the atom% ^{15}N in excess of the soil organic and fixed N in the three soils are plotted versus time in Fig. 5.2, 5.3, 5.4 and 5.5, respectively.

The best overall fit of the data by the FLUAZ-model, based on the MWE criterion, was obtained for the loamy soil (average MWE of 1.6), followed by the loamy sand soil (average MWE of 2.8) and the clay loam soil (average MWE of 4.6). The simulated values of the NH_4^+- and NO_3^--contents (Fig. 5.2) and the atom% ^{15}N in excess of the NH_4^+- (Fig. 5.3) and NO_3^--pool (Fig. 5.4) were generally within or nearly within the variation of the measured values. However, in some cases the simulated values diverged from the measured values. A discrepancy between the simulated and measured NO_3^--contents (Fig. 5.2) was observed at day 30 in the loamy sand and loamy soil (19 mg N kg^{-1} higher or 20 mg N kg^{-1} lower, respectively) and the simulated NO_3^--contents at day 3 in the clay loam soil (56 mg N kg^{-1} lower). A discrepancy between simulated and observed values was also observed for the atom% ^{15}N in excess values of the NH_4^+- pool in the clay

loam soil, after addition of $^{14}NH_4^{15}NO_3$ (Fig. 5.3 C). The FLUAZ-model simulated an increase in the ^{15}N-enrichment of the NH_4^+- pool between day 7 and day 14, but the simulated atom% ^{15}N in excess values on day 14 and 30 were respectively 1.7 and 2.7 times smaller than the measured values.

The atom% ^{15}N in excess of the soil organic and fixed N pool was very well fitted for the whole duration of the experiment in the loamy sand soil (Fig. 5.5 A) and for the first 14 days of the experiment in the loamy soil (Fig. 5.5 B). In the loamy soil, the simulated atom% ^{15}N in excess value of the soil organic and fixed N pool at day 30 was slightly smaller than the measured value after addition of $^{15}NH_4^{14}NO_3$. This suggests that the NH_4^+ immobilization rate during the last time interval may have been underestimated by the FLUAZ-model. In the clay loam soil, the simulated atom% ^{15}N in excess values of the soil organic and fixed N pool after addition of $^{15}NH_4^{14}NO_3$ were systematically lower than the measured values from day 3 till the end of the incubation period (Fig. 5.5 C).

5.3.5. Gross N transformation rates calculated by FLUAZ

The gross N mineralization (m), nitrification (n), NH_4^+ immobilization (ia), NO_3^- immobilization (in), remineralization (r) and denitrification (d) rates, which were calculated by FLUAZ for the five time intervals considered in the three soils are summarized in Table 5.4. In the loamy sand soil, the gross N mineralization rates during the day 0-day 1 and day 1-day 3 intervals were considerably larger than the average gross N mineralization rates in the time intervals between day 3 and day 30. In the loamy and clay loam soil the same trend was observed, but only for the gross mineralization rate in the day 0-day 1 interval in relation to the gross mineralization rates in the rest of the incubation period. This observation indicates that a flush in gross N mineralization (priming effect) may have occurred at the beginning of the incubation experiments after the addition of the labelling solution. The cumulative gross N mineralization at the end of

the incubation period was largest in the loamy sand soil (68 mg N kg^{-1} soil or 81 kg N ha^{-1}), followed by the clay loam soil (58 mg N kg^{-1} soil or 64 kg N ha^{-1}) and the loamy soil (21 mg N kg^{-1} soil or 28 kg N ha^{-1}) (Fig. 5.6 A). These values of cumulative gross N mineralization correspond with 2.5%, 1.2% and 0.8% of the initial total N content in the loamy sand, clay loam and loamy soil, respectively.

Table 5.4. Gross N mineralization (m), nitrification (n), NH_4^+ immobilization (ia), NO_3^--immobilization (in), remineralization (r) and denitrification (d) rates calculated by FLUAZ for the five time intervals considered in the loamy sand, loamy and clay loam soil

Soil	Interval	N transformation rate (mg N kg^{-1} soil d^{-1})					
		m	n	ia	in	r	d
Loamy sand	d_0-d_1	6.82	2.22	4.05	1.65	0.73	0.00
	d_1-d_3	8.19	3.06	1.78	0.30	0.00	0.00
	d_3-d_7	2.70	4.07	1.70	0.18	0.00	0.00
	d_7-d_{14}	3.51	5.11	1.69	0.08	0.00	0.00
	d_{14}-d_{30}	0.59	3.37	1.37	0.08	0.01	0.00
Loamy	d_0-d_1	2.16	3.71	5.20	1.86	0.00	0.00
	d_1-d_3	1.31	3.70	1.09	0.68	0.21	0.00
	d_3-d_7	1.48	4.41	1.69	0.57	0.16	0.00
	d_7-d_{14}	0.05	1.13	1.59	0.64	0.00	0.00
	d_{14}-d_{30}	0.62	0.48	1.56	0.64	0.13	0.00
Clay loam	d_0-d_1	8.14	37.92	8.39	1.61	0.75	0.00
	d_1-d_3	3.39	5.05	3.31	0.60	0.26	0.00
	d_3-d_7	3.04	2.89	0.56	0.36	0.03	0.00
	d_7-d_{14}	3.01	1.50	2.40	0.63	0.92	0.00
	d_{14}-d_{30}	0.60	0.75	0.00	0.33	0.16	1.49

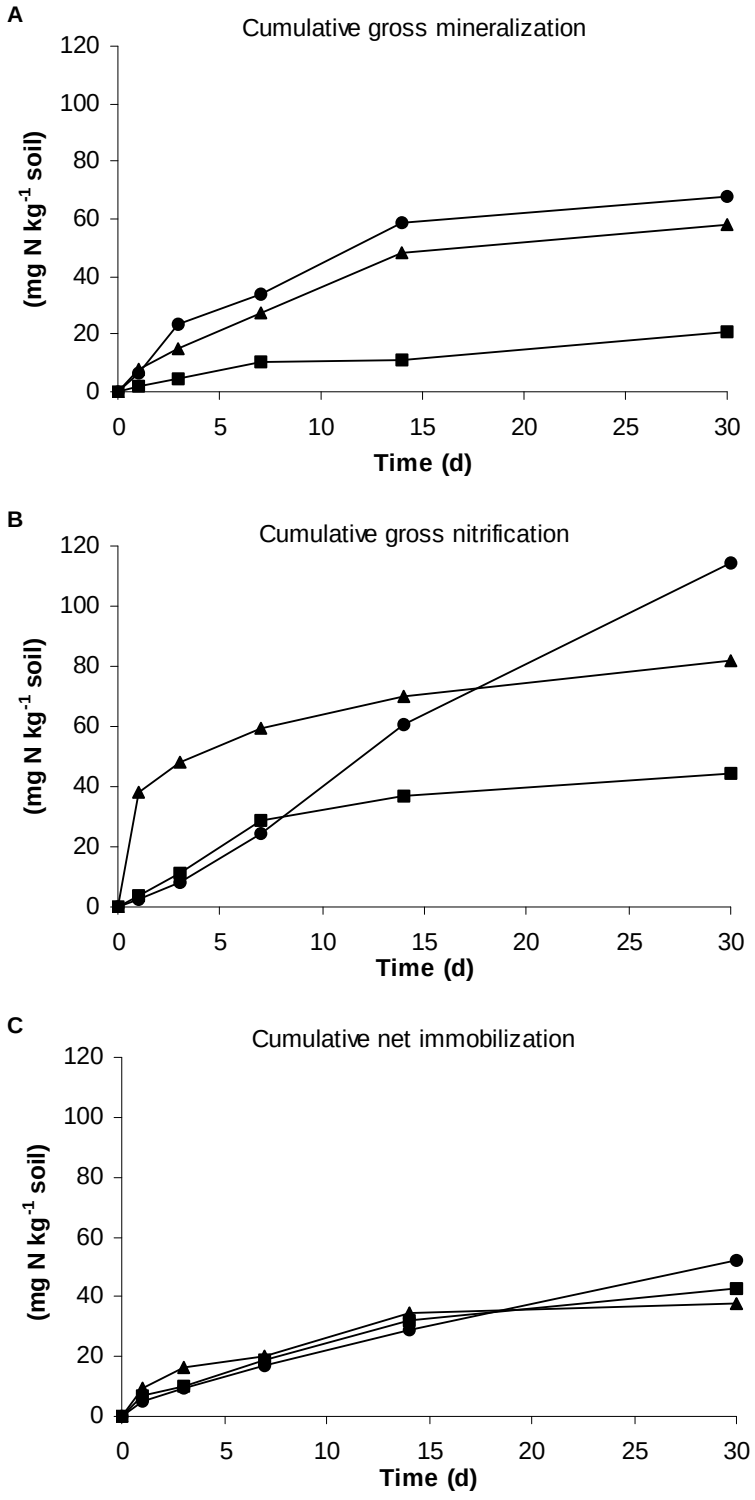


Fig. 5.6. Evolution of the cumulative gross N mineralization (A), cumulative gross nitrification (B) and cumulative net N immobilization (total mineral N immobilization minus remineralization) in the loamy sand , loamy and clay loam soils (values indicated by bullets, squares and triangles, respectively) from day 0 till day 30 after NH_4NO_3-addition

In the loamy sand soil, the gross nitrification rates tended to increase after the NH_4NO_3-addition (2.2 mg N kg^{-1} soil d^{-1} in the day 0-day 1 interval) as the incubation experiment proceeded until day 14 (5.1 mg N kg^{-1} soil d^{-1} in the day 7-day 14 interval), and decreased again after day 14 (3.4 mg N kg^{-1} soil d^{-1}). In the loamy soil, the gross nitrification rates increased from 3.7 mg N kg^{-1} soil d^{-1} in the day 0-day 3 interval to 4.4 mg N kg^{-1} soil d^{-1} in the day 3-day 7 interval, followed by a fast decrease until day 30. In the clay loam soil, a very large gross nitrification rate was observed during the first day after the NH_4NO_3-addition (38 mg N kg^{-1} soil d^{-1}), which was 10 to 17 times larger than the corresponding values in the loamy and loamy sand soils, respectively. The gross nitrification rates tended to decrease very fastly as the incubation experiment proceeded. The largest cumulative gross nitrification at the end of the incubation period was observed in the loamy sand soil (114 mg N kg^{-1} soil or 136 kg N ha^{-1}), followed by the clay loam (82 mg N kg^{-1} soil or 91 kg N ha^{-1}) and the loamy soil (44 mg N kg^{-1} soil or 59 kg N ha^{-1}) (Fig. 5.6 B).

The FLUAZ model indicated that in the three soils NH_4^+ and NO_3^- immobilization occurred simultaneously in each interval considered, except during the day 14-day 30 interval in the clay loam soil where the estimated NH_4^+ immobilization rate was zero (Table 5.4). In nearly all time intervals considered, the estimated NH_4^+ immobilization rates were larger than the NO_3^- immobilization rates and in these cases, the proportion of the NH_4^+ immobilization ranged from 60 to 95% of the total mineral N immobilization. In the three soils investigated, the gross NH_4^+ and NO_3^- immobilization rates were considerably larger during the first day after addition of the NH_4NO_3-solutions in relation to the immobilization rates observed during the rest of the incubations.

In the loamy sand and the clay loam soils, the large NH_4^+ and NO_3^- immobilization rates in the day 0-day 1 interval coincided with significant remineralization rates, which was not the case in the loamy soil. This indicates that a quick recycling of mineral N

occurred in these soils during the first day after the NH_4NO_3-addition. In the clay loam soil, remineralization of immobilized mineral N occurred during the entire incubation period, whereas in the loamy and loamy sand soils remineralization was only observed during two or three time intervals.

The evolution of the cumulative net N immobilization calculated by FLUAZ (total mineral N immobilization minus remineralization) in the three soils is shown in Fig. 5.6 C. At 14 days after the NH_4NO_3-addition, the cumulative net immobilization was comparable in the three soils, and ranged from 29 mg N kg^{-1} soil or 34 kg N ha^{-1} in the loamy sand soil, 32 mg N kg^{-1} soil or 42 kg N ha^{-1} in the loamy soil, to 35 mg N kg^{-1} soil or 39 kg N ha^{-1} in the clay loam soil. Between day 14 and day 30, however, a much lower gross immobilization rate was calculated for the clay loam soil in relation to the loamy sand and loamy soils, and a much higher remineralization rate was calculated for the clay loam and loamy soils in relation to the loamy sand soil (Table 5.4). This resulted in a relatively larger cumulative net N immobilization in the loamy sand soil (52 mg N kg^{-1} soil or 62 kg N ha^{-1}) at 30 days after the NH_4NO_3-addition, in relation to the loamy (42 mg N kg^{-1} soil or 56 kg N ha^{-1}) and clay loam soils (38 mg N kg^{-1} soil or 42 kg N ha^{-1}). The FLUAZ model calculated that denitrification at a rate of 1.49 mg N kg^{-1} soil d^{-1} occurred in the clay loam soil during the last time interval (Table 5.4).

5.3.6. Relationships between C and N fluxes

The relationships between (1) the gross N mineralization rates (Table 5.4) and the C mineralization rates (Table 5.3), and (2) the total gross immobilization rates (sum of the gross NH_4^+ and NO_3^- immobilization rates, Table 5.4) and the C mineralization rates observed in the three soils during the five time intervals were investigated. A significant linear correlation was found between the gross N mineralization rates and the C mineralization rates ($m_N = 0.41m_C - 0.60$, $R^2=0.74$, $p<0.001$), when the outlying fluxes

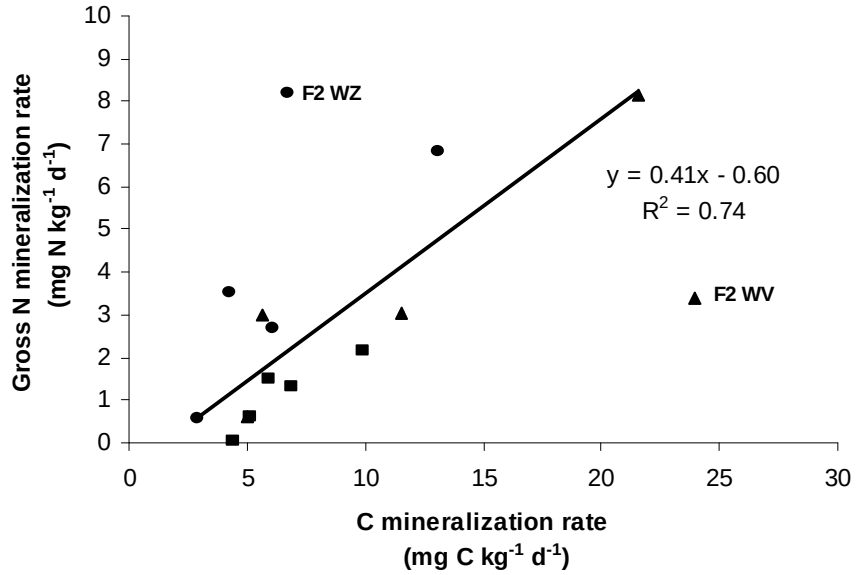


Fig. 5.7. Relationship between gross N mineralization rates and C mineralization rates observed in the loamy sand, loamy and clay loam soils (data indicated by bullets, squares and triangles, respectively) during the five time intervals considered, and linear regression of the relationship excluding the outlying fluxes observed during the second time interval in the loamy sand and clay loam soils (data indicated by F2 WZ and F2 WV, respectively)

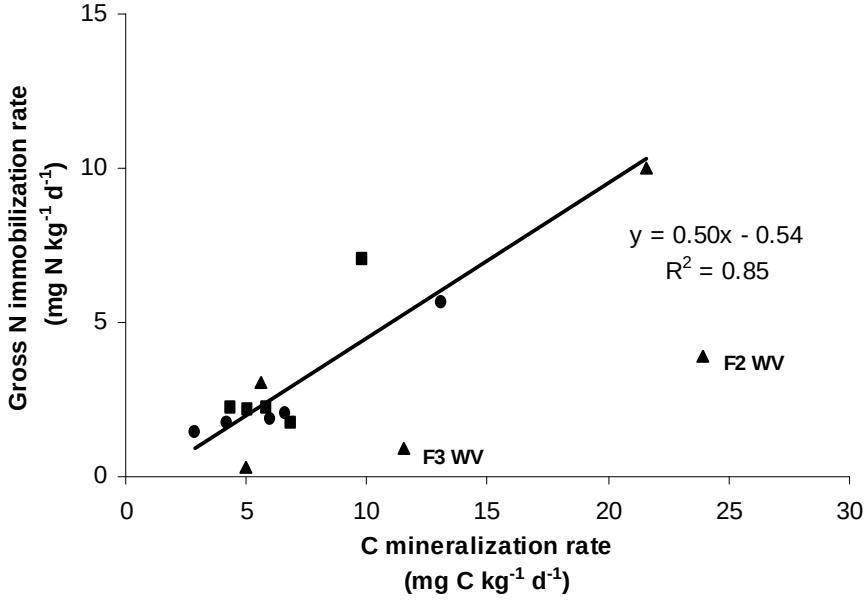


Fig. 5.8. Relationship between the total gross N immobilization rates and C mineralization rates observed in the loamy sand, loamy and clay loam soils (data indicated by bullets, squares and triangles, respectively) during the five time intervals considered, and linear regression of the relationship excluding the outlying fluxes observed during the second and third time interval in the clay loam soil (data indicated by F2 WV and F3 WV, respectively)

observed during the second time interval in the loamy sand and clay loam soils were excluded (Fig. 5.7). A weak, but significant linear correlation was observed between the total gross immobilization rates and the C mineralization rates ($i = 0.28m_C + 0.66$, $R^2=0.46$, $p<0.001$), when all the fluxes were taken into account. A stronger, significant correlation was found ($i = 0.50m_C - 0.54$, $R^2=0.85$, $p<0.01$) when the outlying fluxes observed during the second and third time interval in the clay loam soil were excluded (Fig. 5.8).

5.4. Discussion

5.4.1. ^{15}N-recovery in the soil organic and fixed N pool

Very shortly (15 min.) after addition of both the $^{15}NH_4{}^{14}NO_3$ and $^{14}NH_4{}^{15}NO_3$-solutions, significant amounts of ^{15}N were already recovered in the soil organic and fixed N pool of the three soils investigated (Table 5.2). The rapid ^{15}N-recovery after $^{15}NH_4{}^{14}NO_3$-addition could be attributed to a rapid biological immobilization, a rapid abiotic immobilization or a combination of the two processes occurring simultaneously immediately after the addition of the $^{15}NH_4{}^{14}NO_3$-solution (Davidson et al., 1991). Processes of abiotic immobilization of $^{15}NH_4^+$ which have been described in literature include fixation or adsorption on clay minerals, adsorption to soil organic matter and condensation reactions with humic compounds (Foster et al., 1985; Davidson et al., 1991; Strickland et al., 1992; Compton and Boone, 2002). These processes have been shown to occur very rapidly, in less than 15 min. after addition of NH_4^+-N (Newman and Oliver, 1966). In the loamy and clay loam soil, a small amount of the NH_4^+-N added was not KCl-extractable after 15 min. (4.9 ± 3.9 and 4.3 ± 0.6 mg NH_4^+-N kg^{-1} soil, respectively) which was not the case in the loamy sand soil. This observation, together with the higher clay content in the loamy and clay loam soils in relation to the loamy sand soil (Table 5.1),

may explain why the ^{15}N-recovery in the soil organic and fixed N pool 15 min. after the $^{15}NH_4{}^{14}NO_3$-addition in the loamy and clay loam soil was about 4 times larger than in the loamy sand soil.

A very fast recovery of ^{15}N in the soil organic and fixed N pool after addition of $^{15}NH_4^+$ or $^{15}NO_3^-$ to mineral and organic soils has been reported in several other studies (Recous et al., 1990; Davidson et al., 1991; Mary et al., 1993; Berntson and Aber, 2000; Andersen and Jensen, 2001; Compton and Boone, 2002). Davidson et al. (1991), who compared the ^{15}N recovery in a sterilized and non-sterilized silt loam grassland soil after addition of $^{15}NH_4^+$, concluded that the observed rapid immobilization was completely abiological and occurred within the first 15 min. after addition. In relation to the recoveries in our study after $^{15}NH_4{}^{14}NO_3$-addition (which ranged from 12% to 21% of the $^{15}NH_4$-N added), Recous et al. (1990) observed comparable recoveries of ^{15}N (ranging from 17% to 26% of the $^{15}NH_4$-N added) into the soil organic N pool at 30 min. after addition of $(^{15}NH_4)_2SO_4$ (equivalent to 4.6 mg $^{15}NH_4$-N kg^{-1} soil) to cultivated loam soils. Recous et al. (1990) attributed this rapid ^{15}N immobilization after $^{15}NH_4^+$-addition entirely to abiotic fixation.

The amounts of ^{15}N recovered in the soil organic and fixed N pool immediately after addition of the $^{14}NH_4{}^{15}NO_3$-solution in our study ranged from 2.1% (clay loam soil) to 4.1% (loamy sand soil) of the $^{15}NO_3$-N added. These recoveries are considerably larger than the recoveries observed by Mary et al. (1993) (0.4% of the $^{15}NO_3$-N added) and Recous et al. (1990) (no recovery of the $^{15}NO_3$-N added) within one hour after addition of 8.1 mg $^{15}NO_3$-N kg^{-1} soil (Mary et al., 1993), or within 30 min. after addition of 4.5 mg $^{15}NO_3$-N kg^{-1} soil (Recous et al., 1990) as $K^{15}NO_3$ to cultivated loam soils. Rapid immobilization of $^{15}NO_3^-$-N has also been observed in forest soils (Berntson and Aber, 2000; Compton and Boone, 2002), but the mechanisms and controlling factors of this process are not yet clear (Compton and Boone, 2002). However, Davidson et al. (2003)

recently described a mechanism of abiotic immobilization of $^{15}NO_3^-$ via iron oxidation (the ferrous wheel hypothesis) as an important process in forest soils.

In the loamy sand soil and the loamy soil, the atom% ^{15}N in excess of the soil organic and fixed N pool showed a significant increase after $^{15}NH_4{}^{14}NO_3$-addition from day 0 till day 14, whereas in the clay loam soil the atom% ^{15}N in excess only significantly increased between day 0 and day 1. This can be explained by the fact that the atom% ^{15}N in excess of the NH_4^+-pool in the clay loam soil was very rapidly diluted between day 0 (8.5 atom%) and day 3 (0.45 atom%) and remained very low afterwards in relation the atom% ^{15}N in excess of the NH_4^+-pool in the loamy sand and loamy soils. This very fast dilution of the NH_4^+-pool was mainly caused by the very fast nitrification during the first three days after $^{15}NH_4{}^{14}NO_3$-addition (Table 5.4), which rapidly decreased the size of the NH_4^+-pool, and thus amplified the ^{15}N-dilution of the NH_4^+-pool through gross mineralization. Consequently the eventual NH_4^+ immobilization which occurred after day 3 in the clay loam soil (Table 5.4) did not result in any further significant enrichment of the soil organic and fixed N pool, which was observed in the loamy sand and loamy soils.

5.4.2. Simulation of the data by FLUAZ

When the MWE-values corresponding with the data fits in the different time intervals were considered, the quality of the fit of the data generally tended to decrease (increasing MWE-values) from the first towards the last time interval. This might be partially attributed to the increasing duration of the time intervals between the sampling dates, as the incubation experiments proceeded. All N transformation rates, except the nitrification rate, were estimated assuming zero order kinetics, which implies that these rates are assumed to remain constant during the whole time interval considered. However, when longer time intervals were considered (especially between day 7 and 14, and

between day 14 and 30) this assumption of constant rates may not have been fully met, explaining the larger MWE-values in the longer time intervals.

The simulated values of the size and atom% ^{15}N in excess of the NH_4^+- and NO_3^--pools were generally within or nearly within the variation of the measured values (Fig. 5.2, 5.3 and 5.4). The largest discrepancies between simulated and measured values were observed for the NO_3^--contents at day 3 in the clay loam soil and at day 30 in the loamy sand and loamy soil (Fig. 5.2). This might be attributed to the relatively large standard deviations associated with these measured values, as measured variables with the largest experimental variability have the lowest weight in the optimization procedure of the FLUAZ model, due to the MWE criterion. The considerable underestimation of the NO_3^--contents at day 3 in the clay loam soil might imply that the gross nitrification rate between day 1 and 3 has been underestimated by FLUAZ.

5.4.3. C mineralization and N transformation rates

C and N mineralization

One of the assumptions associated with the use of the ^{15}N isotope dilution technique to quantify gross N transformation rates in soils is that the N pools involved are isotopically homogeneous. Therefore, addition of relatively large amounts of labelling solution accompanied by thorough mixing of the soil is usually necessary to obtain a homogeneous tracer labelling of the soil, with the possible consequence of altering the N transformation rates at the start of ^{15}N isotope dilution experiments (Davidson et al., 1991; Sparling et al., 1995). However, since the three soils in our study have been labelled with the same amount of labelling solution (corresponding with an increase of 6% gravimetric moisture content), it can be assumed that the effect of the labelling on the N transformation rates was the same in the three soils.

In this study, the effect on the microbial activity of the addition of the labelling solution to the soils was apparent from the considerably larger C mineralization rates, which were observed during the first three days of the incubation experiments, in relation to the rates which were observed during the rest of the incubations (Table 5.3). The gross N mineralization rates which were observed during the first days (loamy and clay loam soils) or the first three days (loamy sand soil) of the incubations were approximately two to three times larger than the average gross mineralization rates observed during the rest of the incubation experiments (Table 5.4). This indicates that the gross N mineralization rates were probably also stimulated during the first days through the addition of the labelling solution and subsequent mixing of the soils.

The cumulative gross N mineralization at the end of the incubation period was largest in the loamy sand soil, and 1.2 to 3.2 times larger than in the clay loam and loamy soils, respectively (Fig. 5.6 A). The total N content however was largest in the clay loam soil, and approximately 2 times larger than in the loamy sand and the loamy soils, which showed nearly equal N contents (Table 5.1). When we take into account these differences in total N content among the three soils, the largest proportion of the initial total N content mineralized (ammonified) upon incubation was observed in the loamy sand soil (2.5%), followed by the clay loam soil (1.2%) and loamy soil (0.8%). Hassink (1994) found a significant, negative correlation between the proportion of initial organic N mineralized upon incubation and the clay plus silt content in grassland soils of varying texture. The loamy sand soil had a significantly lower clay plus silt content (14.1%) than the loamy soil (52.1%) and clay loam soil (72.2%) (Table 5.1). Thus we suggest that the observed differences in cumulative gross N mineralization among the three soils investigated may be largely explained by the differences in clay plus silt content, in combination with the total N content.

Nitrification

As NH_4^+ is the substrate for the nitrification process, the relatively large NH_4^+-addition at the start of the incubation experiments has most probably stimulated the nitrification. Therefore, the calculated gross nitrification rates represent the potential nitrifying activity, rather than the naturally occurring nitrification activity in these soils. The nitrification rate observed during the first day after NH_4NO_3-addition in the clay loam soil was 10 to 17 times larger than the rates observed in the loamy and loamy sand soils, respectively (Table 5.4). This indicates that the clay loam soil had a much higher potential nitrifying activity, resulting in a much faster depletion of the NH_4^+-content (after 3 days) than in the loamy soil (after 14 days) and the loamy sand soils (after 30 days). These differences in potential nitrifying activity might be partially explained by differences in soil pH (Table 5.1). The conditions for nitrification might be more favourable in the clay loam soil due to its higher pH (7.2) in relation to the loamy soil (6.3) and loamy sand soil (5.9). Paul and Clark (1989) reported that nitrification is curtailed when the soil pH is below 6.0, which might explain the considerably smaller potential nitrifying activity in the loamy sand soil. The difference in potential nitrifying activity might possibly also be explained by a difference in the naturally occurring, nitrifying microbial population in the three soils. We observed that the initial NH_4^+-content (after pre-incubation) in the loamy sand soil (42.4 mg N kg^{-1} soil) was much higher than in the loamy and clay loam soils (1.6 and 1.5 mg N kg^{-1} soil, respectively), whereas the initial NO_3^--contents increased in the order of loamy sand soil (28.6 mg N kg^{-1} soil) < loamy soil (39.3 mg N kg^{-1} soil) < clay loam soil (57.6 mg N kg^{-1} soil). This indicates that the naturally occurring nitrification rate, without addition of NH_4^+, also tended to be much smaller in the loamy sand soil in relation to the loamy and clay loam soils.

Immobilization and remineralization

In the three soils investigated, the gross NH_4^+ and NO_3^- immobilization rates observed during the first day after addition of the NH_4NO_3-solutions were considerably larger than the immobilization rates observed during the rest of the incubations. This could possibly indicate a stimulation of both the NH_4^+ and NO_3^- immobilization rates, due to the large amounts of NH_4^+- and NO_3^--N which have been added to the soils. The smaller gross NH_4^+ immobilization rate in the loamy sand soil during the first day in relation to the rates observed in the loamy and clay loam soils, might then be partially explained by a smaller stimulation of the NH_4^+ immobilization, due to the larger NH_4^+-content already present in the loamy sand soil before the NH_4NO_3-addition. However, the larger NH_4^+ and NO_3^- immobilization rates may probably also have been associated with the relatively larger C mineralization rates which were observed during the first (loamy sand and loamy soils) or first three days (clay loam soil) of the experiments (Table 5.3). Considering the rates from all time intervals, 46% of the variation in the total gross N immobilization rates could be explained by the variation in C mineralization rates. When two time intervals from the clay loam soil were not considered, the C mineralization rates explained up to 85% of the variation in the total immobilization rates (Fig. 5.8). These results indicate that the total gross N immobilization rates and C mineralization rates were closely related during the incubation experiments.

This relationship between gross N immobilization rates and C mineralization rates has been observed in several other studies (Schimel, 1986; Hart et al., 1994; Mary et al., 1998; Barret and Burke, 2000). Mary et al. (1998) reported a similar regression coëfficient (0.2) for the relationship between potential gross N immobilization rates and C mineralization rates in an arable soil, after addition of similar amounts of ^{15}N-labelled NH_4^+-N and NO_3^--N. Barret and Burke (2000), however, reported an equal R^2-value (0.46) but a considerably smaller regression coëfficient (0.03) for the relationship between gross

N immobilization and C mineralization rates in grassland soils. This might be partially explained by the 10 times smaller amount of $^{15}NH_4^+$-N which was added to the soils in that study.

In nearly all time intervals considered, NO_3^- immobilization occurred simultaneously with NH_4^+ immobilization, but at a smaller proportion of the total immobilization than the NH_4^+ immobilization. This is consistent with the findings of several other studies and reflects the generally observed preferential microbial uptake of NH_4^+-N in relation to NO_3^--N, when both forms are present in soil (Jansson et al., 1955; Rice and Tiedje, 1989; Recous et al., 1990). The large NH_4^+ and NO_3^- immobilization rates during the first day after NH_4NO_3-addition were accompanied by significant remineralization rates during the first day in the loamy sand and clay loam soils, and between day 1 and 3 in the loamy soil (Table 5.4). This indicates that a quick recycling through the microbial biomass of the recently immobilized mineral N already occurred during the first days after NH_4NO_3-addition in the three soils investigated, and that this fast recycling was most pronounced in the loamy sand and clay loam soils. This fast recycling was also observed by Mary et al. (1998).

An increase in the atom% ^{15}N in excess of the NH_4^+-pool after addition of $^{14}NH_4{}^{15}NO_3$ was observed from day 14 in the loamy soil and from day 7 in the clay loam soil (Fig. 5.3). This ^{15}N-enrichment in the NH_4^+-pool when $^{14}NH_4{}^{15}NO_3$ was applied could be explained by the remineralization of previously immobilized ^{15}N, by means of a direct conversion of NO_3^- to NH_4^+ via dissimilatory reduction (DNRA), or by a combination of the two processes occurring simultaneously in these soils. The good FLUAZ-simulation (Fig. 5.3) indicated that this ^{15}N-enrichment of the NH_4^+-pool in the loamy soil could be largely explained by the remineralization after day 14 (Table 5.4) of recently immobilized $^{15}NO_3^-$. For the clay loam soil, however, FLUAZ simulated a smaller increase in the ^{15}N-enrichment of the NH_4^+-pool than the observed enrichment between day 7 and 30

(Fig. 5.3). This ^{15}N-enrichment was simulated by means of a significant remineralization rate between day 7 and 14 and a smaller remineralization rate between day 14 and 30 (Table 5.4). This underestimation of the observed ^{15}N-enrichment in the NH_4^+-pool by the FLUAZ-model, which doesn't consider DNRA, might suggest that this enrichment could be partially attributed to DNRA occurring in the clay loam soil after day 7. However, considering the strict anaerobic nature of the DNRA process (Paul and Clark, 1996) and the assumption that the soils were incubated under aerobic conditions (water filled pore space of 50%), it would be very unlikely that DNRA, nor denitrification would have occurred in the clay loam soil. Nevertheless, a decrease of the NO_3^--content was observed after day 14 (Fig. 5.2) and FLUAZ estimated a significant denitrification rate between day 14 and 30 (Table 5.4). This might indicate that some anaerobic microsites could have been produced throughout the incubation period, enabling denitrification or DNRA to occur in the clay loam soil. Fazzolari et al. (1998) demonstrated that DNRA activity may be less sensitive than denitrification to an inhibitory effect by O_2 and therefore may also occur in aerobic soils. Müller et al. (2004) suggested that DNRA, rather than remineralization, was responsible for the significant ^{15}N-enrichment of the NH_4^+-pool which they observed during aerobic incubation of an old grassland soil after addition of equal amounts of $^{14}NH_4{}^{15}NO_3$.

5.5. Conclusions

In the three soils investigated, significant amounts of ^{15}N were recovered in the soil organic and fixed N pool shortly after addition of both $^{15}NH_4{}^{14}NO_3$ and $^{14}NH_4{}^{15}NO_3$, indicating a fast biotic or abiotic immobilization capacity. The fast immobilization capacity of $^{15}NH_4^+$-N was most pronounced in the loamy and clay loam soils, and could probably be attributed to clay fixation, as these soils showed relatively larger clay contents. The total amounts of ^{15}N recovered in the soil organic and fixed N pool 30 days after addition of $^{15}NH_4{}^{14}NO_3$ were approximately 5 times larger than the amounts recovered after addition of $^{14}NH_4{}^{15}NO_3$, which reflects the generally observed preferential microbial uptake of NH_4^+-N in relation to NO_3^--N.

The large NH_4^+ and NO_3^- immobilization rates, which were observed during the first days after NH_4NO_3-addition, were accompanied by significant remineralization rates. This indicates that a quick recycling through the microbial biomass of the recently immobilized mineral N occurred shortly after NH_4NO_3-addition. This suggests that numerical simulation models, which take into account remineralization, are preferable with regard to analytical solutions for estimation of gross N transformation rates, even for short-term ^{15}N isotope dilution experiments. The total gross N immobilization rates and C mineralization rates were closely related during the incubation experiments.

The loamy sand soil, which had a considerably lower clay plus silt content than the other soils, showed the largest cumulative gross N mineralization and the largest proportion of N mineralized at the end of the incubation period. These results indicate that the observed differences in cumulative gross N mineralization among the three soils investigated may be largely explained by the differences in clay plus silt content, in combination with the total N content. The observed potential nitrification activity after addition of NH_4NO_3 was considerably larger in the clay loam soil in relation to the loamy

and loamy sand soils. These differences in potential nitrification activity might possibly be explained by the difference in soil pH or a difference in the naturally occurring, nitrifying microbial population among the investigated soils.

CONCLUSIONS AND PERSPECTIVES

Conclusions and perspectives

A first objective of this thesis was to investigate the quality of SOM, in terms of degradability and turnover, in cultivated and grassland soils by means of physical fractionation of the SOM and variations in its ^{13}C isotopic signature. A second objective of this thesis was to study the N dynamics in permanent grassland soils, as affected by the quantity and quality of SOM and soil type.

The conclusions from this thesis can be summarized as follows:

1. The $\Delta\delta^{13}C$ values in the surface layers (0-30 cm depth) of the investigated profiles under permanent grassland were strongly correlated with the C decomposition rate constants and might serve as a practical indicator of soil C stability (Chapter 2);
2. Shifts in the C content, ^{13}C isotopic signature and C/N ratio of size and density fractions of SOM from cultivated and grassland soils reflected an increasing degree of microbial degradation and a decreasing C turnover rate (1) with increasing density among the macro-organic matter fractions, and (2) with decreasing particle size among the size fractions considered (Chapter 3);
3. The largest relative increase in C and N contents (with increasing age of the investigated grassland soils) was observed in the HF 150-2000 µm fraction, followed by the 50-150 µm and <50 µm fractions. As SOM accumulation induced by the conversion of cultivation to permanent grassland tends to be a slow process, our results suggest that the HF 150-2000 µm fraction could serve as a good and relatively easily detectable indicator of early soil organic C and N accumulation. The ratio of gross N immobilization to gross N mineralization tended to increase with increasing SOM contents, which indicates that

potential N retention in soils through biotic immobilization tends to be limited by C availability (Chapter 4);

4. As the size and density fractions considered were characterized by different degrees of microbial degradation and turnover rates (Chapter 3), and as their C and N contents were significantly correlated with the potential gross N transformation rates and long-term net N mineralization rates in the investigated grassland soils (Chapter 4), they might represent suitable pools to be used in mechanistic SOM models;

5. The differences in cumulative gross N mineralization and potential nitrification after NH_4NO_3 addition to grassland soils of varying texture, could be explained by differences in silt plus clay content, SOM content, soil pH or the naturally occuring microbial population. The investigated soils showed a fast biotic or abiotic immobilization capacity for both NH_4^+- and NO_3^--N in the soil organic and fixed N pool. Total gross (biotic) N immobilization showed to be closely related to C mineralization (C turnover). Numerical simulation of the data indicated that quick recycling (remineralization) through the microbial biomass of the recently immobilized mineral N may occur, shortly after NH_4NO_3-addition. This suggests that numerical simulation models, which take into account remineralization, are preferable with regard to analytical solutions for estimation of gross N transformation rates, even for short-term ^{15}N isotope dilution experiments (Chapter 5).

The following research perspectives arose from this thesis:

1. The observed relationship between ^{13}C enrichment and C dynamics, and the potential use of $\Delta\delta^{13}C$ values or the isotope enrichment factor ε as an indicator of soil C stability or availability, might be further elucidated by investigating the influence of soil type (in terms of drainage capacity and texture) and grassland age (in terms of SOM content) on the evolution of the ^{13}C enrichment in grassland profiles. This relationship

might also be investigated in other, undisturbed soil profiles with an exclusive C_3 or C_4 vegetation, like arable soils under no-tillage or forest soils;

2. The combined LF+IF 150-2000 μm fraction (density <1.37 g cm^{-3}), rather than the separate LF and IF 150-2000 μm fractions, could be considered in future grassland SOM studies;

3. Evaluation of the relationship between distribution of SOC among the different size and density fractions and the potential C dynamics (laboratory incubation experiments) in grassland soils of different age;

4. Assessment of the potential C mineralization, net N mineralization, and gross mineralization-immobilization turnover during laboratory incubation of separate size and density fractions;

5. Assessment of the biotic or abiotic nature of the fast N-immobilization capacity in grassland soils by comparing the ^{15}N-recovery in the organic and fixed N pool of sterilized and non-sterilized soils after addition of ^{15}N enriched NH_4^+-N and NO_3^--N;

6. Evaluation of the influence of soil characteristics (texture, pH, SOM content) and climatic factors (soil water content and temperature) on the potential environmental N losses (N_2O and NO emission, NH_3 volatilization, NO_3^--leaching) associated with the N transformations occurring in soils;

7. Evaluation of the relationship between $\Delta\delta^{13}C$ values (as a potential indicator of soil C availability) and potential N retention in grassland soils through microbial immobilization, which tends to be dependent on C availability.

SUMMARY

SAMENVATTING

Summary

Soil organic matter (SOM) is a key component in the quality and fertility of agricultural soils, as it affects many of its biological, physical and chemical properties. SOM is a primary nutrient source for plants and soil organisms, and contributes significantly to the formation and stabilization of the soil structure. The SOM in agricultural soils, and more specifically the soil organic C (SOC), is also receiving increased attention in terms of carbon sequestration, as a potential sink for atmospheric CO_2 (which is the most important anthropogenic greenhouse gas). Maintenance and improvement of the SOM content is thus generally accepted as being a major objective for any sustainable agroecosystem. As SOM comprises an enormous array of compounds, ranging from recent plant materials through a continuum of metabolic products of micro-organisms, to components of stable humus, characterization of SOM quality is very complex. Recently, biologically meaningful SOM fractions have been obtained by methods based on physical fractionation of soil (according to particle size or density), which, combined with biological and chemical analysis, allows further insight into the functionality of the separated fractions. Moreover, the use of stable isotope techniques (e.g. ^{13}C natural abundance analysis and ^{15}N isotope dilution) have further advanced process oriented SOM studies, since these methods are well suited to study SOM quality and dynamics.

A first objective of this thesis was to investigate the quality of SOM, in terms of degradability and turnover, in cultivated soils and grassland soils by means of physical fractionation of the SOM and variations in its ^{13}C isotopic signature. A second objective of this thesis was to study the N dynamics in permanent grassland soils, as affected by the quantity and quality of SOM and soil type.

Chapter 1 introduces the subject and focuses on (1) the role of organic matter in agricultural soils, (2) factors affecting organic matter content and organic matter turnover in agricultural soils, (3) assessment of the quality and turnover of soil organic matter, and (4) the major N transformations that occur in agricultural soils.

In chapter 2 we investigated to what extent the potential C dynamics of SOM are related to the degree of ^{13}C enrichment with increasing depth in soil profiles under permanent grassland. The evolution of the C content and the ^{13}C natural abundance ($\delta^{13}C$ value, expressed in ‰) of SOM was investigated in three soil profiles (0-40 cm depth) under permanent grassland of varying texture (loamy sand, loamy and clay loam). The $\delta^{13}C$ value of the SOM showed a gradual increase with increasing depth and decreasing C content in the three profiles, ranging from 1.9‰ (loamy sand soil), to 2.9‰ (clay loam soil) and 4‰ (loamy soil) in relation to the $\delta^{13}C$ value of SOM at the surface. The relation between the ^{13}C enrichment and total organic C content at different depths in the profiles (down to 40 cm depth in the loamy and clay loam soil, down to 25 cm depth in the loamy sand soil) could be fitted by the Rayleigh equation. This reflects that the observed ^{13}C enrichment with increasing depth is mainly driven by isotopic fractionation associated with C mineralization along with the decomposition process. The enrichment factors ε, associated with the Rayleigh approximation of the data, ranged from -1.57‰ (clay loam soil), to -1.64‰ (loamy sand soil) and -1.91‰ (loamy soil). The evolution of the $\delta^{13}C$ signature in the loamy sand profile, which diverged from the Rayleigh approximation below 25 cm depth, suggests that other factors, like differential preservation or accumulation of ^{13}C depleted material, may also influence the $\delta^{13}C$ evolution in poorly drained, chronically wet soil profiles. The potential C mineralization rates (determined by means of an aerobic incubation experiment) and the C decomposition rate constants (ratio of C mineralization rate to total C content) both decreased significantly with increasing

sampling depth (0-10, 10-20, 20-30 and 30-40 cm depth) in the three profiles, reflecting a more enhanced stage of decomposition and stability of SOC in the deeper soil layers. There was a significant, positive correlation ($drc_C = 0.22\Delta\delta^{13}C + 0.019$, $R^2=0.75$, $p<0.001$, $n=12$) between the C decomposition rate constants (drc_C) from the four sampling depths in the three profiles and the corresponding $\Delta\delta^{13}C$ values (average change of the $\delta^{13}C$ value per depth increment, expressed in ‰ cm^{-1}). A stronger, positive correlation between the C decomposition rate constants and the $\Delta\delta^{13}C$ values was observed when only the data from the upper 30 cm in the profiles ($drc_C = 0.22\Delta\delta^{13}C + 0.019$, $R^2=0.86$, $p<0.001$, $n=9$) or from the upper 20 cm in the profiles ($drc_C = 0.21\Delta\delta^{13}C + 0.020$, $R^2=0.78$, $p<0.05$, $n=6$) were considered. These results suggest that the $\Delta\delta^{13}C$ values in the surface layers (0-30 cm depth) of profiles under permanent grassland, and to a lesser extent the $\Delta\delta^{13}C$ values in the deeper soil layers (30-40 cm depth), may be interpreted as a direct indicator of the degradability of the SOM, in terms of the C decomposition rate constant. As $\Delta\delta^{13}C$ values are more easily accessible, whereas incubation experiments to determine potential C mineralization rates from soil samples are generally time-consuming and laborious, the $\Delta\delta^{13}C$ values might serve as a practical tool for getting a rapid indication of soil C stability in the surface layers (0-30 cm depth) of profiles under permanent grassland.

In chapter 3, we compared the quantity and quality of SOM (in terms of turnover) in the surface layer of cultivated and grassland soils. The variation in ^{13}C enrichment due to isotopic fractionation associated with SOM decomposition was investigated among five size and density fractions, water soluble organic C (WSOC) and microbial biomass C (MBC) in the surface layer (0-20 cm depth) of a continuous grassland soil (CG, C_3 vegetation). The five size and density fractions considered were the light (LF 150-2000 µm; d <1.13 g cm^{-3}), intermediate (IF 150-2000 µm; 1.13< d <1.37 g cm^{-3}) and heavy density fraction (HF 150-2000 µm; d >1.37 g cm^{-3}) of the macro-organic matter (150-2000

µm), the size fraction 50-150 µm and the size fraction <50 µm. The distribution of total C and the incorporation of relatively 'young' C_4-C into these SOM fractions was investigated through $\delta^{13}C$ analysis of the same SOM fractions, originating from the surface layer (0-20 cm depth) of a C_3 soil, which had been converted (since 19 years at the time of sampling) to continuous maize cultivation (CM, C_4 vegetation) and a three year rotation of maize cultivation and grassland (R). The CG soil showed the largest total C content (22.5 g kg^{-1} soil), and the C contents in the R and CM soil were, respectively, 34% and 63% lower than in the CG soil. The amounts of WSOC and MBC in the CG soil were both significantly larger than in the R and CM soil. The proportion of total C present in the macro-organic matter decreased in the order CG (14.6%) > R (9.3%) > CM (6.8%). With decreasing total C contents, the C enrichment ratio (ratio of g C kg^{-1} fraction to g C kg^{-1} whole soil) of the size fraction <50 µm increased from 1.1 in the CG soil to 1.8 in the CM soil, while the C enrichment ratio in the size fraction 50-150 µm decreased from 0.6 (CG soil) to 0.3 (CM soil). Consequently, the proportion of total C present in the size fraction <50 µm increased in the order CG (60.2%) < R (67.9%) < CM (82.3%), while the proportion in the size fraction 50-150 µm decreased in the order CG (25.2%) > R (22.8%) > CM (10.8%). This indicates that C in the clay- and silt-sized fraction (<50 µm) was less affected by soil disruption due to tillage, than C in the macro-organic matter and in the size fraction 50-150 µm. In the three soils investigated, the C/N ratios tended to decrease in the order LF 150-2000 µm fraction > IF and HF 150-2000 µm fractions > size fraction 50-150 µm > size fraction <50 µm. In the CG soil, we observed a trend of increasing ^{13}C enrichment in the order LF < IF < HF 150-2000 µm fraction and a higher ^{13}C enrichment in the size fraction <50 µm in relation to the size fractions >50 µm, which may be attributed to isotopic fractionation associated with the microbial decomposition process of plant residues in soils. The amount of C_4-derived C in the R and CM soil (after in total 10 and 19 years of maize cultivation, respectively) equalled 11% (R soil) and 33% (CM soil)

of the total C content. Among the size and density fractions in the R and CM soils, the LF 150-2000 µm fraction showed the largest proportion of C_4-derived C (47% and 77%, respectively). The shifts in the $\delta^{13}C$ values, together with the decrease in C/N ratios, which were observed among the size and density fractions from the CG, R and CM soils, reflected an increasing degree of microbial degradation and a decreasing turnover rate (1) with increasing density among the macro-organic matter fractions, and (2) with decreasing particle size among the size fractions considered.

The accumulation of SOM upon conversion of arable land to permanent grassland, and the influence of quantity and quality of SOM on the gross N transformation rates and long-term net N mineralization in permanent grasslands soils were studied in chapter 4. This was investigated in the surface layers (0-10 and 10-20 cm depth) of soils (sandy loam texture) which had been converted from continuous arable cropping (during at least 20 years) to permanent grassland since respectively 6, 14 and approximately 50 years at the time of sampling. The SOM was fractionated into the LF 150-2000 µm, IF 150-2000 µm and HF 150-2000 µm fractions, the size fraction 50-150 µm and the size fraction <50 µm in order to study the distribution of the total C and N content among these SOM fractions. The potential gross N transformation rates (mineralization (= ammonification), nitrification, NH_4^+ and NO_3^- immobilization) were determined by means of short-term, fully-mirrored ^{15}N isotope dilution experiments (7-day laboratory incubations). The long-term potential net N mineralization and gross N immobilization rates were determined by means of 70-day laboratory incubations. The total C and N contents mainly tended to increase in the 0-10 cm layer with increasing age of the investigated grassland soils. Significant differences in total SOM storage were, however, only detectable in the long-term (50 years old grassland soil) after conversion of arable land to permanent grassland. In the assumption that the initial SOM contents, before conversion to permanent grassland, and the average annual input of organic material in the grassland soils were

comparable, these results indicate that stabilization of SOM and thus sequestration of C upon conversion of arable land to grassland is a slow process. The C and N contents and the proportions of total C and N stored in the SOM fractions generally decreased in the order <50 µm > 50-150 µm > HF 150-2000 µm > IF 150-2000 µm > LF 150-2000 µm. The largest relative increase in C and N contents occurred in the HF 150-2000 µm fraction, followed by the 50-150 µm and <50 µm fractions. Our results suggest that the HF 150-2000 µm fraction could serve as a good and relatively easily detectable indicator of early soil organic C and N accumulation, or early changes in SOM content in general, induced by the conversion of cultivated soils to permanent grassland. The gross N mineralization, nitrification, and (long-term) gross N immobilization rates tended to increase with increasing age of the investigated grasslands, and showed strong, positive correlations with the total C and N contents. The observed gross N mineralization rates (7-day incubations) and net N mineralization rates (70-day incubations) corresponded with a gross N mineralization of 643, 982 and 1876 kg N ha^{-1} y^{-1}, and a net N mineralization of 195, 208 and 274 kg N ha^{-1} y^{-1} in the upper 20 cm of the 6, 14 and 50 years old grassland soils, respectively. Linear regression analysis showed that 93% of the variability of the gross N mineralization rates could be explained by variations in the total N contents, whereas total N contents together with the C/N ratios of the <50 µm fraction explained 84% of the variability of the net N mineralization rates. The relation between long-term net N mineralization rates and gross N mineralization rates could be fitted by means of a logarithmic equation (net m = 0.24Ln(gross m) + 0.23, R^2=0.69, $p<0.05$), which reflects that the ratio of gross N immobilization to gross N mineralization tended to increase with increasing SOM contents. Since the microbial demand for N (immobilization) tended to increase with increasing SOM content in the investigated grassland soils, this indicates that potential N retention in soils through microbial N immobilization tends to be limited by C availability.

In chapter 5, we investigated the evolution of the gross N transformation rates and the potential N retention after mineral fertilizer application in three permanent grassland soils (0-10 cm depth) of varying texture (loamy sand, loamy and clay loam). Differently ^{15}N-labelled NH_4NO_3 (at a rate of 100 mg N kg^{-1} soil) was added to the soils in paired experiments (30-day laboratory incubations). Size and ^{15}N-enrichment of the NH_4^+, NO_3^-, and soil organic and fixed N pools were measured at 0, 1, 3, 7, 14 and 30 days after NH_4NO_3-application. The C mineralization rates were also monitored during the incubation experiments. The experimental data were simulated with the numerical simulation model FLUAZ (Mary et al., 1998)[1] in order to estimate the gross N transformation rates. The cumulative gross N mineralization (ammonification) and the proportion of initial N mineralized at the end of the incubation were largest in the loamy sand soil (68 mg N kg^{-1} soil or 81 kg N ha^{-1}; 2.5% of initial N), followed by the clay loam soil (58 mg N kg^{-1} soil or 64 kg N ha^{-1}; 1.2% of initial N) and the loamy soil (21 mg N kg^{-1} soil or 28 kg N ha^{-1}; 0.8% of initial N). These differences in cumulative gross N mineralization could be largely explained by the lower clay plus silt content in the loamy sand soil (14%) in relation to the loamy (52%) and the clay loam (72%) soils, in combination with the total N contents. The potential gross nitrification activity was considerably larger in the clay loam soil (pH 7.2) in relation to the loamy (pH 6.3) and loamy sand (pH 5.9) soils. These differences in potential gross nitrification activity might be explained by the difference in soil pH, or a difference in the naturally occurring, nitrifying microbial population in the three soils. In the three soils investigated, significant amounts of ^{15}N were recovered in the soil organic and fixed N pool shortly after addition of both $^{15}NH_4{}^{14}NO_3$ and $^{14}NH_4{}^{15}NO_3$, indicating a fast biotic or abiotic immobilization capacity. The fast immobilization capacity of $^{15}NH_4^+$-N was most pronounced in the loamy and clay loam soils, and could probably be attributed to clay fixation. The total

[1] Mary, B., Recous, S., Robin, D., 1998. A model for calculating nitrogen fluxes in soil using ^{15}N tracing. Soil Biology and Biochemistry 30, 1963-1979.

amounts of ^{15}N recovered in the soil organic and fixed N pool at 30 days after addition of $^{15}NH_4{}^{14}NO_3$ were approximately 5 times larger than the amounts recovered after addition of $^{14}NH_4{}^{15}NO_3$, which also reflects the generally observed preferential microbial uptake of NH_4^+-N in relation to NO_3^--N. The large NH_4^+ and NO_3^- immobilization rates, which were observed during the first days after NH_4NO_3-addition, were accompanied by significant remineralization rates in the three soils investigated. This indicates that a quick recycling through the microbial biomass of the recently immobilized mineral N occurred shortly after NH_4NO_3-addition. The total gross N immobilization rates and C mineralization rates were closely related ($i = 0.50m_C - 0.54$, $R^2=0.85$, $p<0.01$) during the incubation experiments.

Samenvatting

Bodem organisch materiaal (BOM) is van cruciaal belang voor de bodemkwaliteit en bodemvruchtbaarheid, vermits het een invloed uitoefent op talrijke biologische, fysische en chemische bodemkarakteristieken. BOM is een primaire bron van nutriënten voor planten en bodemorganismen, en draagt in belangrijke mate bij tot de vorming en stabilisatie van de bodemstructuur. BOM in landbouwgronden, en meer specifiek de bodem organische koolstof (BOC), krijgt ook steeds meer aandacht in het kader van C-sequestratie als een potentiële *sink* voor atmosferische CO_2, het belangrijkste antropogeen broeikasgas. Het in stand houden of verhogen van het gehalte aan BOM wordt daarom algemeen beschouwd als één van de belangrijkste objectieven voor elk duurzaam agro-ecosysteem. Aangezien het BOM een enorme waaier aan componenten omvat, gaande van recent afgestorven plantenmateriaal, via een continuüm van metabolische producten van micro-organismen tot stabiele humusverbindingen, is de karakterisering van de kwaliteit van BOM zeer complex. Recent werden door middel van methodes gebaseerd op fysische bodemfractionering (volgens deeltjesgrootte of -densiteit) biologisch betekenisvolle fracties van het BOM bekomen, die, in combinatie met biologische en chemische analyse, een dieper inzicht opleveren in de functionaliteit van deze fracties. Daarnaast heeft het gebruik van stabiele isotopentechnieken (zoals analyse van de natuurlijke aanrijking in ^{13}C en ^{15}N isotopenverdunning) het proces georiënteerd onderzoek van BOM sterk vooruitgeholpen, aangezien deze technieken zeer geschikt blijken te zijn om de kwaliteit en de dynamiek van BOM te bestuderen.

Een eerste doelstelling van deze thesis was het onderzoeken van de kwaliteit van BOM, meerbepaald de afbreekbaarheid en omzettingssnelheid ervan, in gecultiveerde bodems en graslanden door middel van fysische fractionatie en variaties in de ^{13}C isotopensignatuur van het BOM. Het onderzoeken van de invloed van de kwantiteit en

kwaliteit van BOM en bodemtype op de N-dynamiek in permanente graslanden was een tweede doelstelling van deze thesis.

In hoofdstuk 1 wordt het onderzoeksgebied kort geïntroduceerd, waarbij de aandacht gericht wordt op (1) de rol van organisch materiaal in landbouwgronden, (2) de factoren die het gehalte aan BOM en de omzettingssnelheid ervan beïnvloeden, (3) inschatting van de kwaliteit en omzettingssnelheid van BOM, en (4) de belangrijkste N-transformaties die optreden in landbouwgronden.

In hoofdstuk 2 werd onderzocht in welke mate de potentiële C-dynamiek van BOM gerelateerd is aan de mate van aanrijking in ^{13}C met toenemende diepte in bodemprofielen onder permanent grasland. De evolutie van het C-gehalte en de natuurlijke aanrijking in ^{13}C ($\delta^{13}C$-waarde, uitgedrukt in ‰) van BOM werd onderzocht in drie bodemprofielen (0-40 cm diepte) met verschillende textuur (lemige zandbodem, lemige bodem en klei-leembodem) onder permanent grasland. De $\delta^{13}C$-waarde van het BOM vertoonde een geleidelijke toename met toenemende diepte en afnemend C-gehalte in de drie profielen, variërend tussen 1.9‰ (lemige zandbodem), 2.9‰ (klei-leembodem) en 4‰ (lemige bodem) ten opzichte van de $\delta^{13}C$-waarde van het BOM aan de oppervlakte. Het verband tussen de ^{13}C-aanrijking en het totale organische C-gehalte op verschillende diepten in de profielen (tot op 40 cm diepte in de lemige bodem en klei-leembodem, tot op 25 cm diepte in de lemige zandbodem) kon benaderd worden door middel van de Rayleigh vergelijking. Dit geeft weer dat de waargenomen ^{13}C-aanrijking met toenemende diepte grotendeels kan verklaard worden door isotopenfractionatie geassocieerd met de C-mineralisatie tijdens het afbraakproces van BOM. De C-aanrijkingsfactoren ε, geassocieerd met de Rayleigh benadering van de data, varieerde tussen -1.57‰ (klei-leembodem), -1.64‰ (lemige zandbodem) en -1.91‰ (lemige bodem). De evolutie van de

δ^{13}C-waarde in de lemige zandbodem, die afweek van de Rayleigh benadering vanaf 25 cm diepte in het profiel, suggereert dat andere factoren zoals differentiële bewaring of accumulatie van ^{13}C-verarmd materiaal eveneens de evolutie van de δ^{13}C-waarde kunnen beïnvloeden in matig gedraineerde, chronisch waterverzadigde bodemprofielen. De potentiële C-mineralisatiesnelheden (bepaald door middel van een aëroob incubatie-experiment) en de C-afbraak snelheidsconstanten (verhouding van de C-mineralisatiesnelheid tot het C-gehalte) namen beiden significant af met toenemende diepte (0-10, 10-20, 20-30 en 30-40 cm diepte) in de drie profielen, hetgeen een verder gevorderd afbraakstadium en grotere stabiliteit van de BOC in de diepere bodemlagen weergeeft. Er bestond een significante, positieve correlatie ($drc_C = 0.22\Delta\delta^{13}C + 0.019$, $R^2=0.75$, $p<0.001$, $n=12$) tussen de C-afbraaksnelheidsconstanten (drc_C) in de vier diepte-intervallen van de drie profielen en de corresponderende $\Delta\delta^{13}$C-waarden (gemiddelde toename van de δ^{13}C-waarde met toenemende diepte, uitgedrukt in ‰ cm^{-1}). Een sterkere, positieve correlatie tussen de C-afbraak snelheidsconstanten en de $\Delta\delta^{13}$C-waarden werd waargenomen wanneer enkel de waarden afkomstig van de bovenste 30 cm in de profielen ($drc_C = 0.22\Delta\delta^{13}C + 0.019$, $R^2=0.86$, $p<0.001$, $n=9$) of van de bovenste 20 cm in de profielen ($drc_C = 0.21\Delta\delta^{13}C + 0.020$, $R^2=0.78$, $p<0.05$, $n=6$) werden beschouwd. Deze resultaten suggereren dat de $\Delta\delta^{13}$C-waarden in de oppervlaktelagen (0-30 cm diepte) van profielen onder permanent grasland, en in mindere mate de $\Delta\delta^{13}$C-waarden in de diepere bodemlagen (30-40 cm diepte), kunnen geïnterpreteerd worden als een directe indicator van de C-afbraaksnelheidsconstante of de afbreekbaarheid van het BOM. Vermits de $\Delta\delta^{13}$C-waarden relatief makkelijk toegankelijk zijn, terwijl incubatie-experimenten ter bepaling van de potentiële C-dynamiek in bodems meestal tijdrovend en arbeidsintensief zijn, zouden de $\Delta\delta^{13}$C-waarden potentieel kunnen aangewend worden als een praktische en snelle indicator van de stabiliteit van de bodem-C in de oppervlaktelagen (0-30 cm diepte) van profielen onder permanent grasland

In hoofdstuk 3 werden de kwantiteit en kwaliteit (meer specifiek de omzettingssnelheid) van het BOM in de oppervlaktelaag van gecultiveerde bodems en grasland vergeleken. De variatie in ^{13}C-aanrijking ten gevolge van isotopenfractionatie, geassocieerd met de afbraak van BOM, werd onderzocht voor vijf grootte- en densiteitsfracties, de water oplosbare organische C (WSOC) en de microbiële biomassa C (MBC) in de oppervlaktelaag (0-20 cm diepte) van een permanent grasland (CG, C_3-vegetatie). De vijf grootte- en densiteitsfracties van BOM die beschouwd werden waren de lichte (LF 150-2000 µm; d <1.13 g cm^{-3}), intermediaire (IF 150-2000 µm; 1.13< d <1.37 g cm^{-3}) en zware densiteitsfractie (HF 150-2000 µm; d >1.37 g cm^{-3}) van het macro-organisch materiaal (150-2000 µm), de groottefractie 50-150 µm en de groottefractie <50 µm. De verdeling van de totale C en de incorporatie van relatief 'jonge' C_4-C werd onderzocht door middel van $\delta^{13}C$ analyse van dezelfde BOM fracties, afkomstig van de oppervlaktelaag (0-20 cm diepte) van een bodem met oorspronkelijk enkel C_3-BOC, die omgezet was (sedert 19 jaar op het tijdstip van de staalname) naar permanente maïscultivatie (CM, C_4-vegetatie) en een driejarige rotatie van maïscultivatie en grasland (R). De CG bodem vertoonde het hoogste C-gehalte (22.5 g kg^{-1} grond), en de C-gehaltes in de R en CM bodems waren respectievelijk 34% en 63% lager dan in de CG bodem. De WSOC- en MBC-gehaltes in de CG bodem waren beiden significant hoger dan in de R en CM bodems. Het aandeel van de totale C aanwezig in het macro-organisch materiaal nam af in de volgorde CG (14.6%) > R (9.3%) > CM (6.8%). Met afnemende totale C-gehaltes, nam de C-aanrijkingsverhouding (verhouding van g C kg^{-1} fractie tot g C kg^{-1} grond) in de groottefractie <50 µm toe van 1.1 in de CG bodem tot 1.8 in de CM bodem, terwijl de C-aanrijkingsverhouding in de groottefractie 50-150 µm afnam van 0.6 (CG bodem) tot 0.3 (CM bodem). Bijgevolg nam het aandeel van de totale C aanwezig in de groottefractie <50 µm toe in de volgorde CG (60.2%) < R (67.9%) < CM (82.3%), terwijl het aandeel van de totale C in de groottefractie 50-150 µm afnam in de volgorde CG (25.2%) > R (22.8%) > CM (10.8%). Dit wijst erop dat de C in de klei-leem fractie (<50 µm) minder onderhevig

was aan verstoring van de bodemstructuur ten gevolge van bodembewerking, dan de C in het macro-organisch materiaal en in de groottefractie 50-150 µm. In de drie onderzochte bodems nam de C/N verhouding af in de volgorde LF 150-2000 µm fractie > IF en HF 150-2000 µm fracties > groottefractie 50-150 µm > groottefractie <50 µm. In de CG bodem werd een trend van toenemende ^{13}C-aanrijking in de volgorde LF < IF < HF 150-2000 µm fractie en een hogere ^{13}C-aanrijking in de groottefractie <50 µm ten opzichte van de groottefracties >50 µm waargenomen, hetgeen kan toegeschreven worden aan isotopenfractionatie geassocieerd met de microbiële respiratie tijdens het afbraakproces van plantenmateriaal in bodems. Het aandeel C_4-C in de R en CM bodem (na respectievelijk in totaal 10 en 19 jaar maïscultivatie) bedroeg 11% (R bodem) en 33% (CM bodem) van het totale C-gehalte. Onder de grootte- en densiteitsfracties in de R en CM bodems, vertoonde de LF 150-2000 µm fractie het hoogste aandeel C_4-C (respectievelijk 47% en 77%). De verschuivingen in de δ^{13}C-waarden in combinatie met de afname in de C/N verhoudingen die werden waargenomen voor de grootte- en densiteitsfracties in de CG, R en CM bodems, gaven een toenemende graad van microbiële afbraak en een afnemende omzettingssnelheid weer (1) met toenemende densiteit onder de fracties van het macro-organisch materiaal, en (2) met afnemende deeltjesgrootte onder de beschouwde groottefracties.

De accumulatie van BOM na omzetting van een gecultiveerde bodem naar permanent grasland, en de invloed van kwantiteit en kwaliteit van BOM op de bruto N-transformatiesnelheden en de lange termijn netto N-mineralisatie in bodems onder permanent grasland werden onderzocht in hoofdstuk 4. Dit werd onderzocht in de oppervlaktelagen (0-10 en 10-20 cm diepte) van bodems (met een zandleemtextuur) die waren omgezet van een permanente cultivatie (gedurende ten minste 20 jaar) naar permanent grasland sinds respectievelijk 6, 14 en circa 50 jaar op het tijdstip van de

staalname. Het BOM werd gefractioneerd in de LF 150-2000 µm, IF 150-2000 µm en HF 150-2000 µm fracties, de groottefractie 50-150 µm en de groottefractie <50 µm om de verdeling van het totale C- en N-gehalte over deze fracties van het BOM te bestuderen. De potentiële bruto N-transformatiesnelheden (mineralisatie (= ammonificatie), nitrificatie, NH_4^+- en NO_3^--immobilisatie) werden bepaald door middel van korte termijn, volledig gespiegelde ^{15}N isotopendilutie-experimenten (incubaties gedurende 7 dagen in het laboratorium). De lange termijn potentiële netto N-mineralisatie en bruto N-immobilisatie werden gemeten door middel van incubaties gedurende 70 dagen in het laboratorium. De totale C- en N-gehaltes namen voornamelijk toe in de laag van 0-10 cm diepte met toenemende ouderdom van de onderzochte graslanden. Significante verschillen in het totale gehalte aan BOM waren echter slechts meetbaar op lange termijn na de omzetting naar permanent grasland (in het 50 jaar oude grasland). In de veronderstelling dat de initiële BOM-gehaltes, voor de omzetting naar permanent grasland, en de gemiddelde jaarlijkse input van organisch materiaal vergelijkbaar waren in de drie graslanden, wijzen deze resultaten erop dat de stabilisatie van BOM en aldus ook C-sequestratie na omzetting van gecultiveerde bodems naar grasland een traag proces is. De C- en N-gehaltes en het aandeel van de totale C en N aanwezig in de fracties van het BOM namen in het algemeen af in de volgorde groottefractie <50 µm > groottefractie 50-150 µm > HF 150-2000 µm > IF 150-2000 µm > LF 150-2000 µm. De grootste relatieve toename in C- en N-gehalte werd waargenomen in de HF 150-2000 µm fractie, gevolgd door de 50-150 µm en de <50 µm groottefracties. Deze resultaten suggereren dat de HF 150-2000 µm fractie zou kunnen aangewend worden als een goede en relatief eenvoudig meetbare indicator van vroege accumulatie van bodem organische C en N, of van vroege veranderingen in het BOM-gehalte in het algemeen, geïnduceerd door omzetting van gecultiveerde bodems naar permanent grasland. De bruto N-mineralisatie, nitrificatie en (lange termijn) bruto N-immobilisatie neigden toe te nemen met toenemende ouderdom van de onderzochte graslanden, en waren sterk positief gecorreleerd met de totale C- en N-gehaltes. De

waargenomen bruto N-mineralisatiesnelheden (incubatie gedurende 7 dagen) en netto N-mineralisatiesnelheden (incubatie gedurende 70 dagen) kwamen respectievelijk overeen met een bruto mineralisatie van 643, 982 en 1876 kg N ha^{-1} jr^{-1}, en een netto mineralisatie van 195, 208 en 274 kg N ha^{-1} jr^{-1} in de bovenste 20 cm van het 6, 14 en 50 jaar oude grasland. Lineaire regressie-analyse toonde aan dat 93% van de variabiliteit van de bruto N-mineralisatie kon verklaard worden door de variaties in het totale N-gehalte, terwijl het totale N-gehalte en de C/N verhouding in de groottefractie <50 µm samen 84% van de variabiliteit van de netto N-mineralisatie konden verklaren. Het verband tussen lange termijn netto N-mineralisatiesnelheid en bruto N-mineralisatiesnelheid kon benaderd worden door middel van een logaritmische vergelijking (netto m = 0.24Ln(bruto m) + 0.23, R^2=0.69, $p<0.05$), hetgeen weergeeft dat de verhouding van bruto N-immobilisatie tot bruto N-mineralisatie neigde toe te nemen met toenemende gehaltes aan BOM. De microbiële behoefte aan N (immobilisatie) neigde dus toe te nemen met toenemende C-beschikbaarheid in de onderzochte graslanden, hetgeen erop wijst dat de potentiële N-retentie in bodems door microbiële N-immobilisatie gelimiteerd neigt te zijn door het beschikbare C-gehalte.

In hoofdstuk 5 werd de evolutie van de bruto N-transformatiesnelheden en de potentiële N-retentie na minerale bemesting in drie permanente graslanden (0-10 cm diepte) met een verschillende textuur (lemige zandbodem, lemige bodem en klei-leembodem) onderzocht. Differentieel ^{15}N-gelabeld NH_4NO_3 (dosis van 100 mg N kg^{-1} grond) werd toegevoegd aan de drie gronden in gepaarde experimenten (incubaties gedurende 30 dagen in het laboratorium). De grootte en ^{15}N-aanrijking van de NH_4^+- en NO_3^--pool en de organische en gefixeerde N-pool werden gemeten op 0, 1, 3, 7, 14 and 30 dagen na toevoeging van NH_4NO_3. De experimentele data werden gesimuleerd met het

numeriek simulatiemodel FLUAZ (Mary et al., 1998)[1] om de bruto N-transformatiesnelheden te schatten. De cumulatieve bruto N-mineralisatie (ammonificatie) en het procentueel aandeel van het initiële N-gehalte dat gemineraliseerd was aan het eind van de incubatieperiode waren het grootst in de lemige zandbodem (68 mg N kg^{-1} of 81 kg N ha^{-1}; 2.5% van de initiële N), gevolgd door de klei-leembodem (58 mg N kg^{-1} of 64 kg N ha^{-1}; 1.2% van de initiële N) en de lemige bodem (21 mg N kg^{-1} of 28 kg N ha^{-1}; 0.8% van de initiële N). Deze verschillen in cumulatieve bruto N-mineralisatie konden grotendeels verklaard worden door een lager klei- plus leemgehalte in de lemige zandbodem (14%) in vergelijking met de lemige bodem (52%) en de klei-leembodem (72%), in combinatie met de totale N-gehaltes. De potentiële bruto nitrificatie was aanzienlijk hoger in de klei-leembodem (pH 7.2) in vergelijking met de lemige bodem (pH 6.3) en de lemige zandbodem (pH 5.9). Deze verschillen in potentiële bruto nitrificatie in de drie bodems zouden verklaard kunnen worden door de verschillen in pH, of door een verschil in de natuurlijk voorkomende, nitrificerende microbiële populatie. In de drie onderzochte bodems werden significante hoeveelheden ^{15}N teruggevonden in de organische en gefixeerde N-pool, kort na toevoeging van zowel $^{15}NH_4{}^{14}NO_3$ als $^{14}NH_4{}^{15}NO_3$, hetgeen wijst op een snelle biotische of abiotische immobilisatiecapaciteit. De snelle immobilisatiecapaciteit van $^{15}NH_4^+$-N was meest uitgesproken in de lemige bodem en de klei-leembodem, en kon waarschijnlijk toegeschreven worden aan kleifixatie. De totale hoeveelheden ^{15}N teruggevonden in de organische en gefixeerde N-pool 30 dagen na toevoeging van $^{15}NH_4{}^{14}NO_3$ waren circa 5 keer groter dan de hoeveelheden die teruggevonden werden na toevoeging van $^{14}NH_4{}^{15}NO_3$, hetgeen ook de algemeen waargenomen preferentiële microbiële opname van NH_4^+-N ten opzichte van NO_3^--N weergeeft. De relatief grote NH_4^+- en NO_3^--immobilisatiesnelheden die werden waargenomen gedurende de eerste dagen na toevoeging van NH_4NO_3, waren vergezeld

[1] Mary, B., Recous, S., Robin, D., 1998. A model for calculating nitrogen fluxes in soil using ^{15}N tracing. Soil Biology and Biochemistry 30, 1963-1979.

door significante remineralisatiesnelheden in de drie onderzochte bodems. Dit wijst erop dat een snelle recirculatie optrad door de microbiële biomassa van de recent geïmmobiliseerde minerale N, kort na de toevoeging van NH_4NO_3. De totale bruto N-immobilisatiesnelheden (i) en de C-mineralisatiesnelheden (m_C) waren nauw gerelateerd ($i = 0.50m_C - 0.54$, $R^2=0.85$, $p<0.01$) gedurende de incubatie-experimenten.

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CURRICULUM VITAE

Curriculum vitae - Frederik Accoe

1. Personal information

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Date of birth:	22 december 1974
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2. Profession

Scientific co-worker at Ghent University since March 1, 2000

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3. Education

1996-1999	Ghent University Faculty of Agricultural and Applied Biological Sciences **Bio-engineer in Environmental Technology**
1994-1996	Ghent University Faculty of Civil Engineering **Bachelor of Civil Engineering**
1992-1994	Ghent University Faculty of Agricultural and Applied Biological Sciences **Bachelor of Agricultural and Applied Biological Sciences**
1987 –1992	Secondary School Don Boscocollege, Zwijnaarde **Latin-Mathematics**

<u>Foreign educational experience:</u>

1998	Socrates student at 'Universidad de Córdoba', Spain

4. Publications

4.1. Theses

Bio-engineer in Environmental Technology (1999)
Calibration of dynamic biofilmmodels by off-gas measurements (in Dutch)

4.2. Papers in journals with peer review

Accoe, F., Boeckx, P., Van Cleemput, O., Hofman, G., Hui, X., Bin, H. and Guanxiong, C., 2002. Characterization of soil organic matter fractions from grassland and cultivated soils via C content and $\delta^{13}C$ signature. Rapid Communications in Mass Spectrometry 16, 2157-2164.

Accoe, F., Boeckx, P., Van Cleemput, O., Hofman, G., Zhang, Y., hua Li, R. and Guanxiong, C., 2002. Evolution of the $\delta^{13}C$ signature related to total carbon contents and carbon decomposition rate constants in a soil profile under grassland. Rapid Communications in Mass Spectrometry 16, 2184-2189.

Accoe, F., Boeckx, P., Van Cleemput, O., Hofman, G., 2003. Relationship between soil organic C degradability and the evolution of the $\delta^{13}C$ signature in profiles under permanent grassland. Rapid Communications in Mass Spectrometry 17, 2591-2596.

Accoe, F., Boeckx, P., Busschaert, J., Hofman, G., Van Cleemput, O., 2004. Gross N transformation rates and net N mineralization rates related to the C and N contents of soil organic matter fractions in grassland soils of different age. Soil Biology and Biochemistry (submitted).

Accoe, F., Boeckx, P., Videla, X., Hofman, G., Van Cleemput, O., 2004. Estimation of gross N transformation rates and potential N retention after addition of ^{15}N-labelled NH_4NO_3 to permanent grassland soils. Soil Science Society of America Journal (submitted).

4.3. Chapters in books

Accoe, F., Boeckx, P., Hofman, G**.,** Van Cleemput, O., 2004. The $\delta^{13}C$ signature in grassland profiles as an indicator for soil organic C stability. In: Research Trends (Ed.), Trends in Soil Science, Poojapura, India (submitted as an invited contribution).

4.4. Abstracts

Accoe, F., Boeckx, P., Van Cleemput, O. and Hofman, G., 2001. Characterization of size-density fractions of soil organic matter, microbial biomass carbon and water soluble organic carbon in permanent and temporary grassland via ^{13}C natural abundance variations. Abstract in: Proceedings of the COST-action 627 meeting, Dublin, Ireland, 5-8 April 2001.

Accoe, F., Boeckx, P., Hui, X., Bin, H., Guanxiong, C., Hofman, G. and Van Cleemput, O., 2001. Variation of ^{13}C natural abundance of soil organic carbon in the soil profile and in different organic matter fractions of agricultural soils. Abstract in: Proceedings of the 12th World Fertilizer Congress, Beijing, China, 3-9 August 2001.

Accoe, F., Boeckx, P., Van Cleemput, O. and Hofman, G., 2001. Stable carbon isotopic characterization of organic matter fractions in agricultural soils. Abstract in: Proceedings of the 11th Nitrogen Workshop, Reims, France, 9-12 September 2001.

Accoe, F., Boeckx, P., Van Cleemput, O. and Hofman, G., 2001. Stable carbon isotopic characterization of organic matter fractions in agricultural soils. Abstract in: Proceedings of the COST-action 627 meeting, Foulum, Denmark, 28-29 September 2001.

Accoe, F., Boeckx, P., Van Cleemput, O. and Hofman, G., 2001. Carbon mineralization dynamics related to C distribution among different size fractions and evolution of the $\delta^{13}C$ signature in a grassland soil profile. Abstract in: Proceedings of the COST-action 627 meeting, Merelbeke, Belgium, 22-24 November 2001.

Accoe, F., Boeckx, P., Van Cleemput, O. and Hofman, G., 2002. Evolution of the $\delta^{13}C$ signature related to total C contents and C decomposition rate constants in a soil profile under grassland. Abstract in: Proceedings of the Stable Isotope Mass Spectrometer Users Group Annual Meeting, Belfast, Ireland, 17-18 Januari 2002.

Accoe, F., Boeckx, P., Van Cleemput, O. and Hofman, G., 2003. Evolution of the $\delta^{13}C$ and $\delta^{15}N$ signature and relation between $\Delta\delta^{13}C$ values and potential C dynamics of soil organic matter in soil profiles of different texture under permanent grassland. Abstract in: Proceedings of the Stable Isotope Mass Spectrometer Users Group Annual Meeting, Bristol, United Kingdom, 14-16 April 2003.

Accoe, F., Boeckx, P., Van Cleemput, O. and Hofman, G., 2003. Gross N transformation rates and soil organic matter contents in grassland soils of different age. Abstract in:

Proceedings of the 12th Nitrogen Workshop, Exeter, United Kingdom, 21-24 September 2003.

5. Scientific activities

5.1. Participation in conferences, symposia or workshops with poster or online presentation

2001

February, 14. General Assembly and Young Scientists Day of the Belgian Soil Science Society. Oral presentation: *Stable carbon isotopic characterization of organic matter fractions in agricultural soils.* Belgian Soil Science Society, Ghent, Belgium.

April, 5-8. COST-action 627 meeting. Oral presentation: *Characterization of size- density fractions of soil organic matter, microbial biomass carbon and water soluble organic carbon in permanent and temporary grassland via ^{13}C natural abundance variations.* COST 627, Dublin, Ireland.

August, 3-9. 12th World Fertilizer Congress. Co-author oral presentation: *Variation of ^{13}C natural abundance of soil organic carbon in the soil profile and in different organic matter fractions of agricultural soils.* Beijing, China.

September, 9-12. 11th Nitrogen Workshop. Poster: *Stable carbon isotopic characterization of organic matter fractions in agricultural soils.* INRA, Reims, France.

September, 28-29. COST-action 627 meeting. Poster: *Stable carbon isotopic characterization of organic matter fractions in agricultural soils.* COST 627, Foulum, Denmark.

November, 22-24. COST-action 627 meeting. Poster: *Carbon mineralization dynamics related to C distribution among different size fractions and evolution of the $\delta^{13}C$ signature in a grassland soil profile.* COST 627, Merelbeke, Belgium.

2002

Januari, 17-18. Stable Isotope Mass Spectrometer Users Group Annual Meeting. Poster: *Evolution of the $\delta^{13}C$ signature related to total C contents and C decomposition rate constants in a soil profile under grassland.* SIMSUG, Belfast, Ireland.

2003

April, 14-16. Stable Isotope Mass Spectrometer Users Group Annual Meeting. Poster: *Evolution of the $\delta^{13}C$ and $\delta^{15}N$ signature and relation between $\Delta\delta^{13}C$ values and potential C dynamics of soil organic matter in soil profiles of different texture under permanent grassland.* SIMSUG, Bristol, United Kingdom.

September, 21-24. 12th Nitrogen Workshop. Poster: *Gross N transformation rates and soil organic matter contents in grassland soils of different age.* IGER, Exeter, United Kingdom.

5.2. Participation in conferences, symposia or workshops without poster or online presentation

2000

October, 24. Workshop non-CO_2 trace gas emissions (N_2O, CH_4, NO) from Belgian soils: where research and policy meet. Ghent University, Belgium.

2002

October, 17. Studie- en vervolmakingsdag Technologisch Instituut KVIV: Stikstofproblematiek in de landbouw: evaluatie, maatregelen, consequenties. Technologisch Instituut, Meise, Belgium.

2003

November, 19. Thematic day of the Belgian Soil Science Society: Carbon sequestration in terrestrial ecosystems. Belgian Soil Science Society, Brussels, Belgium.