ORGANIC MATTER DYNAMICS AND N MINERALIZATION IN GRASSLAND SOILS

JAN HASSINK

JAN HASSINK

ORGANIC MATTER DYNAMICS AND N MINERALIZATION IN GRASSLAND SOILS

Promotor:	Dr. L. Brussaard Hoogleraar in de Bodembiologie
Co-promotor:	Dr. Ir. J.J. Neeteson Adjunct Directeur DLO-instituut voor Agrobiologisch en Bodemvruchtbaarheidsonderzoek (AB-DLO)

STELLINGEN

1 Fysieke bescherming van organische stof tegen biologische afbraak vindt in zandgronden uitsluitend plaats door de binding van organische stof aan klei- en siltdeeltjes, terwijl in zavel- en kleigronden organische stof ook fysiek beschermd wordt tegen afbraak door insluiting in kleine poriën en aggregaten.
Dit proefschrift

2 De hoeveelheid organische stof die in de grond fysiek beschermd kan worden tegen biologische afbraak is beperkt en is gecorreleerd met de textuur van de grond.
Dit proefschrift

3 De afbraaksnelheid van gewasresten wordt niet beïnvoed door de bodemtextuur als zodanig, maar door de mate van verzadiging van de beschermingscapaciteit van de grond.
Dit proefschrift

4 Bodem-organische stof kan op een simpele manier op basis van verschillen in grootte en dichtheid gescheiden worden in fracties die verschillen in afbraaksnelheid.
Dit proefschrift

5 Het stikstofleverend vermogen van minerale graslandgronden kan eenvoudig geschat worden uit het verschil tussen het actuele organische-stikstofgehalte van de bodem en het stikstofgehalte dat onder evenwichtsomstandigheden bereikt wordt.
Dit proefschrift

6 Lange-termijnproeven hebben een grote waarde voor het organische-stofonderzoek.
Dit proefschrift

7 Het preventief onderdrukken van bodemgebonden ziekten door een goed organische-stofbeheer en een voldoende ruime vruchtwisseling, is te verkiezen boven het bestrijden van deze ziekten via gemanipuleerde planten en/of geïntroduceerde micro-organismen.

8 Alles wat wijs is, is al eens gedacht. We moeten het alleen weer opnieuw bedenken.
Naar Goethe

9 De minerale wereld bestaat uit levenloze materie; planten bezitten naast levenloze materie ook levenskracht; dieren bezitten naast levenloze materie en levenskracht ook bewustzijn; terwijl mensen naast levenloze materie, levenskracht en bewustzijn ook zelfbewustzijn bezitten. Het essentiële van planten, dieren en mensen is alleen te begrijpen door je te richten op deze hogere niveaus. Het is kortzichtig dat de moderne 'bio-wetenschappen' zich bijna uitsluitend bezighouden met het bestuderen van de materie. Dit leidt tot een "verdinging" van de samenleving.
Naar Schumacher

10 De kwaliteit van een cultuur is af te meten aan de kwaliteit van de zorg voor komende generaties.
Els Lodewijks-Frencken

11 Ons beeld van de werkelijkheid wordt sterk beïnvloed door onze innerlijke overtuigingen, terwijl onze innerlijke overtuigingen weer sterk cultuur-bepaald zijn.
Willis Harman

12 Onderzoekers worden overvoerd met informatie, zijn druk in de weer met het verzamelen van kennis, maar komen te weinig toe aan het ontwikkelen van visie en wijsheid.

13 Verschillen in karakter tussen klei- en zandgrond worden weerspiegeld in de verschillen in karakter tussen Groningers (gesloten, aards, individualistisch, egocentrisch, zwaar; Hofstee, 1937) en Drenten (luchtig, saamhorig, afhankelijk, gelaten; Beks, 1912).

14 De verontrustende ontdekking dat de kwaliteit en hoeveelheid van het mannelijke sperma de laatste 50 jaar steeds verder is achteruitgegaan door milieuvervuiling (Deens milieubureau) zal steeds meer Nederlanders doen inzien dat er inderdaad niets boven (het schone) Groningen gaat.

Stellingen behorend bij het proefschrift "Organic matter dynamics and N mineralization in grassland soils".

Jan Hassink, Wageningen, 14 juni 1995.

JAN HASSINK

ORGANIC MATTER DYNAMICS AND N MINERALIZATION IN GRASSLAND SOILS

Proefschrift

ter verkrijging van de graad van
doctor in de landbouw- en milieuwetenschappen,
op gezag van de rector magnificus,
dr. C.M. Karssen,
in het openbaar te verdedigen
op woensdag 14 juni 1995
des namiddags om half twee in de Aula
van de Landbouwuniversiteit te Wageningen

ABSTRACT

Jan Hassink, 1995. Organic matter dynamics and nitrogen mineralization in grassland soils. DLO Research Institute for Agrobiology and Soil Fertility (AB-DLO), Haren, The Netherlands. PhD thesis Agricultural University, Wageningen, The Netherlands.

The aims of this study are i) to improve our understanding of the interactions between soil texture/soil structure, soil organic matter, soil biota and mineralization in grassland soils, ii) to develop a procedure that yields soil organic matter fractions that can be determined directly and can be used in soil organic matter models, iii) to develop a model that predicts the long-term dynamics of soil organic matter, iv) to develop a simple model that can be used by farmers and advisers to predict the non-fertilizer N supply of grassland soils, and v) to quantify the effect of the non-fertilizer N supply of grassland soils on the optimum N fertilizer application rate.

In mineral soils there is a positive relationship between the amount of soil organic N and the clay + silt content, and a negative relationship between the percentage of soil N mineralizing and the clay + silt content. For soil C the relationships are less clear, as a result of the presence of charcoal in the sandy soils (inert C). The degree of physical protection of organic matter in soil increases with the clay and silt content of the soil. In sandy soils, organic matter apparently becomes physically protected only by the adsorption to or coating by clay and silt particles, while in fine-textured soils, organic matter is also protected by its location in small pores and aggregates. Each soil has a maximum capacity to preserve organic C and N by association with clay and silt particles. The degree of saturation of the protective capacity of a soil with soil organic matter, and not soil texture *per se*, affects the decomposition rate of applied residue C. The biomass of bacteria is closely correlated with pores with neck size diameters between 0.2 and 1.2 µm and nematodes with pores with neck size diameters between 30 and 90 µm, suggesting that most of the nematodes are spatially separated from most of the bacteria in the soil. Food web calculations indicate that the observed C and N mineralization rates can not be explained from differences in microfaunal activity, but must be caused by the observed but hitherto unexplained differences in C:N ratios of the microbes between fine- and coarse-textured soils.

A simple procedure is developed that separates soil organic matter into size and density fractions, using silica suspensions as heavy liquids. The fractions differ in decomposition rate and can be used in organic matter dynamics models. Grass-derived C incorporated into the soil is transferred from soluble and light macroorganic matter fractions to intermediate and heavy macroorganic matter fractions and accumulates in microaggregates. In all fractions grass-derived C decomposes faster than soil-derived C.

Two models are presented. The first model predicts the long-term changes in soil organic matter. It includes the observation that the degree of saturation of the protective capacity determines the degree of physical protection of residue C. The second model is an empirical relationship that can be used by farmers and advisers to estimate the Non Fertilizer Nitrogen Supply (NFNS) of grassland soils. For mineral soils NFNS can be estimated from the difference between the actual soil organic N content and the content under equilibrium conditions; for peat soils NFNS can be estimated from correlation with the average deepest groundwater table. An annual increase in NFNS of 100 kg per ha on mineral soils results in an annual decrease of the optimum N application rate of 80 kg N per ha on mown grassland.

Keywords: N mineralization, organic matter, grassland, soil texture, soil structure, pore size distribution, physical protection, microbial biomass, soil microfauna, size and density fractionation, protective capacity, saturation deficit, optimum N fertilizer application rate

DANKWOORD

Het proefschrift is afgerond. Graag wil ik degenen die mij geholpen hebben ook een beetje in het zonnetje zetten. Het onderzoek dat in dit proefschrift staat beschreven is uitgevoerd op het Instituut voor Agrobiologisch en Bodemvruchtbaarheidsonderzoek (AB-DLO) te Haren. Veel collega's hebben een belangrijke bijdrage geleverd aan het totstandkomen van dit proefschrift.

De collega's van de proeftechnische dienst Jacob, Johannes, Jan, Henk, Jans, Klaas, Dirk, Han, Jan Berend, Henk, Jan, Henk, Henk, Ernst, Andries, Boele, Okko, Jannes, Tina, Willem, Marieke en Meerten hebben een groot deel van het praktische werk uitgevoerd; vertrouwde werkzaamheden zoals aanleg en onderhoud van de veldproeven, het nemen van bodemmonsters, het uitvoeren van bemestingen, het malen van monsters en het maken van proef- en analyseschema's, en nieuwe werkzaamheden zoals het meten van de microbiële biomassa, mineralisatiemetingen en het fractioneren van bodem-organische stof. Ik wil jullie bedanken voor het enthousiasme en de deskundigheid waarmee de proeven werden uitgevoerd, voor jullie waardevolle suggesties om "onderzoekersplannen" te vertalen in praktisch uitvoerbare experimenten, voor jullie openheid om nieuwe werkzaamheden aan te pakken en voor jullie geduld als ik weer met een uitbreiding van het proefschema kwam.

Janet Schrage, Ton Lepelaar, Jacob Dijkstra, Koos van Huis, Anneke Edens en Alie van Wielink, jullie wil ik bedanken voor de deskundigheid waarmee de vele vele analyses zijn verricht. Ik voelde me af en toe wel schuldig als ik al die monsters jullie kant op zag gaan.

Jimmy Robot en Menneke Bouwsema, jullie bedank ik voor het omwerken van al mijn ruwe plaatjes tot de mooie figuren die in dit proefschrift staan. Hendrik Terburg was de onmisbare steun en toeverlaat die mijn Nederlands-Engelse teksten corrigeerde en omtoverde in gedegen Engels- of Amerikaanstalige manuscripten. Aukje Gorter, Trijn Offringa en Aylien Mansholt, jullie wil ik bedanken voor de hulp bij het afwerken van de manuscripten. Aukje, jij nog speciaal bedankt voor de enorme hulp bij de lay out van het proefschrift. Rieks Visscher bedank ik voor het inplakken van de figuren.

De setting op het AB-DLO maakte het mogelijk om samen te werken met onderzoekers van verschillende wetenschappelijke afdelingen. Jaap Bloem, Lucas Bouwman, Kor Zwart, Anje-Margriet Neutel en Peter de Ruiter (afdeling Bodemecologie), Hans Dalenberg (afdeling Bodem- en Milieuchemie) en Frits Meijboom, Meine van Noordwijk, Andy Whitmore en Francisco Matus (afdeling Bodem en Nutriëntenbeheer), jullie wil ik bedanken voor de bijzonder prettige samenwerking. Andy, jij nog speciaal bedankt voor de vasthoudendheid bij het afronden van het organische stof-model, Peter voor je wijze woorden toen je zag dat ik de neiging had te veel hooi op mijn vork te nemen en Francisco voor de warme vriendschap. Meint, Guido, Popko, An en Derk, jullie dank ik voor de ondersteunende werkzaamheden. Koos Nijborg, jou wil ik speciaal bedanken voor je hulp bij het verwerken van de gegevens.

I would also like to thank Claire Chenu of "le Station de Science du Sol" in Versailles for giving me the opportunity to make the world of the microbes 'visible' with the CryoSem. Claire, I hope we can continue our collaboration.

Anneke, jij was vanaf het begin mijn rechterhand. Ik heb het bijzonder gewaardeerd dat we zo plezierig en ontspannen konden samenwerken en dat je de meest diverse werkzaamheden als vanzelfsprekend uitvoerde. Ik heb genoten van de wijze waarop je met

je vrouwelijke charme allerlei dingen wist te regelen.

Jacques en Kor, ik wil jullie bedanken voor het onbegrensde vertrouwen en de enorme ruimte die jullie me gaven om mijn onderzoek in te vullen. Ik weet dat ik met veel en af en toe gekke ideeën aankwam, maar steeds bleek dat er meer mogelijk was dan ik gehoopt had. Jacques, ik wil jou nog speciaal bedanken voor je stimulerende houding als co-promotor.

Last but not least ben ik veel dank verschuldigd aan mijn promotor Lijbert Brussaard. Lijbert, ik dank je voor het vertrouwen in de goede afloop, je deskundige commentaar en de boeiende en verdiepende discussies. Jij zorgde er mede voor dat ik de puntjes op de i kon zetten.

Tenslotte wil ik mijn hardloop- en voetbalmaatjes bedanken voor de ontspannende 'uurtjes' tussen de middag. Even lekker uitrazen tussen de middag komt jezelf en het onderzoek ten goede.

Jan

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Yields of grass and animal products (milk and meat) in the Netherlands have increased considerably since the beginning of this century. This is mainly the result of the enormous increase in the input of fertilizer N and concentrates. The application of fertilizer N to grassland soils currently amounts to approximately 400 kg N ha^{-1} per annum on intensively managed grasslands (Van Burg et al., 1980; Van der Meer, 1991; Ketelaars and Van de Ven, 1992). A drawback of the intensification is the low efficiency of the N inputs. Budget studies showed that the output of N in milk and meat was less than 20% of the input of N on intensive dairy farms during the mid-eighties (Aarts et al., 1988). The difference between N input and N output approximates 450 kg N ha^{-1} per annum, of which most is probably lost to the environment (Van der Meer, 1991). To prevent losses to the environment, the efficiency of the N inputs must be improved. A major part of the total N input originates from purchased inorganic fertilizers (Aarts et al., 1988). The N demand of grass is predominantly satisfied by the uptake of fertilizer N and inorganic N originating from mineralization of organic matter in the soil. Annual net mineralization rates may vary dramatically in time and space. Annual rates between 10 and 900 kg N ha^{-1} have been found in grassland soils (Brockman, 1969; Richards and Hobson, 1977). An improved recommendation for the application of fertilizer N should take the contribution of N mineralization to N availability into account. The application of additional fertilizer N can then be tuned to grass demand. This will result in a reduction of N losses to the environment. A major problem, however, is the prediction of N mineralization in grassland soils.

Grassland soils usually contain 5-15 tonnes N ha^{-1} in the root zone (Ryden, 1984). More than 90% of this N is present in organic form and is not directly available for plant uptake. N mineralization is driven by organic matter (C) decomposition by soil organisms. When organic material is decomposed, organic C is mineralized to CO_2. Inorganic N is released when the assimilated material contains more N than the growth demand of the microorganisms that decompose the organic material (Alexander, 1977). This means that the process of organic matter accumulation and decomposition must be understood to be able to predict N mineralization.

This chapter deals with some of the main problems researchers are faced with when soil organic matter dynamics are to be modeled. The current knowledge on the different problems of modeling soil organic matter dynamics and N mineralization is described and the outline of the thesis is presented.

MODELING SOIL ORGANIC MATTER DYNAMICS

Several models have been constructed to predict organic matter dynamics and N mineralization. The first models describing the processes of organic matter accumulation and turnover under field conditions have been constructed more than 15 years ago (Jenkinson and Rayner, 1977; Van Veen, 1977). The models that have been proposed since then are

essentially all modifications of the initial models and no major progress seems to have been made since then. Generally, models for organic matter dynamics include pools with a rapid turnover rate and pools with a slower turnover rate (Van Veen and Paul, 1981; Molina et al., 1983; Van Veen et al., 1984; Parton et al., 1987; Verberne et al., 1990). The pools with a rapid turnover rate are assumed to play a dominant role in soil nutrient dynamics. Most models consider the microbial biomass as an important pool. Soil microbial biomass has been referred to as "the eye of the needle through which all the natural organic material that enters the soil must pass" (Jenkinson, 1978), which means that the microbial biomass may be regarded as the central transformation station that takes up and converts fresh residues into new products (Van Veen et al., 1984).

There are three major unsolved problems related to soil organic matter models:

i) It has been recognized that soil texture and soil structure have a predominant effect on the activity of soil biota and the rate of organic matter decomposition and N mineralization (Van Veen and Kuikman, 1990; Juma, 1993). It is generally accepted that there is more physical protection of soil organic matter and microorganisms in fine-textured soils than in coarse-textured soils (Van Veen and Kuikman, 1990), and several models have been constructed in which the extent of physical protection of soil organic matter increases with the clay content of the soil (Van Veen et al., 1984, 1985; Verberne et al., 1990). Methodology is lacking, however, to explicitly determine the impact of the soil matrix on biological transformations (Van Veen, 1992) and it is not yet clear which mechanism is responsible for the physical protection of organic matter (Hassink, 1992) and which is the maximum amount of organic matter that can become physically protected in the soil.

ii) It has been observed that the soil microfauna may stimulate C and N mineralization (Woods et al., 1982; Rutherford and Juma, 1992). Organic matter models, however, do not explicitly take the role of the soil microfauna into account (Buurman, 1994). It is not clear whether the outcome of models would improve when the contribution of the microfauna to C and N mineralization is included.

iii) The soil organic matter pool contains plant, animal and microbial products at various stages of decay, together with a diversity of organic substances frequently associated with inorganic soil components (Christensen, 1992). These organic substances are divided over several pools differing in decomposition rates. A major problem is that, except for the microbial biomass, the different pools can not be determined directly by chemical or physical fractionation procedures (Paustian et al., 1992; Van Veen, 1992; Buurman, 1994). Successful development of techniques for direct measurement of pool sizes would represent a major step towards appropriate verification of models and the revision of inherent concepts (Bonde et al., 1992). It would also enable us to better study the fate and transformations of crop residues in the soil.

INTERACTIONS BETWEEN SOIL TEXTURE, SOIL STRUCTURE, SOIL ORGANIC MATTER, SOIL BIOTA, AND MINERALIZATION

Preservation of soil organic matter in soils with different textures

More than 50 years ago it was already recognized that the adsorption of organic compounds to clay minerals makes them resistant to biological decomposition and that the decomposition of organic materials is therefore generally lower in clay soils than in sandy soils (Mattson, 1932; Waksman, 1936). In the last decade it has generally been recognized that the degree of physical protection of soil organic matter is important for the decay rates and hence the dynamics of soil organic matter (Van Veen and Kuikman, 1990). This is partly based on the observation that the turnover rates of easily decomposable compounds are much higher in liquid microbial cultures than in soils (Van Veen and Paul, 1981) and on electron microscopy studies that revealed sites of accumulation of organic residues of clearly cellular origin (Foster, 1988). The observation that drying of soil samples and disruption of soil aggregates can increase C and N mineralization (Richter et al., 1982; Elliott, 1986; Gregorich et al., 1989; Hassink, 1992) is indirect evidence of the existence of physical protection in soil.

The soil structure is very different between fine- and coarse-textured soils. Scanning Electron Microscopy showed that the fabric of a fine-textured soil was very dense, that most pores were smaller than 10 μm in diameter and that porosity was poorly interconnected (Fig. 1). The fabric of a coarse-textured soil was very different; pore size diameters ranged from 1 to 150 μm and were interconnected (Hassink et al., 1995; Fig. 2).

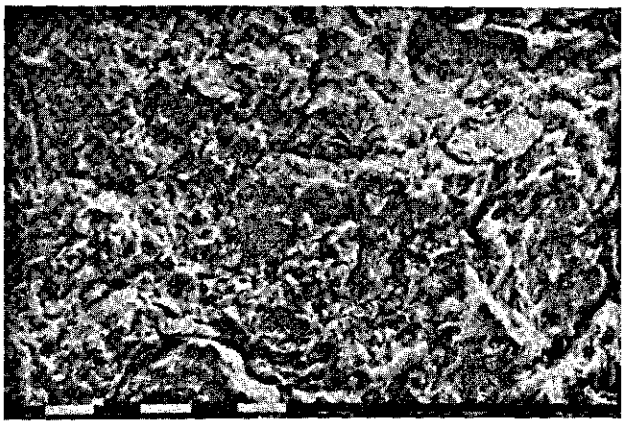


Fig. 1. SEM photograph of the internal fabric of a macroaggregate of a clayey grassland soil. White bar = 10 μm.

Organic matter additions generally decompose more rapidly in sandy soils than in clay soils (Ladd et al., 1985; Amato and Ladd, 1992; Ladd et al., 1993), and with the same input of organic material, clay soils usually contain more organic matter than sandy soils (Kortleven, 1963; Jenkinson, 1988; Spain, 1990). This indicates that the degree of physical protection may increase with the clay content of the soil. Although there is a general trend that the preservation of organic residues in soil is positively correlated with its clay content, conflicting observations have been reported too (Sörensen, 1975; Gregorich et al., 1991; Bremer and Kuikman, 1994).

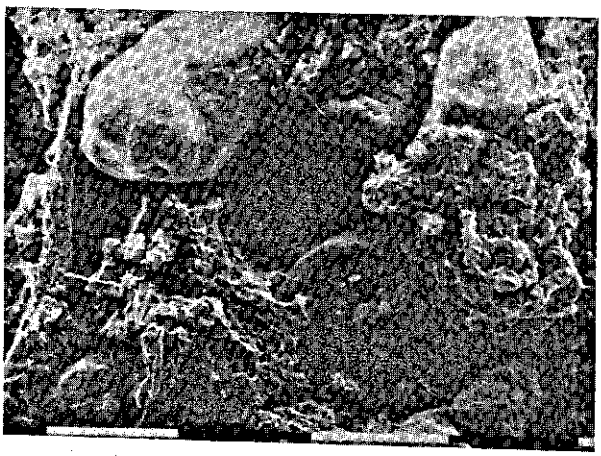


Fig. 2. SEM photograph of the internal fabric of a macroaggregate of a sandy grassland soil. White bar = 100 µm.

This shows that the capacity of the soil to preserve applied organic matter is not always directly related to soil texture. *We therefore need to improve our understanding of the interactions between soil organic matter and soil texture to explain the contradictory results on soil organic matter preservation in different soils. The maximum amount of organic matter that can be preserved in the soil has not been defined. The actual capacity of a soil to preserve applied organic matter could be related to the degree of saturation of the soil's protective capacity.*

Several mechanisms have been suggested to explain the physical protection of soil organic matter against microbial decomposition. Tisdall and Oades (1982) mention the adsorption of organic substances on, or coating by, clay particles. Other explanations are entrapment of organic compounds in small pores and the incorporation into soil aggregates, which renders them inaccessible to the decomposing microbial community (Tisdall and Oades, 1982; Elliott and Coleman, 1988; Golchin et al., 1994[a]). *The extent to which the different mechanisms of preservation of soil organic matter apply in different soils is not clear, however.*

Interactions between soil microfauna/soil microbes/soil structure

Soil structure may indirectly control decomposition processes by its effect on the grazing intensity of the soil fauna on microbes. The soil microfauna may stimulate microbial growth rates through grazing (Clarholm, 1985; Coleman et al., 1978), and predation of microbes by protozoa and nematodes has been suggested as an important mechanism of nutrient turnover in soil (Coleman et al., 1978; Elliott et al., 1980). A large proportion of bacteria may occupy pores < 3 μm (Kilbertus, 1980), while protozoa and nematodes are restricted to larger pores. This means that a large part of the bacterial population will be physically separated from the protozoa and nematodes in soil (Postma and Van Veen, 1990). It has been shown that grazing of microbes by protozoa and nematodes can markedly increase N mineralization (Woods et al., 1982; Verhoef and Brussaard, 1990; Brussaard et al., 1991; Bouwman and Zwart, 1994; Brussaard et al., 1995) and that the grazing activity of the fauna may be affected by the available pore space (Heijnen et al., 1988; Postma and Van Veen, 1990; Rutherford and Juma, 1992; Juma, 1993). *Most observations are, however, from microcosm studies where organisms were added to the soil. It remains to be assessed whether the relation between pore size distribution, soil fauna and N mineralization is similar under field conditions.*

FRACTIONATION OF SOIL ORGANIC MATTER

Soil organic matter can be fractionated by chemical and physical methods. Chemical disciplines have historically had the most apparent impact on the methodology applied in soil organic matter research, and much experimental and theoretical effort has been put into studies on the chemical structure of soil organic matter (Christensen, 1992). In many studies chemical extractants have been utilized to fractionate soil organic matter (Stevenson, 1965). Although chemical fractionation and characterization methods have not been proven particularly useful in following the dynamics of organic material in soils (Duxbury et al., 1989) they are still used (Schnitzer and Schuppli, 1989; Nandasena, 1990).

Physical fractionation of soil organic matter is considered less destructive than chemical fractionation, and the results obtained with physical methods are anticipated to relate better to the structure and function of soil organic matter *in situ* (Golchin et al., 1994[b]). Soil organic matter can be physically fractionated on the basis of size and density (Christensen, 1992). Size fractionation yields micro- and macroaggregates (composed of primary soil particles held together) and primary soil particles (clay, silt and sand). The basic structural units in soils are considered to be microaggregates (Edwards and Bremner, 1967). They protect organic matter against microbial degradation (Tisdall and Oades, 1982). To obtain primary particles, soils are usually dispersed ultrasonically. By this procedure a variable percentage of the microaggregates is destroyed, leading to a considerable increase in mineralization due to the release of organic matter that was originally protected in the microaggregates (Gregorich et al., 1989). Despite this drawback of the method, it is generally found that organic matter in

the sand size fraction decomposes at a higher rate than organic matter in clay and silt size fractions (Dalal and Mayer, 1986; Tiessen and Stewart, 1983; Tiessen et al., 1984). Density fractionation yields a light and a heavy fraction; the light fraction consists largely of non- or partially decomposed plant residues that are not associated with soil minerals (Spycher et al., 1983; Sollins et al., 1984). The heavy fraction includes the organomineral complexed soil organic matter, considered to be further processed decomposition products with a slower turnover rate and a higher degree of physical protection (Greenland and Ford, 1964; Christensen, 1992). In the past organic liquids such as tetrabromethane, bromoform and tetrachloromethane were used for density fractionation (Greenland and Ford, 1964; Turchenek and Oades, 1979). One of the drawbacks of these liquids is the high potential toxicity of halogenated hydorcarbons. During the last decade aqueous solutions of inorganic salts such as sodium iodide (Sollins et al., 1984) and sodium-polytungstate (Cambardella and Elliott, 1992) have been used progressively. However, inorganic salts are expensive and toxic to the environment. As a consequence only small amounts of dried soil samples are used in density fractionation studies (e.g. Janzen et al., 1992; Cambardella and Elliott, 1992). The long incubation period needed to equilibrate the suspended material with the salt (Janzen et al., 1992; Cambardella and Elliott, 1992) and the decrease in mineralization after introducing organic material into the salt solution (Sollins et al., 1984) are other drawbacks. A recently developed relatively simple size and density fractionation procedure, using stable silica as density solutions, seems to have overcome most of these problems (Meijboom et al., 1995). *Because physical fractionation can yield functional soil organic matter fractions, organic matter models should be adapted to contain pools that are based on physical fractionation.*

OUTLINE OF THE THESIS

The aims of this thesis are i) to improve our understanding of the interactions between soil texture, soil structure, organic matter, soil biota and mineralization, ii) to develop a procedure that yields soil organic matter fractions that can be determined directly and can be used in soil organic matter models, iii) to develop a model that predicts the long-term dynamics in soil organic matter, iv) to develop a simple model that can be used by farmers and advisers to predict the non-fertilizer N supply of grassland soils, and v) to quantify the effect of the non-fertilizer N supply of grassland soils on the optimum fertilizer application rate. We try to reduce the gaps in knowledge and integration that were identified in the previous sections and that were indicated in italics.

Chapter 2 of the thesis deals with the effects of soil texture and grassland management on organic C and N, and rates of C and N mineralization. In Chapter 3 we determine whether differences in C and N mineralization between soils of different textures were caused by differences in soil structural and biological characteristics. We also investigate which proposed mechanisms of physical protection are dominant in fine- and coarse-textured soils. In Chapter 4 relations between soil texture and the capacity to protect organic C and N are determined.

The hypothesis that not soil texture *per se*, but the degree of saturation of the protective capacity of a soil determines the preservation of residue C in the soil is tested in Chapter 5. In Chapter 6 we investigate whether differences in the grazing activity of the soil microfauna may be the cause of the observed differences in N mineralization between fine- and coarse-textured soils. The following two chapters (7, 8) deal with the quantification and turnover of soil organic matter fractions that can be determined by simple physical fractionation. In Chapter 9 the fractionation procedure is used to study the fate of crop residues in the soil. In Chapter 10 a model is presented that predicts the long-term dynamics of soil organic matter, and that includes the concept that the degree of saturation of the protective capacity of the soil determines the preservation of residue C in the soil. In Chapter 11 emphasis is placed on the development of a simple model to predict the non-fertilizer N supply of grassland soils. We test whether the gap between the equilibrium contents of soil organic N and microbial N and their actual levels is correlated with the uptake of N on non-fertilized fields. Finally, we study which effect differences in available N have on the optimum fertilizer application rate and how much fertilizer could be saved by taking differences in available N into account.

So the approach in the thesis is both theoretical and practical. On the one hand we try to increase our understanding of soil organic matter dynamics and on the other hand we try to develop an easy method that can be used by farmers and advisers to optimize the fertilizer application rate on grasslands.

REFERENCES

Aarts H. F. M., Biewinga E. E.., Bruin G., Edel B. and Korevaar H. (1988) Dairy farming and environment. (in Dutch with English summary.) Report 79. Centre for Agrobiological Research, Wageningen, 136 pp.

Alexander M. (1977) Introduction to Soil Microbiology. Wiley, New York.

Amato M. and Ladd J. N. (1992) Decomposition of ^{14}C-labelled glucose and legume materials in soil: properties influencing the accumulation of organic residue C and microbial biomass C. Soil Biology & Biochemistry 24, 455-464.

Bonde T. A., Christensen B. T. and Cerri C. C. (1992) Dynamics of soil organic matter as reflected by natural ^{13}C abundance in particle size fractions of forested and cultivated oxisols. Soil Biology & Biochemistry 24, 275-277.

Bouwman L. A. and Zwart K. B. (1994) The ecology of bacterivorous protozoans and nematodes in arable soil. Agriculture, Ecosystems and Environment 51, 145-160.

Bremer E. and Kuikman P. (1994) Microbial utilization of ^{14}C[U]glucose in soil is affected by the amount and timing of glucose additions. Soil Biology & Biochemistry 26, 511-517.

Brockman J. S. (1969) The relationships between total N input and the yield of cut grass. Journal of the British Grassland Society 24, 89-96.

Brussaard L., Kools J. P., Bouwman L. A. and De Ruiter P. C. (1991) Population dynamics

and nitrogen mineralization rates in soil as influenced by bacterial grazing nematodes and mites. In: G.K Veeresh, D. Rajagopal and C.A. Viraktamath (eds), Advances in management and conservation of soil fauna. Oxford & IBH Publ Co, PVT Ldt, New Delhi, pp. 517-523.

Brussaard L., Noordhuis R., Geurs M. and Bouwman L. A. (1995) Nitrogen mineralization in soil in microcosms with or without bacterivorous nematodes and mites. Accepted by Acta Zoologica Fennica.

Buurman P. (Ed.) (1994) Decomposition and accumulation of organic matter in terrestrial ecosystems: final report of a concerted activity organized by the European Commission. Ecosystems Research Report no. 12.

Cambardella C. A. and Elliott E. T. (1992) Particulate soil organic-matter changes across a grassland cultivation sequence. Soil Science Society of America Journal 56, 777-783.

Clarholm M. (1985) Possible role for roots, bacteria, protozoa and fungi in supplying nitrogen to plants. In: A.H. Fitter, D. Atkinson, D.J. Read and M.B. Usher (eds), Ecological Interactions in Soil: Plants, Microbes and Animals. British Ecological Society Special Publications, Oxford, pp. 355-365.

Christensen B. T. (1992) Physical fractionation of soil and organic matter in primary particle size and density separates. Advances in Soil Science 20, 1-89.

Coleman D.C., Cole C. V., Hunt H. W. and Klein D. A. (1978) Trophic interactions in soil as they affect energy and nutrient dynamics. I. Introduction. Microbial Ecology 4, 345-349.

Dalal R. C. and Mayer R. J. (1986) Long-term trends in fertility of soils under continuous cultivation and cereal cropping in Southern Queensland. III. Distribution and kinetics of soil organic carbon in particle-size fractions. Australian Journal of Soil Research 24, 293-300.

Duxbury J. M., Smith M. S. and Doran J. W. (1989) Soil organic matter as a source and a sink of plant nutrients. In: D.C. Coleman, J.M. Oades and G. Uehara (eds), Dynamics of soil organic matter in tropical ecosystems. University of Hawaii Press, Honolulu, pp. 33-67.

Edwards A. P. and Bremner A. P. (1967) Microaggregates in soils. Journal of Soil Science 18, 64-73.

Elliott E. T. (1986) Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Science Society of America Journal 50, 627-633.

Elliott E. T. and Coleman D. C. (1988) Let the soil work for us. In: H. Eijsackers and A. Quispel (eds), Ecological implications of contemporary agriculture. Proc. 4th Eur. Ecol. Symp., Wageningen. Ecological Bulletins 39, pp. 327-335.

Elliott E. T., Anderson R. V., Coleman D. C. and Cole C. V. (1980) Habitable pore space and microbial trophic interactions. Oikos 35, 327-335.

Foster R. C. (1988) Microenvironments of soil microorganisms. Biology and Fertility of Soils 6, 189-203.

Golchin A., Oades J. M., Skjemstad J. O. and Clarke P. (1994[a]) Soil structure and carbon cycling. Australian Journal of Soil Research 32, 1043-1068.

Golchin A., Oades J. M., Skjemstad J. O. and Clarke P. (1994[b]) Study of free and occluded particulate organic matter in soils by solid state ^{13}C CP/MAS NMR Spectroscopy and scanning electron microscopy. Australian Journal of Soil Research 32, 285-309.

Greenland D. J. and Ford W. (1964) Separation of partially humified organic materials from soil by ultrasonic dispersion. Proc. 8th Int. Congr. Soil Sci., Bucharest, Vol. III, pp. 137-148.

Gregorich E. G., Kachanoski R. G. and Voroney R. P. (1989) Carbon mineralization in soil size fractions after various amounts of aggregate disruption. Journal of Soil Science 40, 649-659.

Gregorich E. G., Voroney R. P. and Kachanoski R. G. (1991) Turnover of carbon through the microbial biomass in soils with different textures. Soil Biology & Biochemistry 23, 799-805.

Hassink J. (1992) Effects of soil texture and structure on carbon and nitrogen mineralization in grassland soils. Biology and Fertility of Soils 14, 126-134.

Hassink J., Matus F. J., Chenu C. and Dalenberg J. W. (1995) Interactions between soil biota, soil organic matter and soil structure. Advances in Agroecology (accepted for publication).

Heijnen C. E., Elsas J. D. van, Kuikman P. J. and Veen J. A. van (1988) Dynamics of *Rhizobium leguminosarum biovar trifolii* introduced into soil; the effect of bentonite clay on predation by protozoa. Soil Biology & Biochemistry 20, 483-488.

Janzen H. H., Campbell C. A., Brandt S. A., Lafond G. P. and Townley-Smith L. (1992) Light-fraction organic matter in soils from long-term crop rotations. Soil Science Society of America Journal 56, 1799-1806.

Jenkinson D. S. (1978) The soil biomass. CSIRO Report.

Jenkinson D. S. (1988) Soil organic matter and its dynamics. In: A. Wild (ed), Russel's soil conditions and plant growth, 11th ed., Ch. 18. Longman, New York, pp. 564-607.

Jenkinson D. S. and Rayner J. H. (1977) The turnover of soil organic matter in some of the Rothamsted classical experiments. Soil Science 123, 298-305.

Juma N. G. (1993) Interrelationships between soil structure/texture, soil biota/soil organic matter and crop production. Geoderma 57, 3-30.

Ketelaars J. J. M. H. and Van De Ven G. W. J. (1992) Nitrogen utilization and losses in production grassland. In: H. G. van der Meer and J. H. J. Spiertz (eds), Stikstofstromen in agro-ecosystemen. Agrobiologische Thema's 6. Cabo-DLO Wageningen, pp. 33-49.

Kilbertus G. (1980) Etude des microhabitats contenus dans les agrégats du sol. Leur relation avec la biomasse bactérienne et la taille des procaryotes présents. Revue Ecologie et Biologie du Sol 17, 543-557.

Kortleven J. (1963) Quantitative aspects of humus accumulation and decomposition. Verslagen Landbouwkundige Onderzoekingen nr. 69.1. PUDOC, Wageningen (in Dutch).

Ladd J. N., Amato M. and Oades J. M. (1985) Decomposition of plant material in Australian soils. III. Residual organic and microbial biomass C and N from isotope-labelled legume material and soil organic matter, decomposing under field conditions. Australian Journal of Soil Research 23, 603-611.

Ladd J. N., Foster R. C. and Skjemstad J. O. (1993) Soil structure: carbon and nitrogen metabolism. Geoderma 56, 401-434.

Mattson S. (1932) The laws of soil colloidal behavior: VII. Proteins and proteinated complexes. Soil Science 60, 41-72.

Meijboom F. W., Hassink J. and Van Noordwijk M. (1995) Density fractionation of soil macroorganic matter using silica suspensions. Accepted by Soil Biology & Biochemistry.

Molina J. A., Clapp C. E., Shaffer M. J., Chichester F. W. and Larson W. E. (1983) NCSOIL, A model of nitrogen and carbon transformations in soil: description, calibration, and behavior. Soil Science Society of America Journal 47, 85-91.

Nandasena K. A. (1990) Fractionation and decomposition of soil organic matter. Thesis Catholic University Leuven, Belgium.

Parton W. J., Schimel D. S., Cole C. V. and Ojima D. S. (1987) Analysis of factors controlling soil organic matter levels in great plains grasslands. Soil Science Society of America Journal 51, 1173-1179.

Paustian K., Parton W. J. and Persson J. (1992) Modelling soil organic matter in organic-amended and nitrogen fertilized long-term plots. Soil Science Society of America Journal 56, 476-488.

Postma J. and Van Veen J. A. (1990) Habitable pore space and population dynamics of *Rhizobium Leguminosarum Biovar Trifolii* introduced into soil. Microbial Ecology 19, 149-161.

Richards I. R. and Hobson R. D. (1977) The relationships between nitrogen supply and the nitrogen yield of cut grass swards. Journal of Agricultural Science, Cambridge 88, 289-292.

Richter J., Nuske A., Habenicht W. and Bauer J. (1982) Optimized N-mineralization parameters of loess soils from incubation experiments. Plant and Soil 68, 379-388.

Rutherford P. M. and Juma N. G. (1992) Influence of texture on habitable pore space and bacterial-protozoan populations in soil. Biology and Fertility of Soils 12, 221-227.

Ryden J. C. (1984) The flow of nitrogen in grassland. Proceedings No. 229. The Fertiliser Society, London.

Schnitzer M. and Schuppli P. (1989) Method for the sequential extraction of organic matter from soils and soil fractions. Soil Science Society of America Journal 53, 1418-1424.

Sollins P., Spycher G. and Glassman C. A. (1984) Net nitrogen mineralization from light- and heavy fraction forest soil organic matter. Soil Biology & Biochemistry 16, 31-37.

Sörensen L. H. (1975) The influence of clay on the rate of decay of amino acid metabolites synthesized in soils during decomposition of cellulose. Soil Biology & Biochemistry 7, 171-177.

Spain A. V. (1990) Influence of environmental conditions and some soil chemical properties on the carbon and nitrogen contents of some tropical Australian rainforest soils. Australian Journal of Soil Research 28, 825-839.

Spycher G., Sollins P. and Rose S. (1983) Carbon and nitrogen in the light fraction of a forest soil: Vertical distribution and seasonal patterns. Soil Science 135, 79-87.

Stevenson F. J. (1965) Origin and distribution of nitrogen in soil. In: W.V. Barholomew and F. E. Clark (eds), Soil Nitrogen. American Society of Agronomy, Madison, Wisconsin, pp. 1-42.

Tiessen H. and Stewart J. W. B. (1983) Particle-size fractions and their use in studies of soil organic matter: II. Cultivation effects on organic matter composition in size fractions. Soil Science Society of America Journal 47, 509-514.

Tiessen H., Stewart J. W. B. and Hunt H. W. (1984) Concepts of soil organic matter transformations in relation to organo-mineral particle size fractions. Plant and Soil 76, 287-295.

Tisdall J. M., and Oades J. M. (1982) Organic matter and water-stable aggregates in soils. J. Soil Sci. 33:141-163.

Turchenek L. W. and Oades J. M. (1979) Fractionation of organomineral complexes by sedimentation and density techniques. Geoderma 21, 311-343.

Van Burg P. F. J., 't Hart M. L. and Thomas H. (1980) Nitrogen and grassland - past and present situation in the Netherlands. In: H.W. Prins and G.H. Arnold (eds), The role of nitrogen in intensive grassland production. Proc. internat. symp. European Grassl. Fed., Wageningen. PUDOC, Wageningen, pp. 15-33.

Van der Meer H. G. (1991) Nitrogen balance sheets. In: H.G. van der Meer (ed), Stikstofbenutting en -verliezen van gras- en maisland. DLO, Wageningen, pp. 5-12 (in Dutch).

Van Veen J. A. (1977) The behavior of nitrogen in soil. A computer simulation model. Ph.D Thesis, V.U. Amsterdam.

Van Veen J. A. (1992) Modelling of decomposition and accumulation of organic matter in soils. In: N. van Breemen (ed), Decomposition and accumulation of organic matter in terrestrial ecosystems: research priorities and approaches. CEC Ecosystems Research Report No. 1, pp. 69-72.

Van Veen J. A. and Paul E. A. (1981) Organic carbon dynamics in grassland soils: I. Background information and computer simulation. Canadian Journal of Soil Science 61, 185-201.

Van Veen J. A. and Kuikman P. J. (1990) Soil structural aspects of decomposition of organic matter. Biogeochemistry 11, 213-233.

Van Veen J. A., Ladd J. H. and Frissel M. J. (1984) Modelling C and N turnover through the microbial biomass in soil. Plant and Soil 76, 257-274.

Van Veen J. A., Ladd J. H. and Frissel M. J. (1985) Turnover of carbon and nitrogen through the microbial biomass in a sandy loam and a clay soil incubated with [^{14}C(U)] glucose and [^{15}N]$(NH_4)_2SO_4$ under different moisture regimes. Soil Biology & Biochemistry 17, 747-756.

Verberne E. L. J., Hassink J., Willigen P. de, Groot J. J. R. and Van Veen J. A. (1990) Modelling organic matter dynamics in different soils. Netherlands Journal of Agricultural Science 38, 221-238.

Verhoef H. A. and Brussaard L. (1990) Decomposition and nitrogen mineralization in natural

and agro-ecosystems: the contribution of soil animals. Biogeochemistry11, 175-211.
Waksman S. A. (1936) Humus: origin, chemical composition, and importance in nature. Williams and Wilkins Company, Baltimore.
Woods L. E., Cole C. V., Elliott E. T., Anderson R. V. and Coleman D. C. (1982) Nitrogen transformation in soil as affected by bacterial-microfaunal interactions. Soil Biology & Biochemistry 14, 93-98.

CHAPTER 2

EFFECTS OF SOIL TEXTURE AND GRASSLAND MANAGEMENT ON SOIL ORGANIC C AND N AND RATES OF C AND N MINERALIZATION

By: J. Hassink. 1994. Soil Biology & Biochemistry 26, 1221-1231.

EFFECTS OF SOIL TEXTURE AND GRASSLAND MANAGEMENT ON SOIL ORGANIC C AND N AND RATES OF C AND N MINERALIZATION

ABSTRACT

The effects of soil texture and grassland management, i.e. level of fertilizer N input, mowing versus grazing, and the number of years the site is under grass, on the amounts of soil organic C and N and on the rates of C and N mineralization were investigated.

A positive relationship was found between the amount of organic N in the soil and the clay + silt content. The relationship was affected by the groundwater table. There was a negative relationship between the percentage of soil N mineralizing during incubation and the clay + silt content of the soil. The amount of organic C was only positively correlated with soil texture in the soils with a high water table, but the relationship was less clear. Except for the groundwater table, differences in the C:N ratio of the soil organic matter in sandy soils confused the relationship of soil organic C with soil texture. Organic matter in podzol soils had C:N ratios between 15 and 20 while in other sandy soils the C:N ratio ranged from 10-18; in loams and clays the C:N ratio was approximately 10. The percentage of soil C mineralizing in sandy soils was negatively correlated with the C:N ratio of the soil organic matter. The sandy soils with a C:N ratio > 16 that were used for incubation contained black humus including small charcoal particles; both other sandy soils with a lower C:N ratio contained brown humus without visible charcoal particles. So we hypothesize that sandy soils with a high C:N ratio contained more inert C than sandy soils with a low C:N ratio.

The level of N fertilization had no effect on soil organic C and N nor on the rates of C and N mineralization.

Differences between the effects of grazing and mowing on soil organic C and N and the rate of C and N mineralization were very small and not very consistent.

Both the amounts of soil organic C and N found and the rates of C and N mineralization were significantly higher in old grassland (10 years) than in young grassland (1-3 years). The increases in the mineralization rates were larger than the increases in soil organic C and N.

INTRODUCTION

Grassland soils frequently contain 5 to 15 tonnes nitrogen (N) per ha in the root zone (Ryden, 1984). More than 90% of this nitrogen is present in organic form and is not directly available for plant uptake. Part of the nitrogen demand of grass is satisfied by the uptake of inorganic nitrogen produced by microbial mineralization of organic nitrogenous compounds in soils. Soils differ considerably in the amounts of organic nitrogen that are mineralized. Annual rates of net mineralization were found to be between 10 and 900 kg N per ha in grassland soils

(Brockman, 1969; Richards and Hobson, 1977). To tune fertilizer N supply to grass demand and minimize losses to the environment, the contribution of mineralization of soil organic N to the total supply of inorganic N to the plant and its availability over time should be taken into account.

When organic material is decomposed, organic C is mineralized into CO_2. The rate of C mineralization (CO_2 production) is thus a measure of the rate of decomposition of organic matter. Inorganic nitrogen is released when the assimilated material contains nitrogen in excess of the growth demand of the microorganisms that decompose the organic material (Alexander, 1977).

Organic additions typically decompose more rapidly in sandy soils than in clay soils (Ladd *et al.*, 1985; Sörensen, 1981). With the same input of organic material, clay soils usually contain more organic matter than sandy soils (Jenkinson, 1988).

Management also affects the amounts of soil organic C and N and the rate of C and N mineralization. Returns of organic residues to the soil are usually higher in grasslands than in arable land and higher under grazing than under mowing as a result of the return of dung and urine, and the higher utilization losses of herbage (Ryden, 1984). A higher return of organic residues to the soil should lead to a higher level of soil organic C and N and, hence, a higher mineralization rate. It is not clear whether the level of N fertilization affects organic matter dynamics. It is generally accepted that the accumulation of N is independent of the input of inorganic N (Clement and Williams, 1976; Hassink and Neeteson, 1991). Although the amount of total N is not influenced by fertilizer input to a measurable extent, it has been observed that the amount of mineralizable N may increase considerably with higher rates of fertilizer N input (Williams and Clement, 1965).

In the present study the effect of soil texture and grassland management (age of grassland, grazing vs mowing and different levels of fertilizer N input) on soil organic C and N and rates of C and N mineralization were studied.

MATERIALS AND METHODS

Fields sampled for incubation

In March 1989 and March 1991 samples were collected from soils which had been under grass for at least 8 years. The land was grazed by dairy cattle and received 400-500 kg fertilizer-N per ha per year. Three mixed samples, each consisting of 20 bulked cores, were taken from the 0-10 and the 10-25 cm layer of each location. Some characteristics of the soils are given in Table 1. Some soils were expected to have lower organic C and N contents than other grassland soils: some soils were recently reclaimed from the sea (Swifterbant and Lelystad in 1957 and Zeewolde in 1968); one soil (Mijnsheerenland) had been in use as arable soil and was sown to grass only eight years before sampling.

Table 1. Some soil characteristics of the 0-10 and the 10-25 cm layers of the grassland sites sampled for incubation (pH and granular composition are only given for the top 10 cm because differences between both layers were very small).

Location	Soil type	Exp. year	C (%)		C/N		pH (K-Cl)	Granular comp., % particles <		
			0-10	10-25	0-10	10-25	0-10	2μm	16μm	50μm
Heino 1	sand	1989	1.98	1.97	18.0	17.9	5.0	1.9	2.8	8.7
Jubbenga	sand	1989	2.87	2.92	21.6	21.9	4.6	1.0	1.9	3.7
Heino 2	sand	1989	4.68	3.56	15.6	15.9	5.2	5.6	8.9	29.7
Markelo	sand	1989	3.91	2.60	17.2	20.1	5.2	3.4	5.4	12.4
Achterberg	sand	1989	3.09	2.26	17.5	17.4	4.9	3.0	5.8	9.0
Holten	sand	1989	2.27	1.47	17.5	17.8	5.1	2.5	3.6	12.0
Tynaarlo	sand	1989	4.38	3.48	17.8	21.8	4.4	2.4	4.4	23.5
Finsterwolde	sand	1989	5.37	2.13	10.9	9.9	5.0	8.4	13.3	23.2
Cranendonck	sand	1991	2.96	2.25	17.1	19.3	5.4	3.2	4.8	21.2
Dalfsen	sand	1991	3.88	5.49	20.8	40.5	5.1	1.9	3.1	9.8
Maarheeze	sand	1991	2.61	2.29	18.3	18.6	5.0	2.6	4.5	11.8
Drentse Aa	sand	1991	5.75	4.21	17.4	20.0	4.8	5.3	8.4	36.9
Tynaarlo 2	sand	1991	3.80	2.97	19.7	21.2	4.3	3.1	5.1	22.3
Tynaarlo 3	sand	1991	6.85	6.49	21.7	24.6	4.4	3.8	7.3	31.9
Average standard deviation for each location			0.35	0.12	0.7	1.7				
Swifterbant[r]	loam	1989	1.77	1.87	11.6	10.8	7.0	23.0	38.4	72.2
Aduard	loam	1989	3.55	3.19	10.1	9.5	5.6	29.8	45.5	69.4
Zeewolde[r]	loam	1989	3.53	3.01	12.9	14.3	6.9	26.7	45.4	77.2
Zaltbommel 1	loam	1989	3.99	2.65	9.7	9.2	5.8	25.8	42.6	67.2
Burum	loam	1989	5.37	2.13	9.8	9.1	4.8	24.1	36.5	71.7
Lelystad[r]	loam	1991	3.07	1.68	11.1	12.1	7.1	21.6	35.6	56.4
Mijnsh.land.[m]	loam	1991	2.20	1.40	9.6	9.1	7.2	20.1	33.5	79.9
Average standard deviation for each location			0.15	0.12	0.2	0.1				
Zaltbommel 2	clay	1989	6.07	5.23	9.2	8.9	5.4	51.1	76.0	86.4
Haskerdijk	clay	1991	5.60	4.04	11.1	11.1	4.9	54.0	77.2	88.5
Finsterwolde	clay	1991	3.23	1.80	10.1	9.3	7.1	45.8	65.8	85.1
Zaltbommel 3	clay	1991	3.98	3.28	9.5	8.9	5.4	51.0	74.3	90.6
Average standard deviation for each location			0.19	0.14	0.3	0.1				

r = soils reclaimed from the sea (polder soils); m = soil sown to grass only 8 years before sampling

Fields sampled by the Winand Staring Centre

Between 1956 and 1986 the Winand Staring Centre determined the granular composition and the soil organic C and N contents in the top 15-25 cm of soils which had been under grass for at least 10 years. As at all sites also pH-KCl, groundwater table and soil classification were determined the data set was also used to determine the effects of these characteristics on soil organic C and N.

Groundwater table was defined by a combination of average highest water table and average lowest water table (Table 2). The soils were classified according to the Dutch system of soil classification (De Bakker and Schelling, 1966). As no significant effect of soil class on the relationship between soil texture and organic C and N content was found for the soils sampled by the Winand Staring Centre, the soils were pooled before analysis.

Table 2. Classification of groundwater tables expressed in cm below the surface.

	Class						
	I	II	III	IV	V	VI	VII
Average highest groundwater table	0	0	<40	>40	<40	40-80	>80
Average lowest groundwater table	<50	50-80	80-120	80-120	>120	>120	>120

Selected fields for effect of grassland management

Two sandy soils and two loams were sampled to study the effects of fertilizer input and mowing versus grazing on C and N dynamics. Information about the fields is given in Table 3.

In Cranendonck (sandy soil) and Lelystad (loam) grazed fields were sampled in March 1991 that had been under grass for 1, 3 and 10 years prior to sampling. The average number of cow days of grazing was about 1000. Before being used as grassland all fields had been in use as arable land.

Plots on the mown fields were laid out in triplicate, while the grazed fields were unreplicated. Three mixed samples, each consisting of 20 bulked cores, were taken from the 0-10 cm layer of all the grazed fields. A mixed sample, consisting of 20 bulked cores, was taken from the 0-10 cm layer of every plot on the mown fields. Deeper layers were not sampled because it was assumed that the effects of management would be most pronounced in the top layer.

Table 3. Information on the fields used in the study of the effect of grazing versus mowing and fertilizer N input on soil organic C and N, and C and N mineralization rates.

Location	Soil type	Exp. year	Treatments/ Fertilization (kg N ha^{-1} $year^{-1}$)	Area studied (m^2)	Cow days of grazing per year	Time treatment/ fertilization in effect (years)
Achterberg	sand	1989	mown 250, 550	25	0	3
Achterberg	sand	1989	grazed 550	2000	1500	3
Cranendonck	sand	1991	mown 0, 200, 400	50	0	2
Cranendonck	sand	1991	grazed 400	1200	1000	2
Swifterbant	loam	1989	mown 250, 550	25	0	2
Swifterbant	loam	1989	grazed 550	10000	600	2
Burum	loam	1991	mown 0, 200	54	0	9
Burum	loam	1991	grazed 0, 200	840	1000	9

Determination of potential C and N mineralization rates

Different methods have been used to measure net N mineralization rates. They include incubation of undisturbed soil cores in the laboratory or in the field (Raison *et al.*, 1987) and incubation of homogenized sieved field-moist or rewetted samples in the laboratory (Stanford and Smith, 1972; Nordmeyer and Richter, 1985). Incubation of undisturbed samples from grassland soils leads to a temporary immobilization of N, probably due to dying roots (Hassink, 1992), while the incubation of sieved remoistened soil causes a flush in C and N mineralization during the first weeks of incubation (Nordmeyer and Richter, 1985; Hassink, 1992). In coarse-sieved field-moist samples, N mineralization rates remain constant during incubation (Addiscott, 1983). Therefore, C and N mineralization rates of a wide range of grassland soils were compared by incubating sieved field-moist samples. The water potential was close to -10 KPa at the time of sampling in all soils. Potential C and N mineralization rates were determined in the 0-10 and 10-25 cm layers of the soils of Table 1.

Soil samples were sieved through a 0.008-m mesh screen; roots and stubble were removed. N mineralization was determined by measuring the increase in mineral N after incubation of soil samples in glass jars at 20°C. Drying of the samples was prevented by covering the jars with a polyethylene seal permeable to air, but impermeable to water. Mineral N was extracted with 1N KCl solution for 1 h using a soil:water ratio of 1:2.5 before colorimetric measuring. In 1989 NH_4^+ and NO_3^- were determined 0, 2, 5, 8 and 12 weeks after incubation was started; in 1991 these determinations were carried out after 0, 2 and 12 weeks only.

C mineralization was measured by incubating soil samples in 1.5-l airtight jars containing a vial of 10 ml 0.5 M NaOH. After 1 week and from then on approximately every 2 weeks, the trapped CO_2 was precipitated as carbonate with excess $BaCl_2$ and the excess NaOH was titrated with 0.5 M HCl. In some soils a flush in C mineralization was observed during the first week. Therefore, C mineralization during the first week was not taken into account.

Determination of soil organic C and N

Organic C was defined as dichromate-oxidizable C according to Kurmies (Mebius, 1960). Total soil N was determined according to Deys (1961), after destruction with sulfuric acid and salycylic acid.

Statistical analysis

The time courses of C and N mineralization for the sandy soils, the loams and clays were analyzed separately, using simple regression analysis (Genstat manual, 1987). The relations between soil texture, soil pH-KCl, the C:N ratio of the soil organic matter and soil organic C and N contents, and the percentages of mineralized soil organic C and N were also analyzed using correlation and simple linear and multiple regression techniques (Genstat manual, 1987). The fraction < 50 μm (clay + silt content) explained more of the variance in organic C and N mineralization between soils than the fractions < 2 μm and < 16 μm when using simple linear regression analysis. Therefore, the clay + silt content was taken as a measure of soil texture. The significance of the differences in soil organic C and N contents and in the rates of mineralization between different fields were analyzed with Student's t-test.

RESULTS

Soil organic C and N contents; C:N ratio of the soil organic matter

Effect of soil texture. The soils sampled for incubation (soils of Table 1) showed no significant relationship between soil texture and soil organic C content in both layers (0-10 and 10-25 cm), even when the reclaimed soils (r) and the soil that was sown to grass only 8 years prior to sampling (m) were excluded from the analysis (Table 4).

For the soils sampled by the Winand Staring Centre a significant positive effect of soil texture on the soil organic C content was found for the soils with a high water table (class II and III; soils of class I were not present; $r^2 = 0.62$; Fig. 1). The organic C content of the soils with a lower water table (class IV-VII), however, showed no significant relationship with soil texture (Fig. 1). There was no significant relationship between the soil organic carbon content and the pH of the grassland soils.

Table 4. Relationships between the fraction < 50 µm (clay + silt content) and the contents of soil organic C and N and the percentages of soil organic C and N mineralized during incubation in the top 0-10 cm layer and the 10-25 cm layer of the grassland soils used for incubation (soils of Table 1). The polder soils and the soil recently resown to grass (m) have been excluded when the relationship between soil texture and soil organic C and N contents were determined.

Soils used for incubation	
0-10 cm layer	
C organic (%) non-significant relationship	(r^2 = 0.25)
N organic (%) = 0.125 (0.028) + 0.00454 (0.0005) * % < 50 µm	(r^2 = 0.77)
%C mineralized non-significant relationship	(r^2 = 0.25)
%N mineralized = 6.61 (0.37) - 0.0451 (0.007) * % < 50 µm	(r^2 = 0.65)
10-25 cm layer	
C organic (%) non-significant relationship	(r^2 = 0.00)
N organic (%) = 0.094 (0.027) + 0.00305 (0.00049) * % < 50 µm	(r^2 = 0.63)
%C mineralized non-significant relationship	(r^2 = 0.21)
%N mineralized = 4.07 (0.42) - 0.0355 (0.008) * % < 50 µm	(r^2 = 0.48)

The soils sampled for incubation (soils of Table 1) showed a significant positive relationship between the clay and silt content and the soil organic N content in both the top 0-10 cm and the 10-25 cm layer (Table 4). In sandy soils the N contents ranged from 0.1 to 0.3% in both the 0-10 cm and the 10-25 cm layer. In loams and clays soil N contents were generally higher in the 0-10 cm layer (between 0.15 and 0.65%) than in the 10-25 cm layer (generally between 0.15 and 0.4%, with one exception).

For soils sampled by the Winand Staring Centre with a high or with a low water table a significant positive relationship between clay + silt content and the organic N content was found (Fig. 2). The increase in soil organic N with increasing percentage of clay + silt was greater in the soils with a high water table than in soils with a lower water table (Fig. 2). Soil pH had no significant effect on the soil organic N content.

The C:N ratio of the organic matter in both layers of the sandy soils that were used for incubation was generally > 15; only in one sandy soil the C:N ratio was lower than 15, while in loams and clays the C:N ratio was usually close to 10 (Table 1).

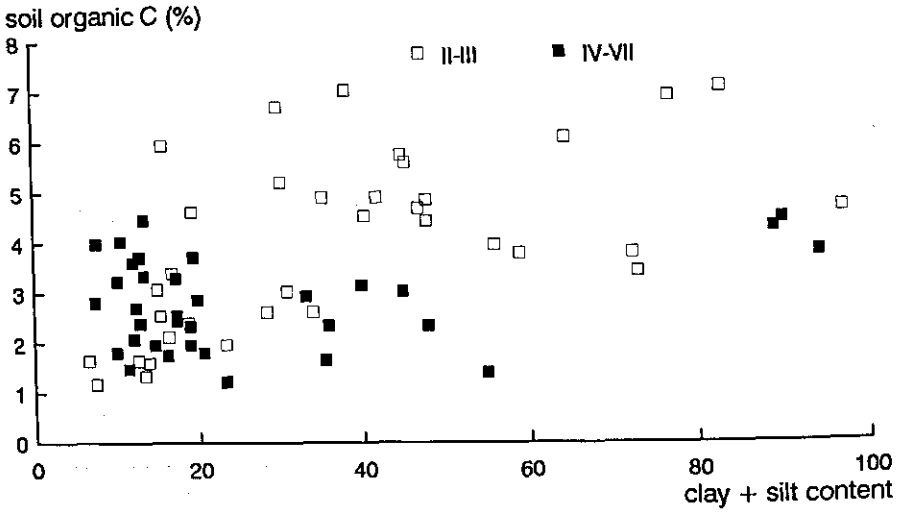


Fig. 1. Relationship between soil organic C content and the fraction < 50 µm (clay + silt content) of grassland soils sampled by the Winand Staring Centre with a high (class II and III) and a low (class IV-VII) water table.
Groundwater table II and III
C organic (%) = 2.25 (0.425) + 0.0436 (0.0097) x % < 50 µm (r^2 = 0.62)
Groundwater table IV - VII
C organic (%) non-significant relationship with soil texture (r^2 = 0.28)

In the soils sampled by the Winand Staring Centre, however, the C:N ratio of sandy soils ranged from 10 to 20 (Fig. 3). All podzol soils had C:N ratios between 15 and 20, while in the other sandy soils the C:N ratio varied between 10 and 18. Loams and clays generally had C:N ratios between 8 and 12. There was no relationship between soil pH and the C:N ratio of the organic matter (Fig. 3).

Effect of grassland management. The level of N fertilization had no effect on organic C and N contents of the soil nor on the C:N ratio of the soil organic matter (Table 5). In comparison with mowing, grazing significantly ($p < 0.05$) increased organic N contents in the sandy soils of Achterberg and Cranendonck, but not in the loams of Swifterbant and Burum (Table 5). In none of the soils were the effects of grazing and mowing on soil organic C content significantly different (Table 5).

Total soil organic C and N contents were significantly higher in old grassland sites (10 years) than in young sites (1-3 years) on both soil types (Table 6). The relative increases in soil organic C and N, respectively, were 70% and 113% in the sandy soil and 47% and 56% in the loam. The C:N ratio of the organic matter clearly decreased with increasing age of the grassland (Table 6).

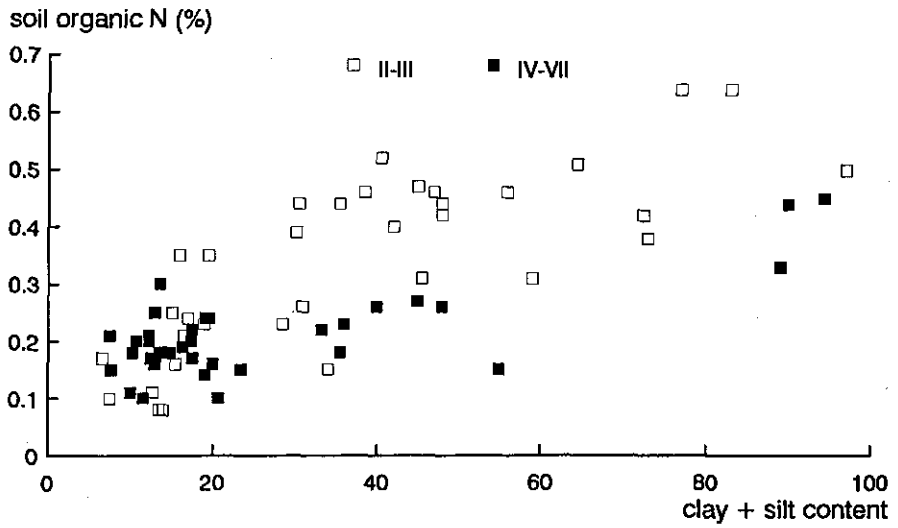


Fig. 2. Relationship between soil organic N content and the fraction < 50 µm (clay + silt content) of grassland soils sampled by the Winand Staring Centre with a high (class II and III) and a low (class IV-VII) water table.
Groundwater table II and III
N organic (%) = 0.132 (0.029) + 0.00517 (0.0007) x % < 50 µm (r^2 = 0.80)
Groundwater table IV - VII
N organic (%) = 0.147 (0.014) + 0.0026 (0.00039) x % < 50 µm (r^2 = 0.76)

C and N mineralization

Course of C and N mineralization during incubation. The rates of C mineralization were not significantly different between different soils. C mineralization rates decreased significantly ($P < 0.05$) during incubation. As an example the course of C mineralization in four of the soils of Table 1 with different soil textures is presented in Fig. 4a. After 12 weeks of incubation C mineralization rates were generally less than 50% of the rate at the start of the incubation.

Contrary to C mineralization, N mineralization rates did not decrease significantly during incubation. There were some fluctuations and they were generally larger in sandy soils than in loams and clays. As an example the course of N mineralization of the same four soils is given in Fig. 4b.

Effect of soil texture on C and N mineralization rates. The average C mineralization rate during 12 weeks of incubation in the laboratory ranged from 8.5 to 41.1 mg C kg^{-1} soil day^{-1} in the 0-10 cm layer and between 4.4 and 14.0 mg C kg^{-1} soil day^{-1} in the 10-25 cm layer of

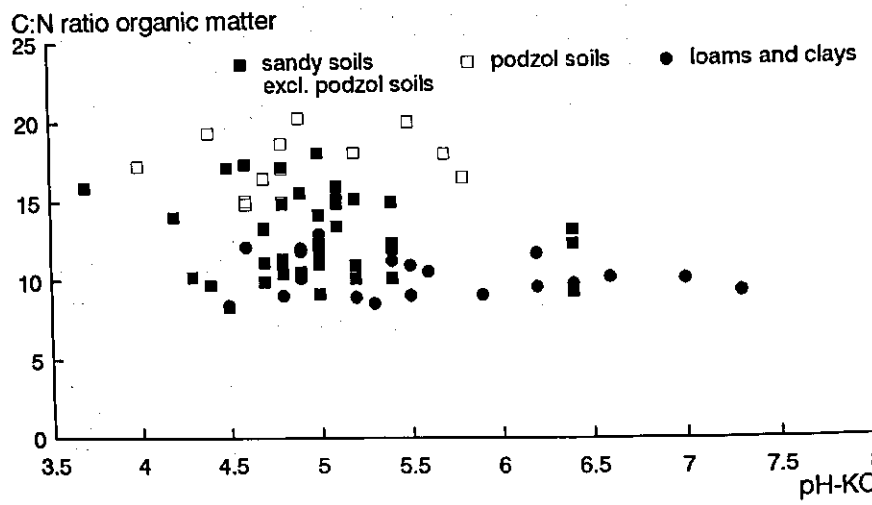


Fig. 3. Relationship between soil pH and the C:N ratio of soil organic matter in podzol grassland soils, other sandy grassland soils, and loamy and clayey grassland soils sampled by the Winand Staring Centre.

the soils of Table 1. There was no significant effect of soil texture on C mineralization rate. Also when C mineralization was expressed as the percentage of soil organic C mineralized during incubation, no significant relationship was found between C mineralization rate and soil texture (clay + silt content) (Table 4). Within the group of sandy soils of Table 1 a significant negative correlation was found between the percentage of soil organic C that mineralized in the top 10 cm and the C:N ratio of the soil organic matter (Fig. 5; Table 7). C mineralization also tended to increase with the pH-KCl of the sandy soils; the correlation, however, was not significant (Table 7). For the 10-25 cm layer no significant correlations with pH-KCl and C:N ratio of the soil organic matter were found.

The average N mineralization rate in the 0-10 cm layer ranged from 0.5 to 2.5 mg N kg^{-1} soil day^{-1} and in the 10-25 cm layer between 0.15 and 1.0 mg N kg^{-1} soil day^{-1}. In both layers there was no relationship between soil texture and N mineralization rate. When N mineralization rates were expressed as the percentages of soil N mineralized during incubation, however, a significant negative relationship was found between the clay + silt content of the soils and N mineralization rate (Table 4). In sandy soils, between 0.045 and 0.09% of the amount of soil N present in the 0-10 cm layer was mineralized per day during laboratory incubation, while in loam and clay soils this ranged from 0.025 to 0.045% (Fig. 6). The percentage of soil N mineralized during incubation was, in general, lower in the 10-25 cm layer than in the 0-10 cm layer. No significant correlations were found between N mineralization and the pH-KCl or the C:N ratio of the soil organic matter.

Table 5. Soil organic C and N contents, C:N ratios of the soil organic matter and C and N mineralization rates (determined in March 1989 or March 1991) in the 0-10 cm layer of grassland soils under different management practices (different N fertilizer input levels and mowing vs grazing).

Treatment/ Fertilization	Organic matter			Mineralization	
(kg N ha^{-1} yr^{-1})	N (%)	C (%)	C:N	N (mg kg^{-1} day^{-1})	C (mg kg^{-1} day^{-1})
Cranendonck (sandy soil). Treatments/fertilization 2 years in effect.					
Mown					
0	0.11	2.10	18.7	0.60	5.76
200	0.13	2.48	18.7	0.58	5.79
400	0.11	1.85	16.9	0.55	5.49
Grazed					
400	0.15*	2.64	18.0	0.60	6.93
Achterberg (sandy soil). Treatments/fertilization 3 years in effect.					
Mown					
550	0.16	2.98	18.6	0.89	12.5
Grazed					
250	0.19	3.10	16.3	1.37	15.2
550	0.20*	3.05	15.3	1.67*	19.9*
Swifterbant (loam). Treatments/fertilization 2 years in effect.					
Mown					
550	0.16	1.88	11.8	0.38	9.29
Grazed					
250	0.18	2.09	11.6	0.54	11.69
550	0.18	2.00	11.1	0.42	10.40
Burum (loam). Treatments/fertilization 9 years in effect.					
Mown					
0	0.50	5.23	10.5	2.42	41.1
200	0.50	5.40	10.7	1.99	40.5
Grazed					
0	0.46	4.70	10.2	2.54	33.7
200	0.51	5.31	10.5	2.25	35.4

* Differences between mown and grazed grasslands are statistically significant ($p < 0.05$)

Table 6. Soil organic C and N contents, C:N ratios of the soil organic matter and C and N mineralization rates in the top 0-10 cm layer of 1-, 3- and 10-year-old grassland soils sampled in March 1991.

Age (years)	Organic			Mineralization	
	N (%)	C (%)	C:N	N (mg kg^{-1} day^{-1})	C (mg kg^{-1} day^{-1})
Lelystad (loam)					
1	0.18	2.14	12.1	1.14	19.04
3	0.21	2.49	11.7	1.09	20.80
10	0.28*	3.15*	11.1*	1.76*	28.16*
Cranendonck (sandy soil)					
1	0.08	1.74	21.8	0.48	6.31
3	0.09	1.87	20.1	0.56	6.11
10	0.17*	2.96*	17.1*	1.31*	11.44*

* Differences between old grassland (10 years) and young grassland (1-3 years) are statistically significant ($p < 0.05$)

Effect of grassland management on C and N mineralization rates. The level of N fertilization had no significant effect on C and N mineralization rates (Table 5).

Grazing compared with mowing increased C and N mineralization rates significantly ($p < 0.05$) in the sandy soil of Achterberg but not in the other soils (Table 5).

C and N mineralization rates were not significantly different between 1-year-old and 3-year-old grassland soils (Table 6). In 10-year-old grassland soils, however, C and N mineralization rates were significantly ($p < 0.05$) higher (Table 6).

DISCUSSION

Effect of soil texture on soil organic matter and mineralization rates

The buildup of soil organic C and N is determined by the amount and quality of the input of organic residues and their decomposition rate. It may be assumed that in intensively managed grasslands in The Netherlands, where the dry matter production is roughly the same on different soil types, the amount and quality of the inputs of organic residues is relatively constant. The decomposition rate of organic residues in the soil is typically lower in fine-textured soils than in coarse-textured soils (Sörensen, 1975; Jenkinson, 1977; Ladd *et al.*, 1985; Jenkinson, 1988). Significant positive correlations were found between soil texture and

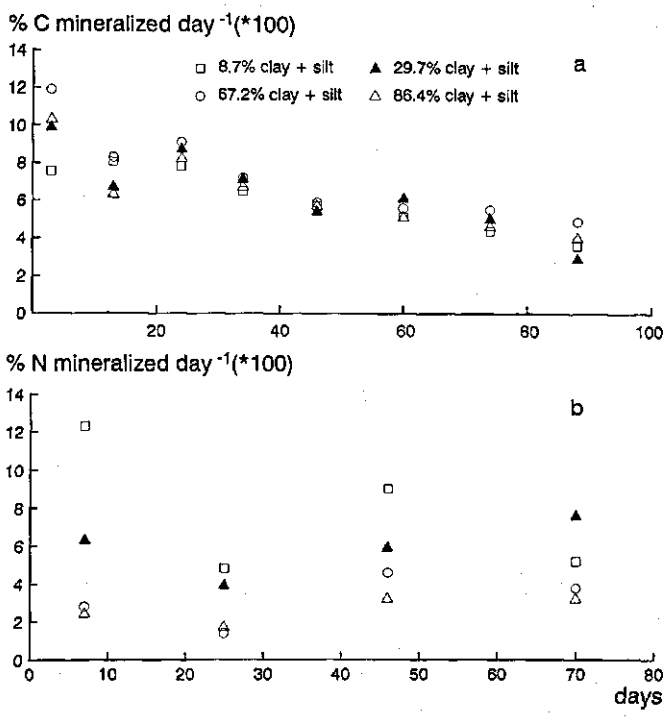


Fig. 4. Course of the percentage of soil organic C (a) and soil organic N (b) mineralized (per day * 100) during laboratory incubation of the 0-10 cm layer of four grassland soils.

Table 7. Correlations and relationships between the percentage of soil organic C mineralized during incubation and soil pH-KCl and C:N ratio of the soil organic matter in the top 10 cm of the sandy grassland soils used for incubation (sandy soils of Table 1).

Coefficients of correlation			
C mineralization	1.00		
C:N ratio org. matter	-0.62	1.00	
pH-KCl	0.46	-0.45	1.00

Significant relationship:
%C mineralized = 9.25 (1.70) - 0.257 (0.094) * C:N ratio org. matter

( ) = standard error of difference

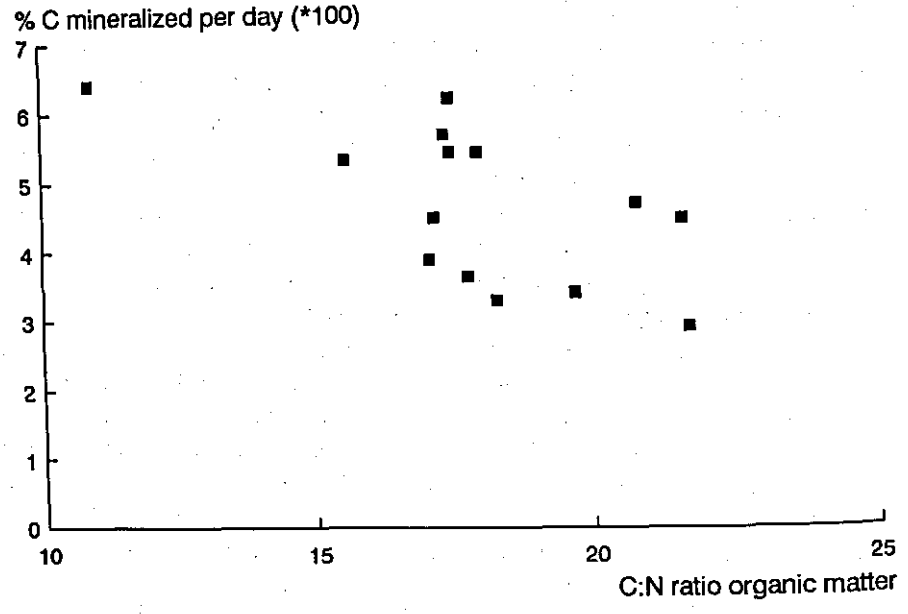


Fig. 5. Relationship between the percentage of soil organic C mineralized (per day * 100) during laboratory incubation of the 0-10 cm layer of the sandy grassland soils of Table 1 and the C:N ratio of their soil organic matter.

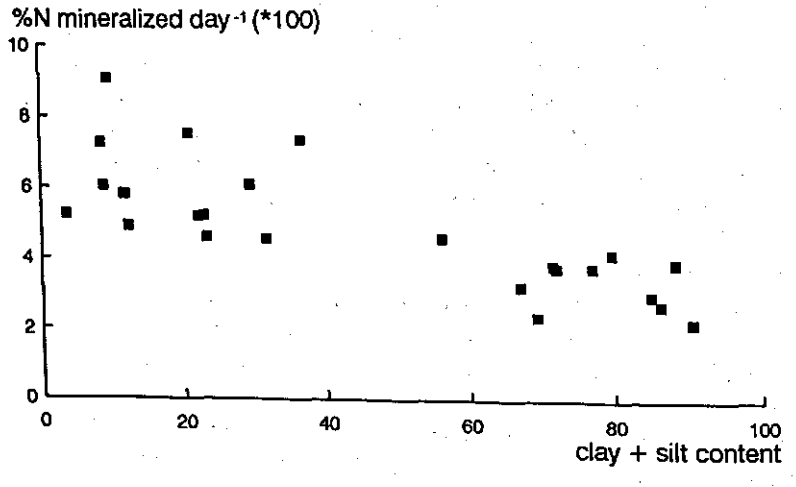


Fig. 6. Relationship between the percentage of soil organic N mineralized (per day x 100) during laboratory incubation of the 0-10 cm layer of the grassland soils of Table 1 and their clay + silt content.

the soil organic C content (Kortleven, 1963; Spain, 1990; Feller *et al.*, 1991). Differences in decomposition rates and amounts of soil organic C have been attributed to differences in physical protection of soil organic matter. The stabilizing effect has been ascribed to adsorption of organics onto surfaces such as clays (Oades, 1989), encapsulation between clay particles (Tisdall and Oades, 1982) or entrapment in small pores in aggregates inaccessible to microbes (Elliott and Coleman, 1988).

Our results indicate that soil texture was not always the dominant factor determining the organic C content of soils and C mineralization rates. Differences in C:N ratio of the soil organic matter and the groundwater table confused the relationship of the soil organic C content and the percentage of soil organic C that mineralized with soil texture.

We observed that the C:N ratio of the soil organic matter in sandy soils ranged roughly from 10 to 20. The C:N ratio of podzol soils was always higher than 15, while the C:N ratio of the other sandy soils was variable. This phenomenon has already been described by Anderson and Byers (1934). The differences in C:N ratio might be due to differences in history. Most of the poor podzol soils have been heathlands (de Bakker and Schelling, 1966). The cultivation of these soils started only 100 years ago. The residues of heathland generally have a high C:N ratio. Other sandy soils have been in use as arable land for a much longer period and received large quantities of residues of different quality (dung or dung mixed with litter of heathland and sand; Kuipers, 1977). Differences in C:N ratio might also result from the presence of inert charcoal in the soil. Charcoal is produced by the burning of the vegetation (Anderson and Byers, 1934). Kortleven (1963) observed that soils might contain inert organic C that cannot be decomposed. The amount of inert organic C was considerably higher in two of his sandy soils than in a loam and a clay.

We tried to obtain more information on the composition and appearance of the soil organic matter of the grassland soils that were used for incubation (soils of Table 1) by shaking the soils with water (soil:water ratio 1:2.5) and observing the suspension after pouring it on a filter. The sandy soils in which the C:N ratio of the soil organic matter was higher than 16 contained black humus including small pieces of charcoal; the two sandy soils with a lower C:N ratio contained brown humus without charcoal, whereas the loams and clays contained a kind of grey-coloured humus without charcoal. The negative correlation observed between the C:N ratio of organic matter and C mineralization in sandy soils might be explained by the fact that sandy soils with a high C:N ratio contained more inert C than other soils. The observation that the ratio C mineralization : N mineralization was not affected by the C:N ratio of the soil organic matter (Fig. 7) also indicates that in soils with increasing C:N ratios a greater part of soil organic C was not decomposed.

Besides previous land use, the groundwater table was also found to affect the relationship between soil texture and the organic C content of the soils. The sandy soils with a low groundwater table tended to have higher organic C contents than sandy soils with a high groundwater table. This was partly caused by the fact that most podzol soils (with a relatively high organic C content) had a low groundwater table. The sandy soils with a low groundwater

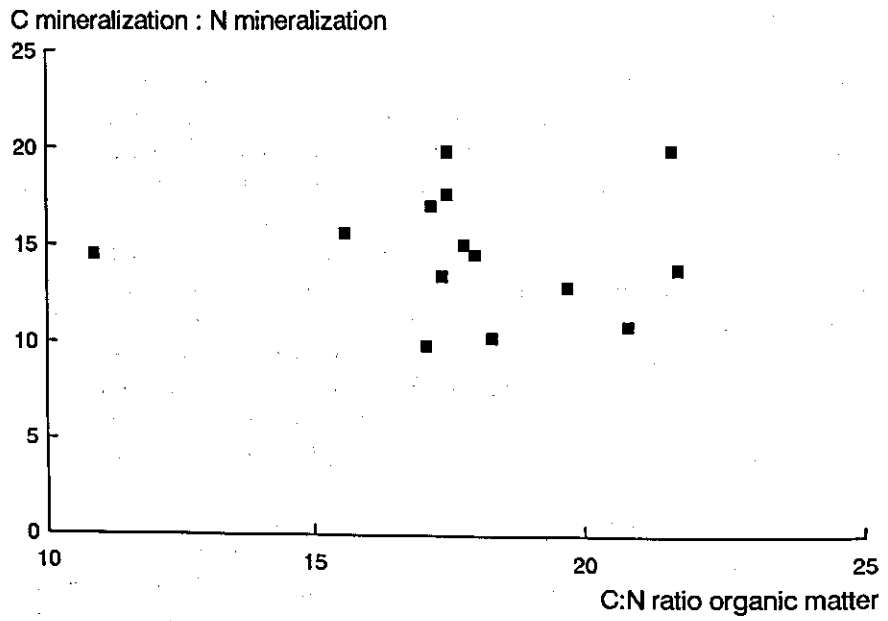


Fig. 7. Relationship between the ratio C mineralization:N mineralization and the C:N ratio of soil organic matter in the 0-10 cm layer of the sandy grassland soils of Table 1.

table might also have a higher soil organic C content because they are more susceptible to drought in summer, leading to lower decomposition rates. In loams and clays the relationship with groundwater table might be the opposite; a high groundwater table might lead more often to anaerobic conditions. Decomposition rates are slower under anaerobic conditions than under aerobic conditions leading to higher organic C contents (Jenkinson, 1988).

Soil pH did not affect total organic C and N, and C and N mineralization significantly. According to Jenkinson (1988) low pH values (around 4) do not necessarily lead to lower decomposition rates of soil organic matter. Apparantly, the pH-KCl of the soil has to be lower than approximately 4.5 to reduce mineralization rates significantly.

The relationships of soil organic N and the percentage of soil organic N that mineralized with soil texture were very significant. This is in agreement with the results of Spain (1990) and Catroux *et al.* (1987). The relationships were not affected by the C:N ratio of the soil organic matter. The groundwater table affected the relationship between soil organic N content and soil texture in the same way as for soil organic C.

Effects of grassland management on soil organic matter and mineralization rates

Differences in fertilizer N input were found not to affect the amount of soil organic C and N (Clement and Williams, 1967; Hassink and Neeteson, 1991). The input of C rather than N is the factor most commonly limiting organic matter and N accumulation under grassland (Ryden, 1984). More grass residues will be returned to the soil at high fertilizer levels. It has been observed, however, that the amount of roots decreases with increasing fertilizer levels (Ennik *et al.*, 1980). The total amount of organic residues returned to the soil may therefore not show a straightforward relationship with the fertilizer N level. In the present study, C and N mineralization rates were not related to fertilizer N input either. This is in agreement with the hypothesis that the input of C is approximately the same under different fertilizer levels. Williams and Clement (1965), however, found a large effect of fertilizer level on N mineralization. This may be explained by the fact that they did not remove roots and stubble from their samples. According to Whitehead *et al.* (1990) the amount of N present in stubble and roots will be affected by the level of fertilizer N input.

Although it has been found that the return of organic residues to the soil is higher under grazing than under mowing due to the return of dung and urine and higher utilization losses of herbage (Ryden, 1984), the amounts of soil C and N, and rates of C and N mineralization were often not significantly different between mowing and grazing. Obviously the extra input of residues due to grazing was too small to establish significant differences.

Soil organic C and N usually increase in soils recently resown to grass with the higher input of organic material to the soil (Jenkinson, 1988). According to Wolf and Janssen (1991) the difference in input of organic material between arable land and grassland is about 100%. This difference in input is considerably larger than the differences in input between mown and grazed fields. The increase in soil N in the sandy soil and the loam was approximately 130 kg N per ha per year (over the 10-year period, assuming a soil bulk density of 1.3). This is in agreement with Ryden (1984), who stated that increases between 50 and 150 kg per ha are usually found. The observation that the relative increase in C and N mineralization was larger than the relative increase in total soil organic C and N is in agreement with the hypothesis that the extra return of crop residues contributes mainly to the active fractions of the soil organic matter pool (Glendining and Powlson, 1990).

ACKNOWLEDGEMENTS

The skillful technical assistance of A. Lahr, M. Wolters, K. Kampen, K. van Huis, J. Schrage and A. Edens is gratefully acknowledged.
Ir. F. de Vries from the Winand Staring Centre is gratefully acknowledged for supplying the soil data set. L. Brussaard and J.J. Neeteson are acknowledged for their support and helpful discussions.

REFERENCES

Addiscott T.M. (1983) Kinetics and temperature relationships of mineralization and nitrification in Rothamsted soils with differing histories. Journal of Soil Science **34**, 343-353.

Alexander M. (1977) *Introduction to soil microbiology.* John Wiley and Sons, New York.

Anderson M.S. and Byers H.G. (1934) The carbon-nitrogen ratio in relation to soil classification. *Soil Science* **38**, 121-138.

Bakker H. de and Schelling J. (1966) *System of Dutch soil classification: the higher levels.* Pudoc, Wageningen. (in Dutch)

Brockman J.S. (1969) The relationships between total N input and the yield of cut grass. *Journal of the British Grassland Society* **24**, 89-96.

Catroux G., Chaussod R. and Nicolardot B. (1987) Assessment of nitrogen supply from the soil. Comptes Rendus de l'Académie d'Agriculture de France **3**, 71-79.

Clement C.R and Williams T.E. (1967) Leys and soil organic matter. II. The accumulation of nitrogen in soils under different leys. *Journal of Agricultural Science (Cambridge)* **69**, 133-138.

Deys W.B. (1961) *The determination of total nitrogen in herbage samples.* (in Dutch). IBS Jaarboek Mededelingen **89**, 158, 90.

Elliott E.T. and Coleman D.C. (1988) Let the soil work for us. In: *Ecological Implications of Contemporary Agriculture* (H. Eijsackers and A. Quispel Eds.), pp. 23-32. Proceedings 4th European Ecology Symposium, Wageningen. Ecological Bulletins **39**.

Ennik G.C., Gillet M. and Sibma L. (1980) Effect of high nitrogen supply on sward deterioration and root mass. In: *Proceedings International Symposium European Grassland Federation.* (W.H. Prins and G.H. Arnold, Eds.), pp. 67-76. Pudoc, Wageningen, Netherlands.

Feller C., Fritsch E., Poss R. and Valentin C. (1991) Effect of the texture on the storage and dynamics of organic matter in some low activity clay soils (West Africa, particularly). Cahier ORSTOM, serie Pedologie XXVI, 25-36.

Genstat Manual (1987) Genstat. *A General Statistical Program.* Clarendon, Oxford.

Glendining M.J. and Powlson D.S. (1990) 130 Years of inorganic nitrogen fertilizer applications to the Broadbalk wheat experiment: the effect on soil organic nitrogen. In: *Proceedings 14th International Congress Soil Science, Kyoto, Japan*, Vol. 4 pp 9-13.

Hassink J. (1992) Effects of soil texture and structure on C and N mineralization in grassland soils. *Biology and Fertility of Soils* **14**, 126-134.

Hassink J. and Neeteson J.J. (1991) Effect of grassland management on the amounts of soil organic N and C. *Netherlands Journal of Agricultural Science* **39**, 225-236.

Jenkinson D.S. (1977) Studies on the decomposition of plant material in soil. V. The effects of plant cover and soil type on the loss of carbon from ^{14}C labelled ryegrass decomposing under field conditions. *Journal of Soil Science* **28**, 424-434.

Jenkinson D.S. (1988) Soil organic matter and its dynamics. In: *Russels's Soil Conditions and*

Plant Growth (A. Wild, Ed.) 11th edition, chapter 18, pp. 564-607 Longman, New York.

Kortleven J. (1963) *Quantitative aspects of humus accumulation and decomposition.* Verslagen Landbouwkundige onderzoeken nr. 69.1. PUDOC, Wageningen (in Dutch).

Kuipers S.F. (1977) Organische bestanddelen (Organic components) (chapter 4). In Bodemkunde (Soil Science), 274 pp. Tjeenk Willink, Culemborg, the Netherlands (in Dutch).

Ladd J.N., Amato M. and Oades J.M. (1985) Decomposition of plant material in Australian soils. III. Residual organic and microbial biomass C and N from isotope-labelled legume material and soil organic matter, decomposing under field conditions. *Australian Journal of Soil Research* **23**, 603-611.

Mebius L.J. (1960) A rapid method for the determination of organic carbon in soil. *Analytica Chimica Acta* **22**, 120-124.

Nordmeyer H. and Richter J. (1985) Incubation experiments on nitrogen mineralization in loess and sandy soils. *Plant & Soil* **83**, 433-445.

Oades J.M. (1989) An introduction to organic matter in mineral soils. In: *Minerals in soil environments* (J.B. Dixon and S.B. Weed, Eds.) Chapter 3, pp. 89-159. Soil Science Society of America, Madison.

Raison R.J., Connell M.H. and Khanna P.K. (1987) Methodology for studying fluxes of soil mineral-N in situ. *Soil Biology & Biochemistry* **19**, 521-530.

Richards I.R. and Hobson R.D. (1977) The relationships between nitrogen supply and the nitrogen yield of cut grass swards. *Journal of Agricultural Science Cambridge* **88**, 289-292.

Ryden J.C. (1984) *The Flow of Nitrogen in Grassland.* Proceedings No. 229. The Fertiliser Society, London.

Sörensen L.H. (1975) The influence of clay on the rate of decay of amino acid metabolites synthesized in soils during decomposition of cellulose. *Soil Biology & Biochemistry* **7**, 171-177.

Sörensen L.H. (1981) Carbon-nitrogen relationships during the humification of cellulose in soils containing different amounts of clay. *Soil Biology & Biochemistry* **13**, 313-321.

Spain A. (1990) Influence of environmental conditions and some soil chemical properties on the carbon and nitrogen contents of some tropical Australian rainforest soils. *Australian Journal of Soil Research* **28**, 825-839.

Stanford G. and Smith S.J. (1972) Nitrogen mineralization potentials of soils. *Soil Science Society of America Proceedings* **36**, 465-472.

Tisdall J.M. and Oades J.M. (1982) Organic matter and water stable aggregates in soils. *Journal of Soil Science* **33**, 141-163.

Whitehead D.C., Bristow A.W. and Lockyer D.R. (1990) Organic matter and nitrogen in the unharvested fractions of grass swards in relation to the potential for nitrate leaching after ploughing. *Plant & Soil* **123**, 39-49.

Williams T.E. and Clement C.R. (1965) Accumulation and availability of nitrogen in soils

under leys. In: *Proceedings of the First General Meeting of the European Grassland Federation* (P.F.J. Van Burg and G.H. Arnold, Eds.), pp. 39-45. Pudoc, Wageningen.

Wolf J. and Janssen L.H.M. (1991) Effects of changing land use in the Netherlands on net carbon fixation. *Netherlands Journal of Agricultural Science* **39**, 237-246.

CHAPTER 3

RELATIONSHIPS BETWEEN SOIL TEXTURE, SOIL STRUCTURE, PHYSICAL PROTECTION OF ORGANIC MATTER, SOIL BIOTA, AND C AND N MINERALIZATION IN GRASSLAND SOILS

Extended version of: J. Hassink, L.A. Bouwman, K.B. Zwart, J. Bloem and L. Brussaard. 1993. Geoderma 57, 105-128.

RELATIONSHIPS BETWEEN SOIL TEXTURE, SOIL STRUCTURE, PHYSICAL PROTECTION OF ORGANIC MATTER, SOIL BIOTA, AND C AND N MINERALIZATION IN GRASSLAND SOILS

ABSTRACT

The effect of soil type on carbon (C) and nitrogen (N) mineralization rates in grassland soils was investigated along with the physical and biological soil characteristics that may have caused the observed differences in mineralization rates between soil types.

The percentage of mineralized organic N was higher in sandy soils than in loams and clays; this was not observed for C. The soil structure was very different between fine- and coarse-textured soils: they differ not only in the distribution of primary particles, but also in pore and aggregate size distribution. In loams and clays small pores constituted a higher percentage of the total pore space than in sandy soils. Different mechanisms of physical protection of organic C and N were distinguished. In sandy soils organic material was physically protected by its association with clay particles leading to high concentrations of C and N in the clay size fraction. In fine-textured soils entrapment of organic C and N in small pores inside or between aggregates was found to be an important mechanism of physical protection. The protected organic material associated with clay particles consisted of amorphous undefined material that did not stain with acridine orange, indicating a high degree of decomposition, while the non-protected organic material present in the sand fraction consisted of plant debris that stained intensely with acridine orange. Physically protected organic matter had a lower C/N ratio than organic matter that was not physically protected.

Grazing pressure on bacteria by bacterivorous nematodes was higher in sandy soils than in loams and clays. This coincided with a higher N mineralization rate per bacterium. The C/N ratio of the microbial biomass was higher in sandy soils than in loams and clays and was positively correlated with the N mineralization rate per unit of microbial biomass N. This is an agreement with the concepts of food webs that N mineralization is positively correlated with the C/N ratio of the consumer (bacteria) for a given C/N ratio of the substrate (organic matter). It is not yet clear which of the factors investigated are the most important in determining N mineralization rates in grassland soils.

INTRODUCTION

Net mineralization of soil organic matter and decomposition of plant material is generally more rapid in sandy soils than in clay soils (Sörensen, 1975; Jenkinson, 1977; Catroux et al., 1987; Hassink et al., 1990; Ladd et al., 1990; Juma, 1993; Ladd et al., 1993). The lower net mineralization in clay soils may be partly caused by a greater physical protection of soil organic matter and microbial biomass (Verberne et al., 1990). Different mechanisms have

been suggested to explain the phenomenon of physical protection in soils: i) adsorption of organics to surfaces of clays or coating of organics by clay particles (Tisdall and Oades, 1982; Fig. 1), and ii) entrapment of organics in small pores mostly in microaggregates inaccessible to microbes (Elliott and Coleman, 1988; Golchin et al., 1994; Fig. 2).

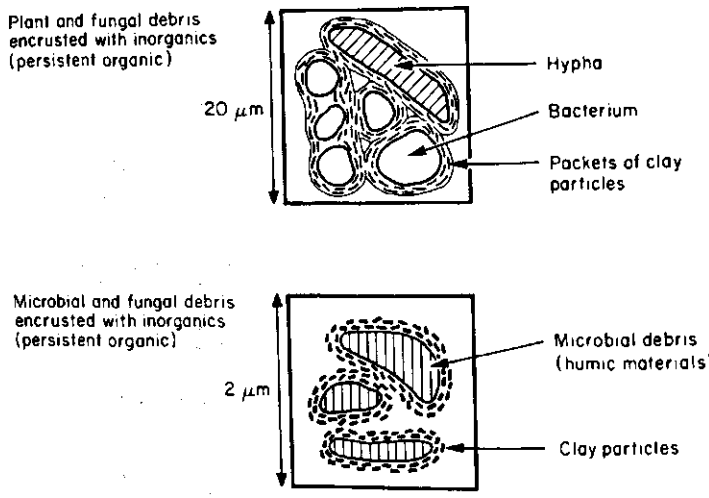


Fig. 1. Part of the model of aggregate organization as given by Tisdall and Oades (1982)

Soil structure may also control decomposition and mineralization processes by its effect on grazing intensity on microbes. Predation of microbes by protozoa and nematodes has been suggested as an important mechanism of nutrient turnover in soil (Coleman et al., 1978; Elliott et al., 1980). A large proportion of bacteria may occupy pores < 3 μm (Kilbertus, 1980), while protozoa and nematodes are restricted to larger pores. This means that a large part of the bacterial population will be physically separated from the protozoa and nematodes in soil (Postma and Van Veen, 1990).

As the relative amount of large pores is usually higher in sandy soils than in loamy and clay soils (Papendick and Campbell, 1981), predation is expected to be more intense in sandy soils than in loamy and clay soils (Heijnen et al., 1988). The results of Hassink et al. (1993) indicated that the higher grazing pressure in sandy soils might strongly affect the N mineralization rate.

Soil structure might also affect the relative contribution of bacteria and fungi to the total microbial biomass. Fungi can be present in pores with sizes different from those where bacteria are found as hyphae can vary considerable in diameter and are not restricted to water films. It is generally assumed that the C/N ratio of fungi is higher than that of bacteria (Hunt et al., 1987). The amount of N mineralized by microbes will generally be higher in soils which contain a microbial population with a high C/N ratio than in soils with a microbial

population with a low C/N ratio. This means that the contribution of fungi to the total microbial biomass may affect N mineralization rates.

The aim of the present study is to describe to what extent sandy soils, loams and clays differ in the above-mentioned soil physical and biological characteristics and how this affects C and N mineralization rates.

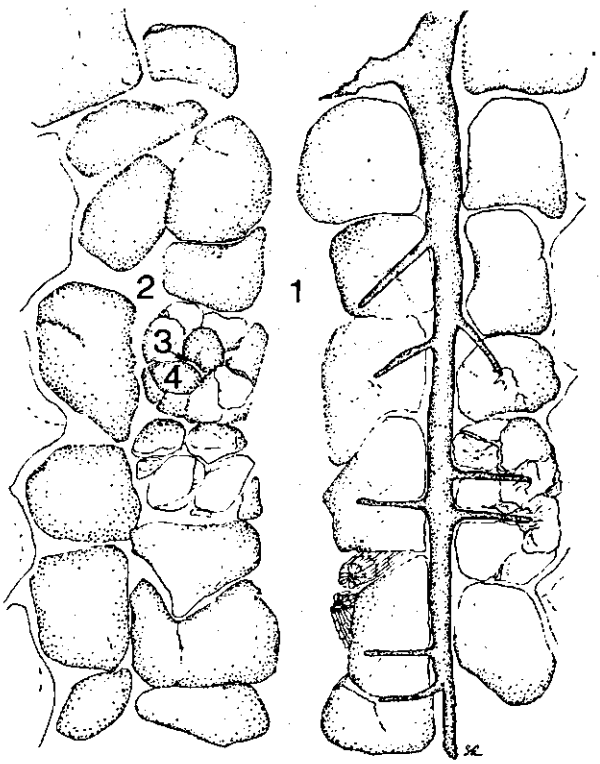


Fig. 2. Hierarchical class of soil pore space according to Elliott and Coleman (1988). 1 = macropores, 2 = pores between macroaggregates, 3 = pores between microaggregates, 4 = pores within microaggregates.

METHODS

Field description

In March 1989 and May 1991 samples were collected from grassland soils which had been under grass for at least 30 years. In Swifterbant the old sward was reseeded in 1985. The

grasslands were grazed by dairy cattle and received 400-500 kg fertilizer-N per ha per year. Samples were taken from the 0-10 cm layer. Some characteristics of the soils are given in Table 1.

Table 1. Some soil characteristics of the 0-10 cm layer of the grassland sites studied

	C	N	C/N	pH-	Granular composition, % particles <			Bulk density
	-g kg^{-1}-			K-Cl	2μm	16μm	50μm	g cm^{-3}
Sand								
Heino	19.8	1.1	18.0	4.8	1.9	2.8	8.7	1.33
Tynaarlo	43.8	2.5	17.5	4.4	2.4	4.4	23.5	1.25
Loam								
Swifterbant	17.7	1.5	11.8	7.0	23.0	38.4	72.2	1.22
Burum	53.7	5.5	9.8	4.9	24.1	36.5	71.7	1.09
Clay								
Zaltbommel	60.7	6.6	9.2	5.4	51.1	76.0	86.4	1.05
Haskerdijk	56.0	5.1	11.0	4.9	54.0	77.2	88.5	1.00

Measurements

The following measurements were performed in May 1991:
1) potential C and N mineralization rates,
2) pore size distribution,
3) water-stable aggregates, the clay and C contents of the aggregates, and mineralization of aggregate-C,
4) increase in C and N mineralization rates due to disruption of soil structure,
5) amount and microscopic characteristics of C and N associated with particles < 2 μm (clay) and of C and N present in the sand fraction > 200 μm (macro organic matter),
6) biomass of bacteria, fungi, protozoa and nematodes (direct counts) and
7) microbial biomass C and N according to the fumigation incubation method

In March 1989 only the potential mineralization rates and microbial biomass according to the fumigation method were determined.

Description of the methods

Three mixed samples, each consisting of 20 bulked cores, were taken from every location at each sampling date for the measurements of microbial biomass, bacterial, fungal and protozoal biomass and C and N mineralization. The samples were sieved through a 0.008-m mesh screen; roots and stubble were removed and the samples were analyzed separately. In all soils the moisture content was close to 60% of the water holding capacity at both sampling times. Field-moist samples were incubated.

Potential C and N mineralization rates

N mineralization was determined by measuring the increase in mineral N after incubation of soil samples in glass jars at 20 °C for 12 weeks. Drying of the samples was prevented by covering the jars with a seal permeable to air, but impermeable to water. Mineral N was measured colorimetrically after extraction with 1N KCl solution for 1 h using a soil:water ratio of 1:2.5.

C mineralization was measured by incubating soil samples in 1.5-l airtight jars containing a vial of 10 ml 0.5 M NaOH. At the sampling dates, the trapped CO_2 was measured after precipitating the carbonate with excess $BaCl_2$. The CO_2 produced in the period between 10 and 20 days after the start of the incubation was used to calculate the C mineralization rate. A flush in CO_2 production was observed during the first 10 days after incubation, probably caused by the disturbance of the soil.

Pore size distribution

The relationship between soil water potential and moisure content was determined according to Klute (1986) in undisturbed soil samples from the 2.5-7.5-cm layer. The effective pore neck diameter (d) was estimated from the water retention curve as

$d = 2r = -30.0 * 10^{-6} h^{-1}$ (m), (1)

where h = pressure head, r = radius of curvature of the capillary pore (m). Equation 1 has been derived for a water temperature of 15 °C (Vargas and Hattori, 1986). The corresponding pF values can be obtained by taking the logarithm of the absolute value of the pressure head: $Pf = {}^{10}\log [h]$. The pore volumes corresponding with different pore neck diameters can then be calculated from the retention curve.

Water-stable aggregates

The amount of water-stable aggregates was determined in one sandy soil, one loam and one clay (Tynaarlo, Burum and Zaltbommel, respectively). The amount of water-stable aggregates was determined by wet-sieving 100 g of field-moist samples on 250, 50 and 20 µm sieves that were placed on a vibrator machine for 4 minutes under a continuous water flow of 1.1

liter per minute (Matus, 1995). The organic C content of the aggregates was defined as dichromate-oxidizable C according to Kurmies (Mebius, 1960). The clay content of the aggregates was determined after oxidation of organic matter with H_2O_2. The soil was dispersed and shaken end over end in a 1-l cylinder. A table showing the settling-time for 2-µm particles was constructed by applying Stoke's law and a particle density of 2.675 g cm^{-3}. After the correct settling time, the particles < 2 µm were isolated by siphoning the suspension at the appropriate depth. Mineralization of aggregate-C was determined as described above. The aggregate samples were incubated at a water potential of -10 kPa. The CO_2 produced in the period between 10 and 20 days after the start of the incubation was used to calculate the C mineralization rate.

Increase in C and N mineralization after disruption of soil structure

The C and N mineralization rates were measured in soil samples that had been pressed through a 0.001 m mesh size screen (fine sieving) and in soil samples that had been sieved through a 0.008 m mesh size screen (coarse sieving). It was assumed that the increase in mineralization observed after fine sieving is caused by the release of a part of the physically protected organic matter, located in small pores in (or between) aggregates. The relative increase in mineralization due to fine sieving in comparison with the mineralization rate in coarsely sieved samples during the same period then gives a qualitative index of the extent of physical protection of organic matter in pores in (or between) aggregates. For C the increase was determined during the first five days after the start of the incubation; for N a period of two weeks was taken (the lengths of the periods the increases in mineralization after fine sieving remained for C and N; Hassink, 1992).

Amount and characteristics of C and N associated with soil particles < 2 µm and present in macro-organic matter

Dry soil (50g) was suspended in 250 ml water for 24 hours. The samples were treated ultrasonically for 15 minutes with a probe-type ultrasound generating unit (Soniprep 150). Probe output was calibrated by measuring the temperature produced upon ultrasonifying a known mass of water for a specified time (North, 1976). Output power assessed was 30 W. Heat buildup in the soil suspension was controlled by a water cooling jacket. The dispersed soil suspension was transferred to a 1-l glass cylinder. The cylinder was shaken end over end until the soil was suspended. A table showing the settling-times for 2 µm particles at temperatures between 15 and 25 °C was constructed by applying Stoke's law and a partice density of 2.675 g cm^{-1}. After the correct settling time, particles < 2 µm were isolated by siphoning the suspension at the appropriate depth. The fractions were dried for 24 hours at 105 °C.

Macro-organic matter is defined as organic material not closely associated with the soil mineral fraction. The macro-organic matter was separated from 100 g portions of soil that had

been sieved through a 0.008 m size screen and was cleared from visible root and plant parts. The samples were dispersed in pyrophosphate solution for 2 hours. The suspensions were shaken for 1 h and sieved through a 200 µm mesh screen. The sand and the macro organic matter fraction was dried overnight, ground and analysed for total C and N using a Carlo Erba NA 1500 analyzer.

Microscopic characterization of organic matter associated with soil particles < 2 µm and present in the macro-organic matter

Subsamples of both the < 2 µm soil particles and the macro-organic matter fractions were examined using a Confocal Laser Scanning Microscope (CLSM, Leica, Heidelberg, Germany). Of each fraction a few mg was suspended in 2 ml demineralized water, fixed with 4% (wt/vol, final concentration) formaldehyde, and stained for 5 min with 0.05% (wt/vol, final concentration) acridine orange (Faegri et al., 1977), for 20 min with 0.0001% 5-(4,6-dichlorotriazin-2-yl) aminofluorescein (DTAF) in 0.05 M Na_2HPO_4 buffer at pH 9. The particles were collected on a 0.2 µm pore size, 25 mm diam aluminium oxide membrane filter (Anodisc 25, Anotec Separations Ltd, Banbury, Oxon, England), and rinsed with 5 ml distilled water. The filter was mounted with immersion oil between a glass slide and a cover glass. The cover glass was sealed with nail polish. Under the CLSM the specimen was excited at 488 and 514 nm by an argon ion laser (2-50 mW). On the first channel the fluorescence of acridine orange or DTAF was detected using a beam splitter of 510 nm and a 525 nm bandpass barrier filter. The reflected light was measured simultaneously on the second channel. With reflected light both fluorescent and non fluorescent particles were detected. The acridine orange or DTAF fluorescence was used to obtain images of organic residues. The obtained video images were photographed using a Polaroid FreezeFrame Video Recorder (Polaroid Co., Cambridge, MA, USA).

Biomass of bacteria, fungi, protozoa and nematodes

Bacteria. Bacteria were counted in europium-chelate-stained soil smears using a Leitz epifluorescence microscope (magnification * 1000) equipped with a HBO-50 mercury lamp. A 1:10 diluted soil suspension was homogenized in a blender for 30 seconds. Details about the method are given by Hassink et al. (1991). The average volume of a bacterium was assumed to be 0.2 μm^3 (Bloem, unpublished results). It was assumed that the bacteria contained 320 * 10^{-15} g C μm^{-3} (Van Veen and Paul, 1979).

Fungi. Fungal biomass was estimated by measurements of hyphal length in agar films, made by mixing 1 ml of a 1:10 diluted soil suspension and 6 ml of agar, stained with fluorescent brightener (Hassink et al., 1991). A conversion factor of 0.33 (ratio between dry mass and wet volume) was used for biomass calculations and a carbon content of 40% of the dry weight (Van Veen and Paul, 1979).

Protozoa. Protozoa were enumerated by the most probable number method after Darbyshire et al. (1974). Details are given by Brussaard et al. (1990).

Nematodes. Three 200-g samples were taken from every location with a spade. Roots were separated by hand and nematodes were isolated from field-moist soil by elutration (Oostenbrink, 1960). For the isolation of nematodes from the roots, roots were spread over a nematode filter and nematodes were allowed to creep through the filter during 48 hours at 18 °C. The nematode suspensions from soil and roots were counted separately under a stereo microscope and 60 specimens per sample were identified under a high power microscope. Four different feeding categories were distinguished: bacterivorous, omnivorous, fungivorous and phytophagous nematodes. Details about conversion factors are given by Brussaard et al. (1990).

Microbial biomass C and N. The amounts of C and N in the microbial biomass were determined by the chloroform fumigation incubation technique (Jenkinson and Powlson, 1976). The exact procedure has been described by Hassink et al. (1991). For C a k value of 0.45 and for N a k value of 0.4 was used to calculate the biomass C and N from the flush (Jenkinson and Ladd, 1981; Schnürer et al., 1985).

Statistical treatment

Differences in mineralization rates between soils and treatments (fine sieving in comparison with coarse sieving) were analysed with the t-test. The relations of pore size distribution with increase in mineralization after fine sieving; biomass of bacteria, fungi, protozoa and nematodes with pore size distribution; mineralization per unit of bacterial or microbial biomass with grazing intensity and C/N ratio of the microbial biomass were analysed with regression analysis (Genstat manual, 1987).

RESULTS

C and N mineralization rates

C mineralization rates were generally higher in loams and clays than in sandy soils, however the percentage of mineralized organic C was not significantly different for the different soil types (Table 2). The percentage of organic C mineralized per day fluctuated considerably between sampling dates and locations. The percentage of mineralized organic C was highest in Swifterbant, the value for this location may, however, be an overestimation because it is a calcareous soil and part of the CO_2 will originate from carbonate.

The N mineralization rates ranged between 0.7 and 3.1 kg ha^{-1} day^{-1} (Table 2). The percentage of mineralized soil organic N per day was significantly ($p < 0.05$) higher in sandy

soils than in loams and clays at both sampling dates.

Table 2. Mineralization rates of C (period 10-20 days after the start of incubation) and N (period 0-84 days after the start of the incubation) at 20 °C (kg ha^{-1} day^{-1} and % of total soil C and N mineralized per day x100)

	Carbon				Nitrogen			
	kg ha^{-1} day^{-1}		%(x10^2)		kg ha^{-1} day^{-1}		%(x10^2)	
	'89	'91	'89	'91	'89	'91	'89	'91
Sand								
Heino	20.6	11.6	7.8	4.4	1.1	1.2	7.3	8.3
Tynaarlo	26.2	19.6	4.9	3.6	1.4	1.9	4.5	6.0
Loam								
Swifterbant	23.1	24.6	10.5	11.2	0.7	0.7	3.8	3.6
Burum	44.3	51.0	7.2	8.3	2.3	2.6	3.6	4.1
Clay								
Zaltbommel	60.0	28.8	8.2	4.0	2.1	1.8	2.7	2.2
Haskerdijk	-	41.6	-	6.2	-	3.1	-	5.0

Pore size distribution

The total pore space was lowest in the sandy soils and highest in the clayey soils. In the loamy and clay soils, the pores with diameters < 0.2 µm were predominant (Table 3). The pores with diameters between 0.2-1.2 µm were more abundant in the loamy and clay soils than in the sandy soils. In the sandy soils, however, the pores with diameters between 6 and 30 µm were most abundant; the pore size class between 30 and 90 µm also made up a larger part of soil volume in the sandy soils than in the loamy and clay soils (Table 3).

Water-stable aggregates

The aggregate size distribution was different between the sandy soil, the loam and the clay. In the sandy soil microaggregates with diameters between 50 and 250 µm were dominant, while in the loamy and clay soil macroaggregates (diameters > 250 µm) and microaggregates with diameters < 20 µm were dominant (Table 4). In the sandy soil the C content of the aggregates was significantly positively correlated with their clay content (Table 4; Fig. 3). In the loam the correlation between the clay content of the aggregates and their C content was weak, while in the clay soil there was no correlation between the clay content of the

Table 3. Pore size distribution of soils

	Pore size distributions (% of soil volume); size classes in µm							
	< 0.2	0.2-1.2	1.2-6	6-30	30-90	90-150	>150	total
Sand								
Heino	6.0	2.1	8.3	18.8	5.4	1.3	3.6	42.2
Tynaarlo	10.5	5.1	10.1	17.0	3.7	0.2	2.7	49.3
Loam								
Swifterbant	18.4	8.3	13.4	0.0	3.0	1.5	3.6	48.1
Burum	20.1	11.4	16.1	0.0	2.6	2.0	6.3	58.7
Clay								
Zaltbommel	29.2	10.9	12.9	1.9	0.0	0.0	7.2	62.0
Haskerdijk	30.7	10.3	13.3	0.0	0.7	1.2	5.6	61.9

aggregates and their clay content (Table 4). We also observed that in the sandy soil the decomposition rate of aggregate-C was significantly ($p < 0.05$) negatively correlated with its clay content (Table 4; Fig. 3), while in the loamy and clay soils this correlation was not found (Table 4).

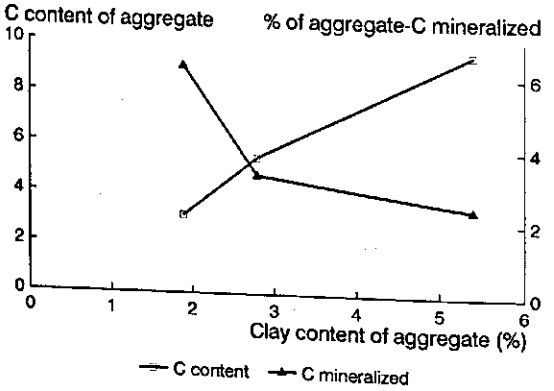


Fig. 3. Relationship between the C content (%) and C mineralization rate (% day^{-1} x 100) of aggregates of different size classes in the sandy soil and their clay content.

Table 4. Composition of water-stable aggregates (% of total soil), C and clay contents (%) of aggregates and mineralization of aggregate-C (% day^{-1} x 100) of a sandy, loamy and clay grassland soil.

	< 20 µm	20-50 µm	50-250 µm	> 250 µm
		Sand (Tynaarlo)		
Composition of aggregates	8.8	8.6	58.0	24.6
C content	14.7	9.5	3.1	5.4
Clay content	38.2	5.4	1.9	2.8
C Mineralization	n.d.	2.4	6.3	3.3
		Loam (Burum)		
Composition of aggregates	31.9	12.2	19.8	36.1
C content	6.9	3.2	3.7	4.9
Clay content	66.5	12.4	11.8	25.4
C mineralization	n.d.	5.8	4.8	3.4
		Clay (Zaltbommel)		
Composition of aggregates	39.3	5.2	5.7	49.8
C content	5.9	4.8	6.6	4.6
Clay content	69.7	27.0	32.6	45.9
C mineralization	n.d.	10.1	14.4	6.1

n.d. = not determined

Relative increase in mineralization rate after fine sieving; estimation of the amount of physically protected organic matter in small pores

In the loams and clays a relative increase in C and N mineralization due to fine sieving in comparison with coarse sieving was observed (Table 5). In the sandy soils, however, the mineralization rates of both C and N were decreased by approximately 20% (Table 5) after fine sieving; the decrease was not significant ($p < 0.05$). The increase in N mineralization due to fine sieving was largest in the clays. Here the relative increase in N mineralization after fine sieving was larger than the relative increase in C mineralization. This was not the case in the loams. The increases in N mineralization was significant in both loams and both clays ($p < 0.05$); the increase in C mineralization was only significant in one of the loams and one of the clays ($p < 0.05$). There was a good correlation between the relative increase in N mineralization after fine sieving and the percentage of soil pore space occupied by pores with diameters < 0.2 µm ($R^2 = 0.81$; Fig. 4). The correlation with any other pore size class was poor. For C this relationship with pores < 0.2 µm was less clear.

Table 5. Relative increase in N and C mineralization rates after fine sieving in comparison with coarse sieving (%)

	Increase in mineralization rate	
	C	N
Sand		
Heino	-25	-18
Tynaarlo	-20	-26
Loam		
Swifterbant	16	66*
Burum	65*	21*
Clay		
Zaltbommel	131*	249*
Haskerdijk	13	240*

* = statistical significant different from 0 ($p < 0.05$)

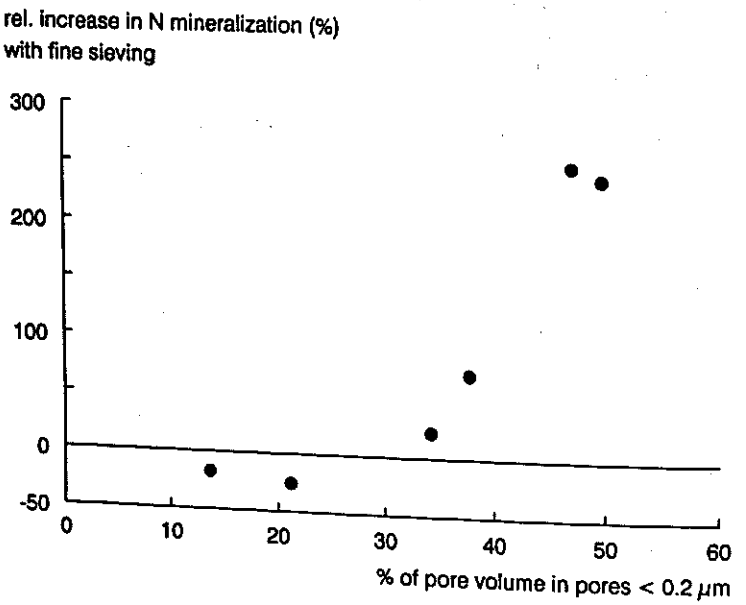


Fig. 4. Relation between the relative increase in N mineralization after fine sieving and the % of soil pore space enclosed by pores with diameters < 0.2 µm. Relative increase = 25 (6.8) + 0.10 (0.02) x % pore space < 0.2 µm; $r^2 = 0.81$ (Hassink, 1992).

C and N associated with particles < 2μm and present in the macro organic matter

The C and N content of the clay fraction (< 2μm) was much higher in the two sandy soils than in the loams and the clay (Table 6). Although the total C and N content of the sandy soils differed considerably (Table 1), the C and N content of their clay fraction was the same. In the loams this was different; the loamy soil from Burum which had a higher organic C and N content than the loamy soil from Swifterbant (Table 1), also had a higher C and N content in the clay fraction (Table 6). In all soils the organic material associated with the clay fraction had a C/N ratio between 8.4-10.3. In the sandy soils, the C/N ratio of this fraction was considerably lower (significant at $p < 0.05$) than the C/N ratio of the total organic matter. In the loams and the clay soil, however, the C/N ratio of the organic material associated with the clay fraction had almost the same C/N ratio as the C/N ratio of the total organic matter. In the sandy soils the N content of the clay fraction was 5.8-14.7 times higher than the N content of the total soil (Table 6). In the loams this was only 1.4-1.8 times higher and in the clay soil the N content of the clay fraction was even lower than the N content of the total soil.

In the loams and clays, the N content of the sand fraction > 200 μm, containing the macro organic matter, was higher than the N content of the total soil (Table 6). In the sandy soils, however, it was the opposite (Compare Tables 1 and 6). In all soils the C/N ratio of the macro organic matter present in the fraction > 200μm, was between 16 and 19, except for the soil from Haskerdijk (Table 6).

Table 6. C and N contents and the C/N ratios of the fraction < 2 μm, and the sand fraction > 200μm (containing macro organic matter); and the ratio N content of the fraction < 2 μm:N content of the total soil and N content of the fraction > 200μm:N content of the total soil

	Fraction < 2μm			Ratio	Fraction > 200μm			Ratio
	C	N	C/N	N	C	N	C/N	N
	g kg^{-1}				g kg^{-1}			
Sand								
Heino	145.4	16.2	9.0	14.7^{a}	15.4	0.9	18.4	0.8^{c}
Tynaarlo	149.6	14.6	10.3	5.8^{a}	20.8	1.2	16.9	0.5^{c}
Loam								
Swifterbant	27.4	2.7	10.2	1.8^{b}	58.8	3.6	16.2	2.4^{b}
Burum	70.3	7.7	9.1	1.4^{b}	123.4	6.4	19.4	1.2^{b}
Clay								
Zaltbommel	37.0	4.4	8.4	0.7^{b}	117.8	7.3	16.3	1.1^{b}
Haskerdijk	-	-	-	-	104.7	8.2	12.7	1.6^{b}

Values with a different character differ significantly ($p < 0.05$).

Microscopic characterization of organic matter associated with soil particles < 2 μm and present in the macro organic matter

The organic material associated with the clay fraction did not show any staining by acridine orange or DTAF. Images obtained with reflected light showed that this fraction consisted of particles with a diameter of about 0.3 μm (Fig. 5).

The macro organic matter present in the sand fraction consisted of recognizable plant residues (Fig. 6). This plant debris stained intensely with acridine orange and DTAF.

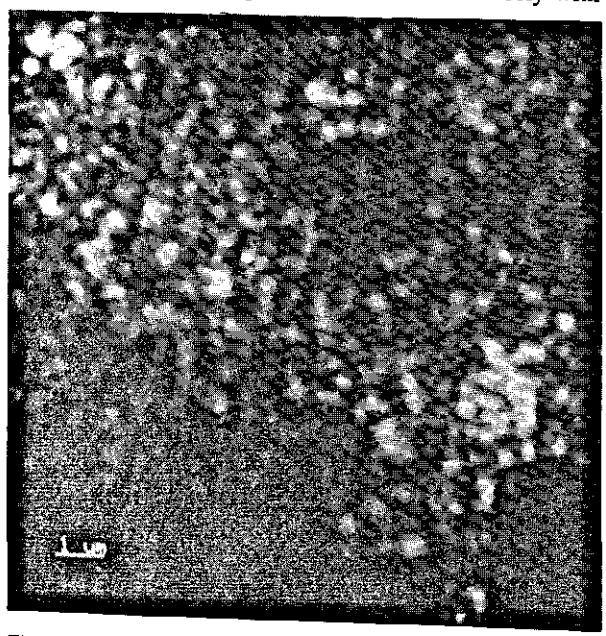


Fig. 5. Image obtained with reflected light of the clay fraction with associated organic matter isolated from the clay soil of Zaltbommel, consisting of undefined particles with a diameter of about 0.3 μm.

Biomass C present in various functional groups and its relation with pore size distribution

Bacteria constituted by far the largest biomass pool. In the sandy soils the amount of bacterial biomass was between 126 and 208 kg C ha^{-1}. In the loams and clays, the amount of bacterial biomass was higher: between 250 and 432 kg C ha^{-1} (Table 7). The amount of C in the fungal biomass was very low: less than 3% of the amount present in the total microbial biomass. There was no difference in the contribution of fungal biomass to total microbial biomass between the sandy soils, the loams and the clays. The biomass of protozoa was also small, it was generally higher in the loams and clays than in the sandy soils. The biomass of nematodes was even lower than the biomass of protozoa. The biomass of bacterivorous

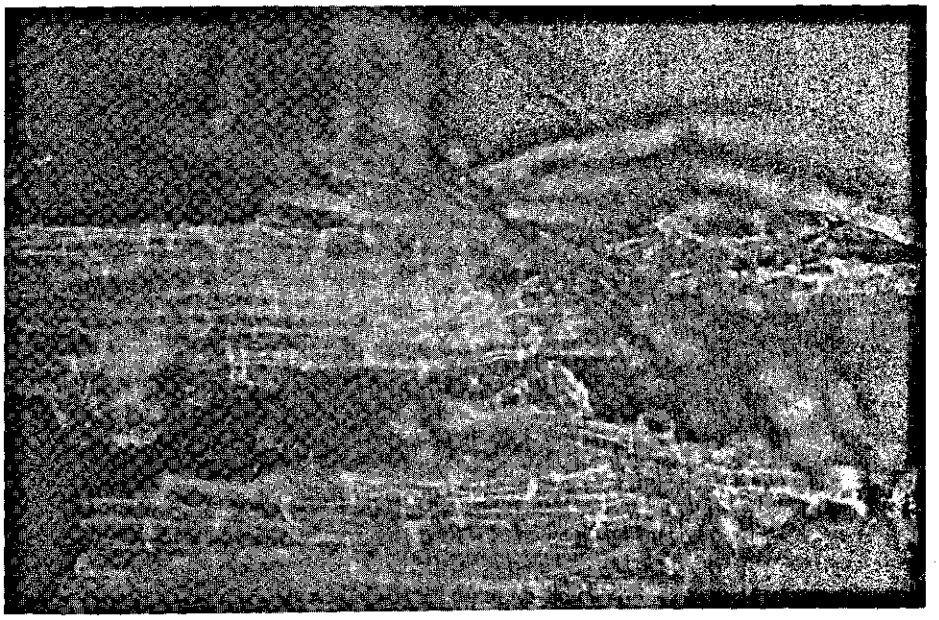


Fig. 6. Image obtained after staining with acridine orange and DTAF of the macro organic matter present in the sand fraction > 200 µm isolated from the clay soil of Zaltbommel. The macro organic matter consists of recognizable plant residues.

nematodes was relatively high in the sandy soils; the ratio biomass of bacterivorous nematodes:biomass of bacteria was higher in the sandy soils than in the loams and clays. There was a good correlation between the amount of bacterial biomass and the soil volume made up by pores with diameters between 0.2-1.2 µm (R^2 = 0.91) and between the biomass of nematodes and the soil volume made up by pores with diameters between 30-90 µm (R^2 = 0.84; Fig. 7). The biomass of fungi and protozoa showed a poor correlation with any of the pore size classes.

Composition and quality of the microbial biomass determined by the fumigation incubation method

The amount of C present in the microbial biomass ranged from 419 to 1869 kg C ha^{-1} (Table 8). These values are much higher than those obtained by microscopic counting. It fluctuated considerably between the two sampling dates. The amount of N present in the microbial biomass ranged from 52 to 553 kg N ha^{-1} (Table 8). The amount of biomass N was lowest in the sandy soils and highest in the clays. The fluctuation between the two sampling dates was much smaller than for C. At both sampling dates the C/N ratio of the microbial biomass was significantly higher ($p < 0.05$) in the sandy soils (6.2-13.8) than in the loams and clays (2.3-5.7).

Table 7. Amount of biomass (kg C ha^{-1}) of functional groups at different sites in the top 10 cm in May 1991

	Bacteria	Fungi	Protozoa		Nematodes			
			a[1]	fl[2]	b[3]	f[4]	p[5]	o[6]
Sand								
Heino	126.0	3.69	2.95	0.32	0.70	0.08	0.19	0.16
Tynaarlo	207.8	3.80	2.90	0.43	0.40	0.04	0.26	0.03
Loam								
Swifterbant	250.0	5.47	5.38	0.90	0.08	0.26	0.20	0.08
Burum	432.8	5.00	4.83	0.87	0.28	0.07	0.23	0.03
Clay								
Zaltbommel	425.8	5.33	5.27	0.96	0.26	0.07	0.05	0.01
Haskerdijk	407.8	4.95	10.81	1.47	0.43	0.07	0.00	0.00

[1]a = amoebae, [2]fl = flagellates, [3]b = bacterivorous, [4]f = fungivorous, [5]p = phytophagous, [6]o = omnivorous

Table 8. Amount of microbial biomass C and N (kg ha^{-1}) and the C/N ratio of the microbial biomass determined with the fumigation incubation method

	Biomass C		Biomass N		C/N ratio	
	'89	'91	'89	'91	'89	'91
Sand						
Heino	714	419	52	68	13.8	6.2
Tynaarlo	577	1143	65	113	8.9	10.1
Loam						
Swifterbant	803	507	205	218	3.9	2.3
Burum	811	1084	265	215	3.1	5.0
Clay						
Zaltbommel	1027	1869	375	327	2.7	5.7
Haskerdijk	-	1776	-	553	-	3.2

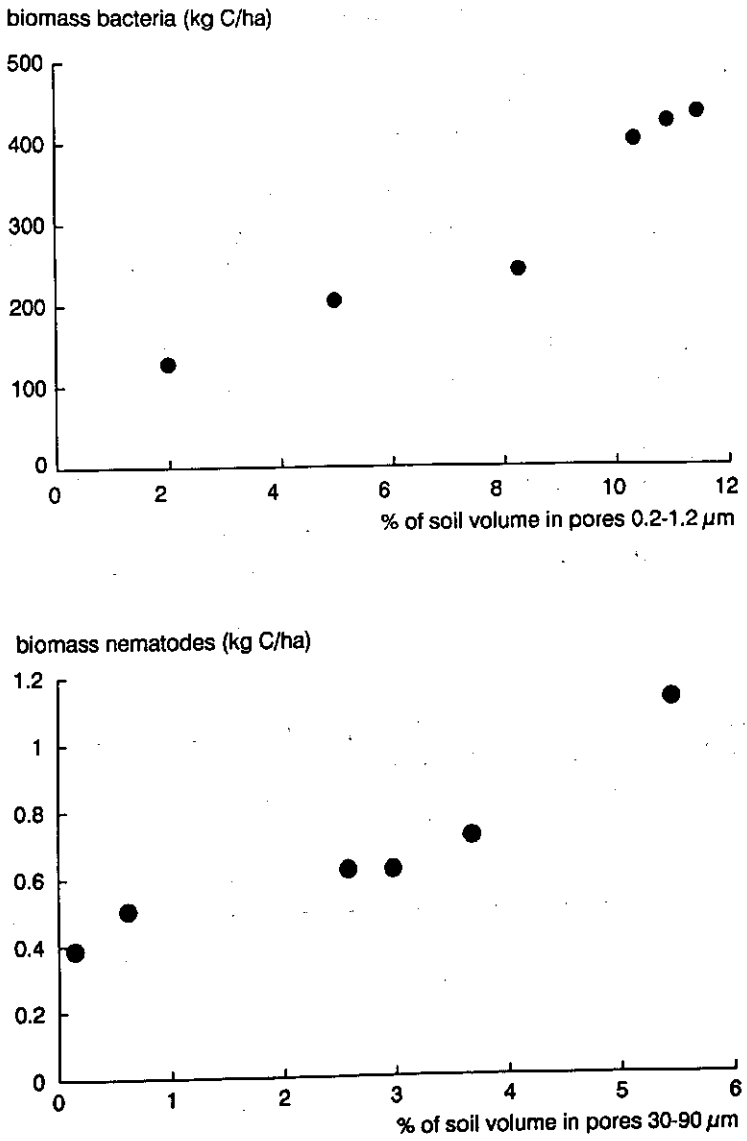


Fig. 7. Relation between the biomass of bacteria (kg C ha^{-1}) and the % of soil volume enclosed by pores with diameters between 0.2-1.2 µm (7a; Bacterial biomass = 35.4 (40.4) + 34.2 (4.6) x % of soil volume in pore class 0.2-1.2 µm; $r^2 = 0.91$; Hassink et al., 1993); and the relation between the biomass of nematodes (kg C ha^{-1}) and the % of soil volume enclosed by pores with diameters between 30-90 µm (7b; biomass of nematodes = 0.35 (0.07) + 0.12 (0.02) x % of soil volume in pore class 30-90 µm; $r^2 = 0.84$; Hassink et al., 1993).

Mineralization rates of C and N per unit of microbial biomass and bacterial biomass; relation with grazing intensity and C/N ratio of the biomass

To gain information about the activity of the bacterial and microbial population, the C mineralization rate (kg ha^{-1} day^{-1}) was divided by the amount of bacterial and microbial biomass (kg C ha^{-1}). The ratios for the bacterial biomass ranged from 6.8 to 11.8 (Table 3.9). There were no differences between sandy soils, and loams and clays. The ratios for the microbial biomass ranged from 1.5 to 5.8 (Table 9). Again no differences between soil types were observed.

The N mineralization rate (kg ha^{-1} day^{-1}) per amount of bacterial biomass C (kg ha^{-1}) was significantly ($p < 0.05$) higher in sandy soils than in loams and clays (Table 9). This was also the case for the N mineralization rate (kg ha^{-1} day^{-1}) per amount of microbial N (kg ha^{-1}; Table 9).

A positive correlation was observed between the N mineralization rate per biomass of bacteria and the grazing intensity of bacterivorous nematodes on bacteria (Fig. 8). Such a correlation was not found for the amoebae and flagellates. There was a positive correlation between the N mineralization rate per unit of microbial biomass and the C/N ratio of the microbial biomass ($R^2 = 0.67$; Fig. 9). Such correlations were not observed for C.

Table 9. Mineralization rates of C and N (kg ha^{-1} day^{-1} x 10^2) divided by the amount of bacterial biomass (microscopic counting in kg C ha^{-1}); and mineralization rates of C and N (kg ha^{-1} day^{-1} x 10^2) divided by the amount of microbial biomass C or microbial biomass N (fumigation incubation in kg C or N ha^{-1}).

	C and N mineralization rates					
	:Bacterial biomass C		:Microbial biomass C		:Microbial biomass N	
	'91 C	'91 N	'89 C	'91 C	'89 N	'91 N
	day^{-1} x 10^2		day^{-1} x 10^2		day^{-1} x 10^2	
Sand						
Heino	9.2	0.96	2.9	2.8	2.0	1.8
Tynaarlo	9.4	0.89	4.5	1.7	2.1	1.6
Loam						
Swifterbant	9.8	0.27	2.9	4.9	0.3	0.3
Burum	11.8	0.59	5.5	4.7	0.9	1.2
Clay						
Zaltbommel	6.8	0.42	5.8	1.5	0.6	0.5
Haskerdijk	10.2	0.75	-	2.3	-	0.6

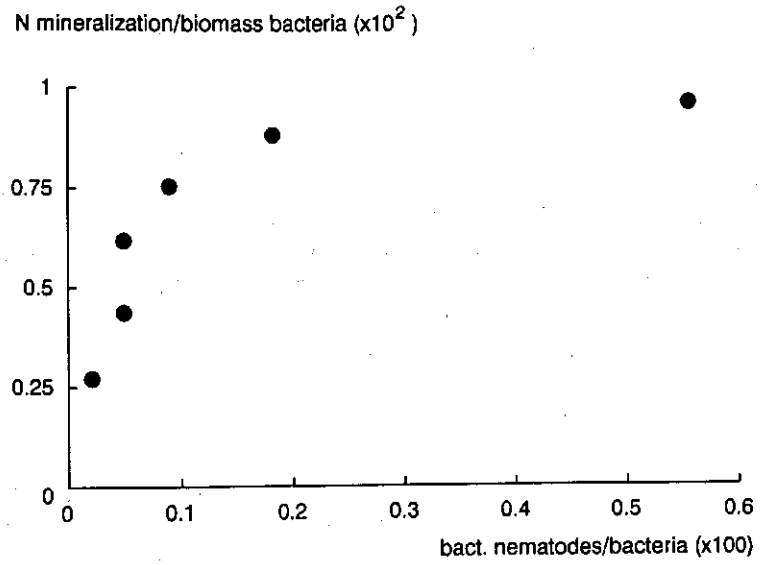


Fig. 8. Relation between the N mineralization rate (kg N ha^{-1} day^{-1}) x 10^2 per unit of biomass of bacteria (kg C ha^{-1}) and the biomass of bacterivorous nematodes (kg C ha^{-1}) per unit of biomass of bacteria (kg C ha^{-1}). (N mineralization/biomass bacteria = 0.96 (0.05) - 1.15 (0.17) x 2.37 x 10^{-7} (8.55 x 10^{-7})^x. ^{x} = biomass of bact. nematodes/biomass of bacteria; r^2 = 0.95).

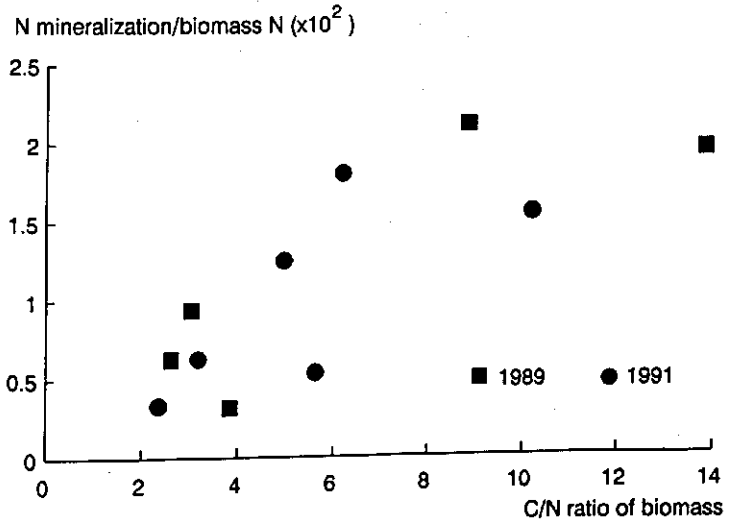


Fig. 9. Relation between the N mineralization rate (kg N ha^{-1} day^{-1}) x 10^2 per unit of microbial biomass N (kg N ha^{-1}) and the C/N ratio of the microbial biomass in 1989 and 1991. (N mineralization / biomass N = 0.17 (0.42) + 0.15 (0.04) x C/N microbial biomass; r^2 = 0.67.)
() = standard error of difference.

DISCUSSION

General

In this study we tried to determine whether sandy soils, loams and clays differ in C and N mineralization rates and whether observed differences are caused by differences in soil structural and biological characteristics. We investigated soils that had been used as intensively managed grassland for a long time. It may be assumed that the amount and quality of organic residues annually incorporated into the soil were similar and were not confounded with the effects of different textures and structural and biological characteristics.

Generally, it is found that both the organic C and N contents are higher in soils with a higher clay content (Jenkinson, 1988; Spain, 1990). The loams and clays in our study were found to have a higher organic N content than the sandy soils. The only exception is the soil from Swifterbant. This location is a young sedimentary calcareous loam reclaimed from the sea only 30 years ago. At this location a large buildup of soil organic N is measured (Hassink and Neeteson, 1991). As the difference in organic C content between the soil types is much smaller than the difference in organic N content, the C/N ratio of the organic matter is considerably higher in the sandy soils than in the loams and clays (Table 1). It has been observed that the sandy soils with a high C/N ratio contain inert charcoal pieces (Hassink, 1994).

The percentage of organic N mineralized during incubation was higher in sandy soils than in loams and clays (Table 2). This is in agreement with earlier observations (Catroux et al., 1987; Hassink et al., 1990). The percentage of organic C that mineralized, however, was not different for the soil types. This may be due again to the presence of inert charcoal pieces in the sandy soils.

Physical protection of organic C and N

We hypothesized that differences in C and N mineralization rates between soil types could be caused by differences in soil structural and biological characteristics. We assumed that in clay soils more organic C and N was physically protected against decomposition than in sandy soils. It may be concluded from our results that the mechanism of physical protection is different for different soil types. In sandy soils fine sieving did not increase the C and N mineralization rate. This suggests that no organic C and N was physically protected in small pores in or between aggregates. The clay fraction in the sandy soils, however, had a much higher C and N content than the total soil. In agreement with Matus (1995), we also observed that the C contents of aggregates in the sandy soil were positively correlated with their clay contents, while the mineralization rate of aggregate-C was negatively correlated with the clay content of the aggregates in the sandy soil. These results suggest that in sandy soils organic C and N is physically protected by the mechanism of adsorption to clay minerals or encrustation by clay minerals (mechanism suggested by Tisdall and Oades, 1982; Fig. 3.1).

In the clay and loamy soils the situation was different. Here the increase in C and N mineralization after fine-sieving was often considerable, the C and N contents of the clay fraction were not much higher than the C and N contents of the total soil, and the C contents and C mineralization rates of aggregates were not correlated with the clay content of the aggregates. Apparently organic compounds are also physically protected by their location in small pores in or between aggregates (mechanism suggested by Elliott and Coleman, 1988; Fig. 2) in loamy and clay soils. The observation that the enrichment factor of the clay fraction (C or N content of the clay fraction / C or N content of the total soil) decreased with the clay content of the soil is in agreement with the results of Christensen (1985), Elustondo et al. (1990) and Hassink et al. (1995; Fig. 10). Scanning Electron Microscopy observations showed that clay particles were present as individual particles in coarse-textured soils, while in fine-textured soils the clay particles were coagulated (Hassink et al., 1995). This might be the cause of the difference in C and N content of the clay particles, as in fine-textured soils less C and N can be adsorbed per unit of weight than in coarse-textured soils.

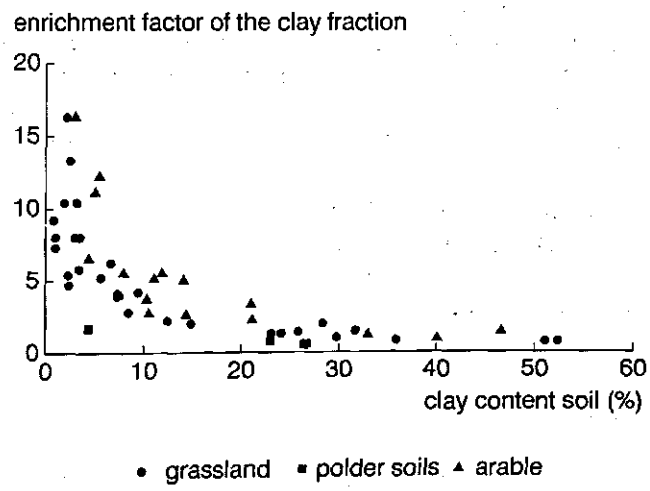


Fig. 10. Relationship between the clay content of different soils and the C enrichment factor (% C in clay fraction / % C in whole soil). Based on data of Christensen (1985), Elustondo et al. (1990) and Hassink et al. (1995).

Acridine orange is used to stain nucleic acids of living and dead organisms, but it is also bound by other macromolecules such as glycosaminoglycans, galactosaminoglycans, liposomes, negative phospholipids and colloids (reviewed by Paul, 1982). DTAF is used to stain proteins (Blakeslee and Baines, 1976). In our study, plant residues could easily be recognized using acridine orange or DTAF. The soil particles < 2μm did not show any staining with acridine orange or DTAF. The lack of stainable macromolecules in this fraction may reflect a higher degree of decomposition of the organic matter in this fraction. This

indicates that this clay-associated protected material has been present in the soil for quite some time. Its low C/N ratio, small size and undefined structure suggests that it consists of microbial decay products.

Biological characteristics

The biomasses of the different groups were similar to those found in an arable soil in the Netherlands (Brussaard et al., 1990). In the arable soil fungal biomass was also very low in comparison with the bacterial biomass (Brussaard et al., 1990). Besides differences in physical protection, we also observed differences in biological characteristics among soil types. The loams and clays had a higher bacterial biomass than the sandy soils. The biomass of bacteria was correlated with the volume of soil made up by the pores between 0.2 and 1.2 µm. The most numerous soil microorganisms have been found to have a diameter < 0.3 µm (Bae et al., 1972; Lundgren, 1984; Foster, 1985). Since the ratio between the diameter of pores and the bacteria they contain is approximately 3:1 (Kilbertus, 1980), a large proportion of bacteria may be expected to occupy pores < 1.2 µm, especially if the grazing pressure keeps bacterial biomass low in larger pores. The biomass of nematodes was correlated with the volume of pores between 30-90 µm. This is in agreement with the results of Jones et al. (1969) that most nematodes have diameters between 15 and 60 µm.

We have shown that the grazing intensity on bacteria by bacterivorous nematodes was higher in sandy soils than in loams and clays. The data suggest that the grazing of bacteria by bacterivorous nematodes increased N mineralization rates (Fig. 8). In general, the effect of a grazer on N mineralization is high when the C/N ratio of its food is low, its own C/N ratio is high and its production efficiency is low (Hunt et al., 1987). Nematodes have a relatively high C/N ratio (7-10) compared to bacteria and a low production efficiency (Hunt et al., 1987). This means that they may potentially increase N mineralization. The absence of a correlation between the grazing intensity of amoebae and flagellates and the mineralization per bacterium may have been caused by the fact that biomass of amoebae and flagellates fluctuates considerably in sandy soils (Hassink et al., 1993).

We also observed that the C/N ratio of the microbial biomass was higher in sandy soils than in loams and clays (Table 8). There was a positive correlation between the N mineralization per unit of microbial biomass N and the C/N ratio of the microbial biomass (Fig. 9). This is in line with the concepts on which food webs are based. When bacteria decompose organic matter, more inorganic N is released from the organic matter when the bacteria have a higher C/N ratio and thus a lower requirement for N. C mineralization is not affected by the C/N ratio of the bacteria (Hunt et al., 1987; De Ruiter et al., 1993). The cause of the higher C/N ratio of the microbes in the sandy soils is not clear. It is assumed that fungi have a higher C/N ratio than bacteria. The fraction of fungi in the microbial biomass, however, is very small in all soils. According to Tezuka (1990), the C/N ratio of the microbes is also influenced by the C/N ratio of their food. One may assume however, that the C/N ratio of the organic residues returned to the soil should have approximately the same C/N ratio

since all soils in our experiments are intensively managed grasslands. According to Scholefield et al (1991) the C/N ratio of 1 year old residues of dead plant material in intensively managed grassland soils is 12.5.

Conclusions and suggestions for further research

Differences in clay content, pore size distribution and aggregation between soil types affect the relative importance of different mechanisms of physical protection of organic matter. Pore size distribution also determines the grazing intensity on bacteria. The C/N ratio of the microbial biomass was lower in clays and loams than in sandy soils. We have no explanation for this difference.

At the moment it is not clear whether all factors are important or whether just one of these is the key factor that determines the C and N mineralization rates in grassland soils. The measurements described here should be tested on a wider range of soils, including soils with the same texture but with a different pore and aggregate size distribution. This would show whether observed differences are related to pore and aggregate size distribution or to clay content of the soil. Ultrastructural studies focusing on the exact location of organic matter and microbes are necessary to obtain additional evidence for our hypothesis. Simulation modelling should then indicate which of the observed differences are the key factors in determining the C and N mineralization rates.

ACKNOWLEGEMENTS

We acknowledge the skillful technical assistance of A. Lahr, G. Hoenderboom, A. Vos, M. Wolters and P. Bolhuis. We also thank F. Matus for the measurement of the energy output of the probe during sonification.

REFERENCES

Bae, H.C., Cota-Robles, E.H. and Casida, L.E., 1972. Microflora of soil as viewed by transmission electron microscopy. Appl. Microbiol., 23: 637-648.

Blakeslee, D. and Baines, M.G., 1976. Immunofluorescence using dichlorotriazinylamino-fluorescein (DTAF). I. Preparation and fractionation of labelled IgG. J. Immunological Meth, 13: 305-320.

Brussaard, L., Bouwman, L.A., Geurs, M., Hassink, J. and Zwart, K.B., 1990. Biomass, composition and temporal dynamics of soil organisms of a silt loam soil under conventional and integrated management. Neth. J. Agric. Sci., 38: 283-302.

Catroux, G., Chaussod, R. and Nicolardot, B., 1987. Assessment of nitrogen supply from the soil. Ac. Agric. Francais, 3: 71-79.

Christensen, B.T., 1985. Carbon and nitrogen in particle size fractions isolated from Danish arable soils by ultrasonic dispersion and gravity-sedimentation. Acta Agic. Scand. 35: 175-185.

Christensen, B.T., 1992. Physical fractionation of soil and organic matter in primary particle size and density separates. Adv. Soil Sci. 20: 1-89.

Coleman, D.C., Cole, C.V., Hunt, H.W. and Klein, D.A., 1978. Trophic interactions in soils as they affect energy and nutrient dynamics. I. Introduction. Microb. Ecol., 4: 345-349.

Darbyshire, J.F., Robertson, L. and Mackie, L.A., 1974. A comparison of two methods of estimating the soil pore network available to protozoa. Soil Biol. Biochem., 17: 619-624.

Elliott, E.T. and Coleman, D.C., 1988. Let the soil work for us. In: H. Eijsackers and A. Quispel (Editors), Ecological implications of contemporary agriculture. Proceedings 4th European Ecology Symposium, Wageningen. Ecol. Bull., 39: 23-32.

Elliott, E.T., Anderson, R.V., Coleman, D.C. and Cole, C.V., 1980. Habitable pore space and microbial trophic interactions. Oikos, 35: 327-335.

Elustondo, J., Angers, D.A., Laverdière, M.R. and N'Dalyegamiye, A. 1990. Êtude comparative de l'agrégation et de la matière organique associée auz fractions granulaométriques de spet soils sous culture de mais ou en prairie. Can J. Soil Sci. 70: 395-402.

Faegri, A., Torsvik, V.L. and Goksöyr, J., 1977. Bacterial and fungal activities in soil: separation of bacteria and fungi by a rapid fractionated centrifugation technique. Soil Biol. Biochem., 9: 105-112.

Foster, R.C., 1988. Microenvironments of soil microorganisms. Biol. Fert. Soils, 6: 189-203.

Genstat Manual 1987. Genstat. A general statistical program. Clarendon, Oxford, 749 pp.

Golchin, A., Oades, J.M, Skjemstad, J.O. and Clarke P., 1994. Soil structure and carbon cycling. Aust. J. Soil Res. 32: 1043-1068.

Hassink, J., 1992. Effects of soil texture and structure on C and N mineralization in grassland soils. Biol. Fert. Soils.,14: 126-134.

Hassink, J., 1994. Effects of soil texture and grassland management on soil organic C and N and rates of C and N mineralization. Soil Biol. Biochem., 26: 1221-1231.

Hassink, J. and Neeteson, J.J., 1991. Effect of grassland management on the amounts of soil organic N and C. Neth. J. Agric. Sci., 39: 225-236.

Hassink, J., Scholefield, D. and Blantern, P., 1990. Nitrogen mineralization in grassland soil. In: N. Gaborcik, V. Krajcovic and M. Zimkova (Editors), Proceedings 13th General Meeting of the European Grassland Federation, vol II. Banscka Bystrica, pp. 25-32.

Hassink, J., Lebbink, G. and Veen, J.A. van, 1991. Microbial biomass and activity of a reclaimed-polder soil under a conventional or a reduced-input farming system. Soil Biol. Biochem., 23: 507-513.

Hassink, J., Bouwman, L.A., Zwart, K.B. and Brussaard, L., 1993. Relationships between habitable pore space, biomass and activity of bacteria and fauna and mineralization rates in grassland soils. Soil Biol. Biochem., 25: 47-55.

Hassink, J., Matus, F.J., Chenu, C. and Dalenberg, J.W., 1995. Interactions between soil biota,

soil organic matter and soil structure. Accepted by Adv. Agroecol.

Heijnen, C.E., Van Elsas, J.D., Kuikman, P.J. and Veen, J.A. van, 1988. Dynamics of *Rhizobium leguminosarum biovas trifolii* introduced into soil; the effect of bentonite clay on predation by protozoa. Soil Biol. Biochem., 20: 483-488.

Hunt, H.W., Coleman, D.C., Ingham, E.R., Elliott, E.T., Moore, J.C., Rose, S.L., Reid, C.P.P. and Morley, C.R., 1987. The detrital food web in a shortgrass prairie. Biol. Fert. Soils, 3: 57-68.

Jenkinson, D.S., 1977. Studies of the decomposition of plant material in soil. V. The effects of plant cover and soil type on the loss of carbon from ^{14}C labelled ryegrass decomposing under field condition. J. Soil Sci., 28: 424-434.

Jenkinson, D.S., 1988. Soil organic matter and its dynamics. In: A. Wild (Editor), Russel's soil conditions and plant growth, 11th edition, chapter 18. Longman, New York, pp. 564-607.

Jenkinson, D.S. and Powlson, D.S., 1976. The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. Soil Biol. Biochem., 8: 209-213.

Jenkinson, D.S. and Ladd, J.N., 1981. Microbial biomass in soil: measurement and turnover. In: E.A. Paul and J.N. Ladd (Editors), Soil Biochemistry, Vol. 5. Marcel Dekker, Inc., New York and Base, pp. 415-471.

Jones, F.G.W., Larbey, D.W. and Parrott, D.M., 1969. The influence of soil structure and moisture on nematodes, especially Xiphinema, Longidorus, Trichodorus and Heterodera spp. Soil Biol. Biochem., 1: 153-165.

Juma, N.G., 1993. Interrelationships between soil structure/texture, soil biota/soilorganic matter and crop production. Geoderma 57: 3-30.

Kilbertus, G., 1980. Etude des microhabitats contenus dans les aggregats du sol, leur relation avec la biomasse bacteriënne et la taille des procaryotes présents. Rev. Ecol. Biol. Sol, 17: 543-557.

Klute, A., 1986. Water retention: Laboratory methods. In: A. Klute (Editor), Methods of soil analysis, Part I. Physical and mineralogical Methods. Agronomy Monograph no. 9. American Society of Agronomy, Madison.

Ladd, J.N., Amato, M., Jocteur-Monrozier, L. and Van Gestel, M., 1990. Soil microhabitats and carbon and nitrogen metabolism. In: Proceedings 14th International Congress Soil Science, Kyoto, Japan, pp 82-87.

Ladd, J.N., Foster, R.C. and Skjemstad J.O., 1993. Soil structure: carbon and nitrogen metabolism. Geoderma 56: 401-434.

Lundgren, B., 1984. Size classification of soil bacteria: Effects on microscopically estimated biovolumes. Soil Biol. Biochem., 16: 283-284.

Matus, F. M., 1995. The distribution of soil organic matter of various aggregate size classes in arable soil. I. Relationships between clay content of aggregates of a sand and a clay soil and carbon mineralization, nitrogen mineralization and microbial biomass carbon. Subm. to Soil Biol. Biochem.

Mebius, L.J., 1960. A rapid method for the determinationof organic carbon in soil. Anal.

Chim. Acta, 22: 120-124.

North, P.F., 1976. Towards an absolute measurement of soil structural stability using ultrasound. J. Soil Sci., 27: 451-459.

Oostenbrink, M., 1960. Estimating nematode populations by some selected methods. In: J.N. Sasser and W.R. Jenkins (Editors), Nematology. University of North Carolina Press, Columbia, pp. 165-205.

Papendick, R.I. and Campbell, G.S., 1981. Theory and measurement of water potential. In: J.F. Pan, W.R. Gardner and L.F. Elliott (Editors), Water potential relations in soil microbiology. ASA Special Publ. No. 9, American Society of Agronomy, Madison, pp. 1-22.

Paul, J., 1982. Use of Hoechst dyes 33258 and 33342 for enumeration of attached and planktonic bacteria. Appl. Environ. Microbiol., 43: 939-944.

Postma, J. and Veen, J.A. van, 1990. Habitable pore space and population dynamics of *Rhizobium Leguminosarum Biovar Trifolii* introduced into soil. Microb. Ecol., 19: 149-161.

Ruiter, P.C. de, Moore, J.C., Zwart, K.B., Bouwman, L.A., Hassink, J., Bloem, J., Vos, J.A. de, Marinissen, J.C.Y., Didden, W.A.M., Lebbink, G. and Brussaard, L., 1993. Simulation of nitrogen mineralization in belowground food webs of two winter wheat fields. J. Appl. Ecol., 30: 95-106.

Schnürer, J., Clarholm, M. and Rosswall, T., 1985. Microbial biomass and activity in an agricultural soil with different organic matter contents. Soil Biol. Biochem., 17: 611-618.

Scholefield, D., Lockyer, P.R., Whitehead, D.C. and Tyson, K.C., 1991. A model to predict tranformations and losses of nitrogen in UK pastures grazed by beef cattle. Plant and Soil, 132: 165-177.

Sörensen, L.H., 1975. The influence of clay on the rate of decay of amino acid metabolites synthesized in soils during decomposition of cellulose. Soil Biol. Biochem., 7: 171-177.

Spain, A.V., 1990. Influence of environmental conditions and some soil chemical properties on the carbon and nitrogen contents of some tropical Australian Rainforest soils. Austr. J. Soil Res., 28: 825-839.

Tezuka, Y., 1990. Bacterial regeneration of ammonium and phosphate as affected by the carbon:nitrogen:phosphorous ratio of organic substrates. Microb. Ecol., 19: 353-361.

Tisdall, J.M. and Oades, J.M., 1982. Organic matter and water-stable aggegates in soils. J. Soil Sci., 33: 141-163.

Vargas, R. and Hattori, T., 1986. Protozoan predation of bacterial cells in soil aggregates. FEMS Microbiol. Ecol. 38: 233-242.

Veen, J.A. van and Paul, E.A., 1979. Conversion of biovolume measurements of soil organisms, grown under various moisture tensions, to biomass and their nutrient content. Appl. Environ. Microbiol., 37: 686-692.

Verberne, E.L.J., Hassink, J., Willigen, P. de, Groot, J.J.R. and Veen, J.A. van, 1990. Modelling organic matter dynamics in different soils. Neth. J. Agric. Sci., 38: 221-238.

CHAPTER 4

THE CAPACITY OF SOILS TO PRESERVE ORGANIC C AND N BY THEIR ASSOCIATION WITH CLAY AND SILT PARTICLES

By: J. Hassink. Submitted to Soil Science Society of America Journal.

THE CAPACITY OF SOILS TO PRESERVE ORGANIC C AND N BY THEIR ASSOCIATION WITH CLAY AND SILT PARTICLES

ABSTRACT

The aim of this study was to quantify the relationships between soil texture and the maximum amounts of C and N that can be preserved in the soil by their association with clay and silt particles (particle size fraction < 20 μm). To estimate the maximum amounts of C and N that can be associated with clay and silt particles we determined clay- and silt-associated C and N in old Dutch grassland soils and compared them with clay- and silt-associated C and N in uncultivated soils of temperate and tropical regions. We observed close positive relationships between the proportion of primary particles < 20 μm in a soil and the amounts of C and N that were associated with this fraction ($r^2 = 0.81$). The observed relationships were assumed to estimate the capacity of a soil to preserve C and N by their association with clay and silt particles. The observed relationships held for soils of the temperate and tropical regions. The only exception were Australian soils, which had lower amounts of C and N associated with clay and silt particles than other soils. The amount of C and N in the fraction > 20 μm was not correlated with soil texture. Cultivation decreased the amount of C and N in the fraction > 20 μm to a greater extent than C and N in the fraction < 20 μm, indicating that C and N associated with the fraction < 20 μm is better protected against decomposition. The consequences of the phenomenon that each soil has a maximum capacity to preserve organic C and N are discussed.

INTRODUCTION

Fine-textured soils have higher organic C and N contents than coarse-textured soils when supplied with similar input of organic material (Jenkinson, 1988; Amato and Ladd, 1992; Hassink, 1994; Hassink et al., 1995). The difference is assumed to result from the greater physical protection of soil organic matter in fine-textured soils (Jenkinson, 1988; Van Veen and Kuikman, 1990). Soil organic matter is physically protected by incorporation into microaggregates and adsorption on or coating by clay and silt particles which renders the organic matter to be unaccessible for microbial transformation (Tisdall and Oades, 1982; Golchin et al., 1994). In most physical fractionation studies, the distribution of C and N over primary particles, rather than over aggregate size classes, has been determined (Christensen, 1992). However, as microaggregates consist mainly of clay and silt particles (Skjemstad et al., 1993), the amounts of C and N associated with clay and silt particles should correspond with the amounts of C and N protected in microaggregates.

It has been observed that the C and N contents of clay and silt particles are much higher in sandy soils than in loams and clays (Christensen, 1992; Elustondo et al., 1990; Hassink et

al., 1995). It has been suggested that this is due to the fact that in sandy soils clay and silt particles are mainly present as individual particles, while in loams and clays the clay and silt particles are coagulated (Hassink et al., 1995). Therefore, less C can be adsorbed per unit of clay and silt mass in loams and clays than in sandy soils. It was also observed that the C content of the clay fraction was similar for grassland soils and arable soils in spite of their differences in total C content and that the C content of the clay fraction was lower in young soils (Hassink et al., 1995). In contrast to the amounts of C in the clay and silt fraction, the amounts of C and N in coarser size fractions depend primarily on the amount of residues incorporated into the soil and seem not to be affected by soil texture (Garwood et al., 1972; Christensen, 1992; Hassink, 1995). These observations suggest that the amount of C and N that can become associated with the clay and silt fraction is limited and is related to soil texture, whereas the amount of organic matter in larger size fractions is not.

Although it has been recognized that physical protection mechanisms are important determinants of the stability of organic matter in soil (Van Veen and Kuikman, 1990) and that there is more physical protection of organic matter in fine-textured soils than in coarse-textured soils (Jenkinson, 1988; Van Veen and Kuikman, 1990; Hassink et al., 1995), it has not been studied whether the capacity of soils to physically protect organic matter is limited and whether the capacity of soils to preserve organic matter can be quantified. The aim of the present paper is to determine the relationship between soil texture and the maximum amounts of organic C and N that can be preserved in the soil by their association with clay and silt particles. As the amounts of soil organic C and N are higher in old grassland and uncultivated soils than in cultivated arable soils (Stevenson, 1982; Jenkinson, 1988; Zhang et al., 1988; Feller et al., 1991; Bonde et al., 1992; Lugo and Brown, 1993), we assume that the relationship between soil texture and clay- and silt-associated C and N in old grassland and uncultivated soils represents the capacity of soils to preserve organic C and N.

MATERIALS AND METHODS

We sampled the top 10 cm of grassland soils which had been under grass for at least 30 years. The grasslands were grazed by dairy cattle and received 400-500 kg fertilizer-N per ha per year. At two locations we also sampled adjacent arable fields which had been under a 4-year rotation of winter wheat, sugar beet, barley and ware potatoes. Some characteristics of the soils are given in Table 1.

Three mixed samples, each consisting of 20 bulked cores, were taken from every location. The samples were sieved through a 0.008-m mesh screen; roots and stubble were removed and the samples were analyzed separately. Dry soil (50 g) was suspended in 250 ml water for 24 hours. The samples were treated ultrasonically for 15 minutes with a probe-type ultrasound generating unit (Soniprep 150). Probe output was calibrated by measuring the temperature produced upon ultrasonifying a known mass of water for a specified time (North, 1976). Output power assessed was 30 W. Heat build-up in the soil suspension was controlled by a

water cooling jacket. The dispersed soil suspension was transferred to a 1-l glass cylinder. The cylinder was shaken end over end until the soil was suspended. A table showing the settling-times for 20 μm particles at temperatures between 15 and 25°C was constructed by applying Stoke's law and a particle density of 2.675 g cm^{-3}. After the correct settling time, particles < 20 μm were isolated by siphoning the suspension at the appropriate depth. The fractions were dried for 24 hours at 105 °C. They were ground and analyzed for total C and N using a Carlo Erba NA 1500 analyzer. Particle size distribtion was determined after oxidation of organic matter with H_2O_2 and removal of $CaCO_3$ with HCl. The amounts of C and N associated with the particle size fraction < 20 μm were calculated by using the percentage of particles < 20 μm obtained after removal of organic matter and $CaCO_3$ and the C and N contents of the fraction < 20 μm obtained after sonification.

Table 1. Some characteristics of the top 10 cm of the Dutch grassland soils that were sampled.

Location	C (%)	C/N	pH (KCl)	Granular composition % particles <		
				2 μm	20 μm	50 μm
Grasslands						
Jubbenga	2.87	21.6	4.6	1.0	1.9	3.7
Heino 1	1.98	18.0	5.0	1.9	2.8	8.7
Holten	2.27	17.5	5.1	2.5	3.6	12.0
Tynaarlo	4.38	17.8	4.4	2.4	4.4	23.5
Achterberg	3.09	17.5	4.9	3.0	5.8	9.0
Markelo	3.91	17.2	5.2	3.4	5.4	12.4
Heino 2	4.68	15.6	5.2	5.6	8.9	29.7
Finsterwolde	5.37	10.9	5.0	8.4	13.3	23.2
Lelystad	3.07	11.1	7.1	21.6	35.6	56.4
Burum	5.37	9.8	4.8	24.1	36.5	71.7
Aduard	3.55	10.1	5.6	29.8	45.5	69.4
Finsterwolde	2.61	9.2	5.5	46.1	65.7	85.5
Zaltbommel 1	3.99	9.7	5.8	25.8	42.6	67.2
Zaltbommel 2	6.07	9.2	5.4	51.1	76.0	86.4
Arable land						
Tynaarlo	3.50	17.5	4.6	3.9	6.2	27.8
Lelystad	1.50	11.2	7.0	17.3	30.1	65.8

Total C in soil was defined as dichromate-oxidizable C according to Kurmies (Mebius, 1960). Total N was determined according to Deys (1961) by digestion with sulfuric acid and salycylic acid. The amounts of C and N not associated with clay and silt particles were

defined as total soil C and N minus C and N in the fraction < 20 μm.

We compared total soil C and N and the distribution of C and N over the fractions < 20 μm and > 20 μm of the grassland soils and arable soils with published results of corresponding measurements in uncultivated soils and in corresponding soils after different periods of conversion to cultivated arable land. We chose 20 μm as the upper size limit for the clay and silt fraction (instead of the previously used 50 μm; Hassink, 1994), because in most studies 20 μm was taken as the upper size limit. We found data of the top layer (approximately 10 cm) of the following uncultivated soils and cultivated grasslands: a grassland soil in Germany (Leinweber and Reuter, 1992), prairie soils in Canada (Elustondo et al., 1990) and North America (Tiessen and Stewart, 1983; Balesdent et al., 1988; Zhang et al., 1988), savanna and forest soils in Africa (Nigeria, Senegal, Togo and Ivory Coast; Bates, 1960; Feller et al., 1991), forest soils in Central and South America (Guadeloupe and Brasil; Feller et al., 1991; Bonde et al., 1992) and virgin soils in Australia (Turchenek and Oades, 1979; Dalal and Mayer, 1986, 1987). In most of these studies, total soil C and N and the amounts associated with the particle size fraction < 20 μm were determined both in grasslands and uncultivated soils and in adjacent soils with similar characteristics that had been cultivated and used as arable land for different periods of time (5-120 years).

Statistical analysis

The relationships between the percentage soil particles < 20 μm and total soil C and N, and C and N associated with the particle size fractions < 20 μm and > 20 μm were analyzed with correlation and regression techniques (Genstat, 1987).

RESULTS

Relationship between soil texture and total amounts of soil C and N in uncultivated and grassland soils

The total amounts of C and N in the top 10 cm of uncultivated and grassland soils ranged from 8 to 60 and 1 to 6.5 g kg^{-1} soil, respectively. There was no clear relationship between the C and N content of a soil and its clay and silt content (Fig. 1 and 2). C and N contents varied considerably between soils with similar clay and silt content and were generally higher in the Dutch grassland soils than in the uncultivated soils of North, Central and South America and Africa. Australian soils generally had the lowest C and N contents (Fig. 1 and 2).

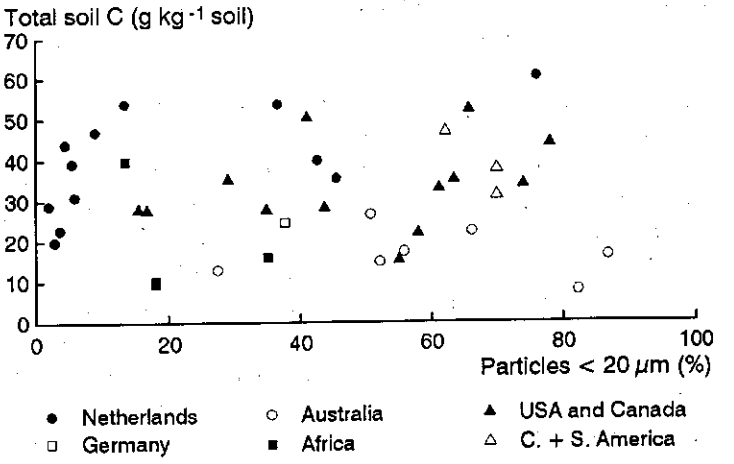


Fig. 1. Relationship between total soil C g kg^{-1} soil and the percentage soil particles < 20 µm in uncultivated and grassland soils of temperate and tropical regions.

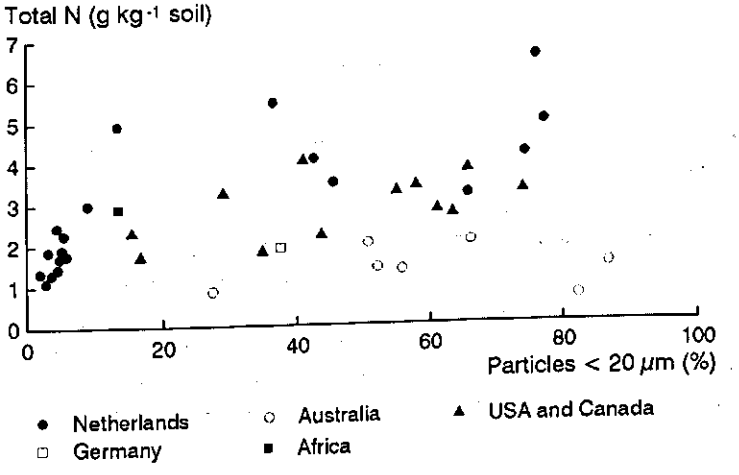


Fig. 2. Relationship between total soil N g kg^{-1} soil and the percentage soil particles < 20 µm in uncultivated and grassland soils of temperate and tropical regions.

Relationship between soil texture and the amounts of soil C and N associated with clay and silt particles in uncultivated and grassland soils

In contrast with total C and N, there were highly significant positive correlations between the clay and silt content of a soil and the amounts of C and N associated with this fraction (Fig. 3 and 4; C (g kg^{-1}) = 4.09 + 0.37 x % particles < 20 μm; N (g kg^{-1}) = 0.40 + 0.037 x % particles < 20 μm; excluding the soils from Australia). The relationship between the clay and silt content of a native soil and the amount of C and N associated with this fraction held for soils from the Netherlands, North, Central and South America, and Africa. The amount of C associated with the clay and silt fraction was less than 10 g kg^{-1} soil in coarse-textured soils and up to 30 g kg^{-1} soil in fine-textured soils. For N, the corresponding amounts were 10 times lower. Only the Australian soils showed different results; lower amounts of C and N were associated with the clay and silt fraction than in other uncultivated soils and no correlation between their clay and silt content and the associated amounts of C and N was found (Fig. 3 and 4).

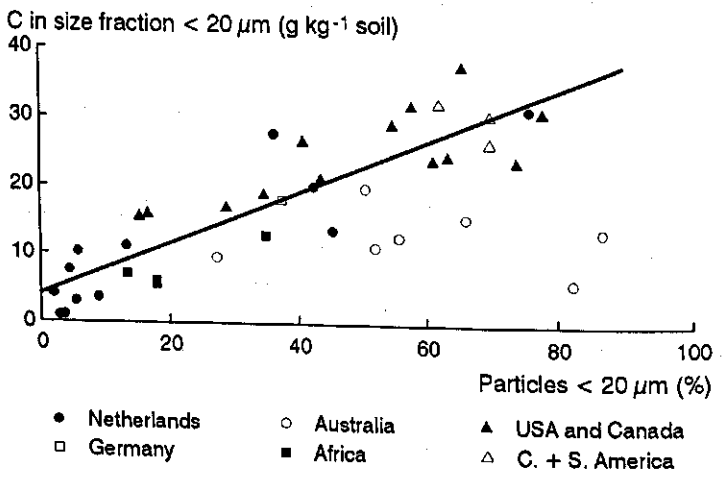


Fig. 3. Relationship between C in the particle size fraction < 20 μm (clay and silt in g kg^{-1} soil) and the percentage soil particles < 20 μm in uncultivated and grassland soils of temperate and tropical regions. C in fraction < 20 μm (g kg^{-1} soil, excluding Australian soils) = 4.09 (1.59) + 0.37 (0.04) x % particles < 20 μm (r^2 = 0.79; () = standard error of difference).

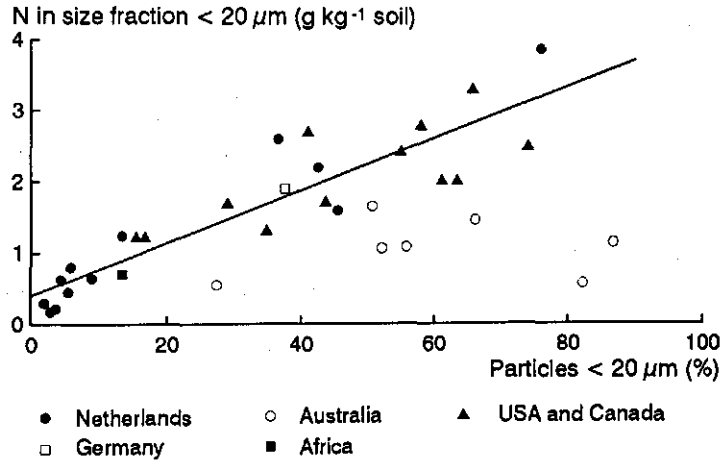


Fig. 4. Relationship between N in the particle size fraction < 20 μm (clay and silt in g kg^{-1} soil) and the percentage soil particles < 20 μm in uncultivated and grassland soils of temperate and tropical regions. N in fraction < 20 μm (g kg^{-1} soil, excluding Australian soils) = 0.40 (0.15) + 0.037 (0.004) x % particles < 20 μm. (r^2 = 0.81; () = standard error of difference)

Relationship between soil texture and the amounts of soil C and N in the fraction > 20 μm in uncultivated and grassland soils

The amounts of C and N in the fraction > 20 μm did not correlate with soil texture and varied considerably between soils with similar clay and silt content (Fig. 5 and 6). The amounts of C and N in the fraction > 20 μm were generally higher in the Dutch grassland soils (20-40 and 1-3 g kg^{-1} soil for C and N, respectively) than in the other soils (3-20 and 0.2-1.5 g kg^{-1} soil for C and N, respectively) and lowest in the soils from Australia (Fig. 5 and 6).

Effect of converting uncultivated and grassland soils to cultivated arable land on the amounts of C and N in the fractions < 20 μm and > 20 μm

The amounts of soil C and N were compared in pairs of soils with similar texture that had either been uncultivated or under grassland, or converted to cultivated arable land for different periods of time (5-120 years) in different experiments. Conversion to arable land generally led to a decrease in the amounts of soil C and N, and the relative decreases in soil C and N were comparable.

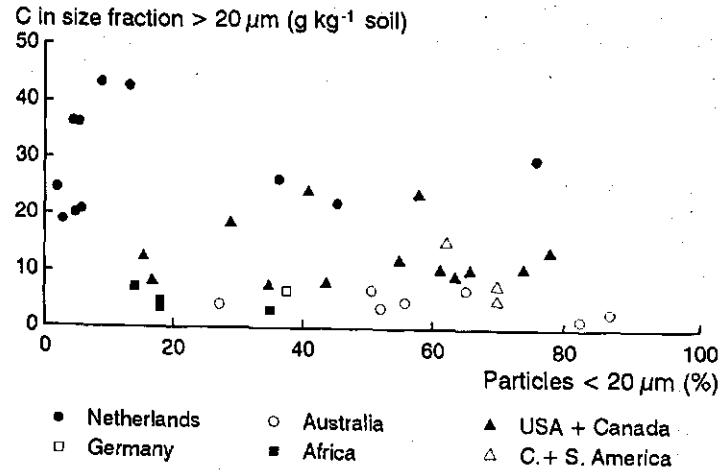


Fig. 5. Relationship between C in the particle size fraction > 20 μm (g kg^{-1} soil) and the percentage soil particles < 20 μm in uncultivated and grassland soils of temperate and tropical regions.

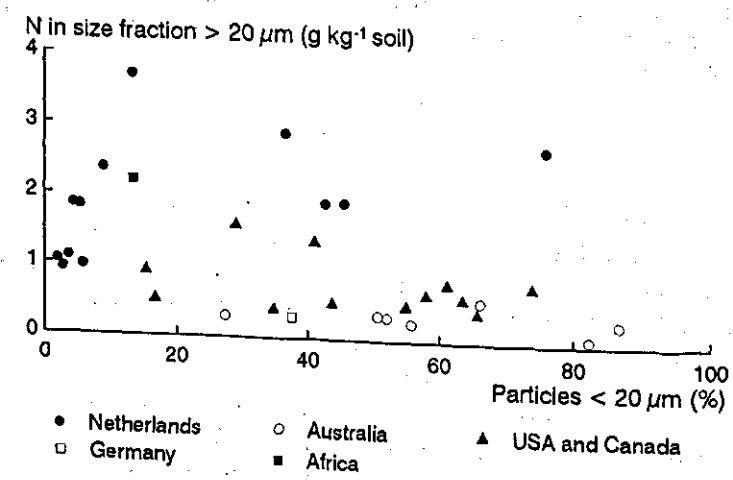


Fig. 6. Relationship between N in the particle size fraction > 20 μm (g kg^{-1} soil) and the percentage soil particles < 20 μm in uncultivated and grassland soils of temperate and tropical regions.

In more than 90% of the soils, the relative decrease in the amount of C or N associated with the fraction < 20 μm was smaller than the relative decrease in the amount of C or N in the fraction > 20 μm (Fig. 7). Cultivated arable soils generally contained less than 50% of the amount of C or N in the fraction > 20 μm of uncultivated and grassland soils; for the fraction < 20 μm the proportion of C or N left after conversion to arable land was generally more than 60%.

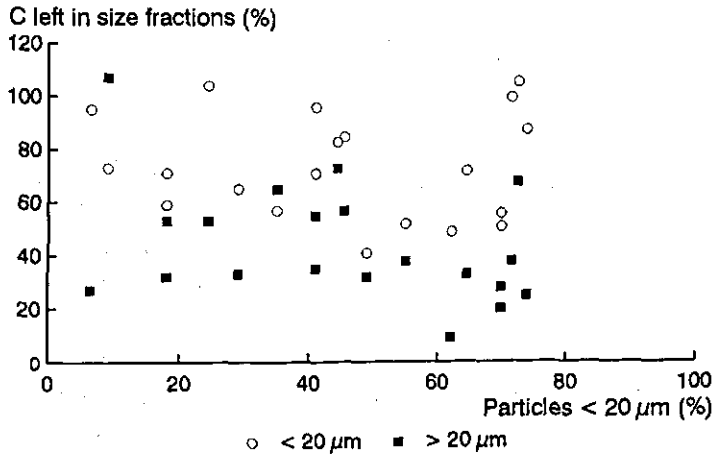


Fig. 7. Relationship between the percentage soil particles < 20 μm and the percentage soil C or N left in the particle size fractions < 20 μm and > 20 μm after conversion of grasslands or uncultivated soils to cultivated arable land.

DISCUSSION

The aim of this paper was to test whether there was an upper limit to the amounts of C and N that can become associated with the clay and silt fraction and to quantify the relationship between soil texture and the maximum capacity of a soil for C and N to be associated with clay and silt particles. Therefore, we determined clay- and silt-associated C and N in old grassland soils and compared that with clay- and silt-associated C and N in uncultivated soils. Grasslands and uncultivated soils generally have higher C and N contents than cultivated arable soils due to the higher incorporation of organic C and the absence of soil tillage (Jenkinson, 1988; Christensen, 1992; Lugo and Brown, 1993; Elliott et al., 1993). We assumed that in all old grassland and uncultivated soils the amounts of C and N were at equilibrium and maximal under the given conditions.

Despite the fact that the clay and silt particles in coarse-textured soils have much higher C and N contents than the clay and silt particles in fine-textured soils (Christensen, 1992; Hassink et al., 1995), fine-textured soils contain more C and N associated with clay and silt

particles than coarse-textured soils. This is due to the fact that the clay and silt content of fine-textured soils is so much higher than that of coarse-textured soils.

We found close correlations between the amounts of C and N associated with the clay and silt fraction and the percentage of soil particles in this size fraction. Although Dutch grassland soils contained relatively high amounts of total soil C and N, and C and N in the fraction > 20 μm, the amounts of C and N associated with the fraction < 20 μm were similar to those for the uncultivated soils. This suggests that the amounts of C and N that can become associated with the clay and silt fraction reached a maximum in all soils. The observation that the C content of clay particles was similar for grassland and arable soils, despite their differences in total C content (Hassink et al., 1995), is also in line with this assumption. Apparently, the input of organic C into the soil was high enough to saturate the clay particles under arable farming. The extra input of C under grassland management could not be bound to clay particles and accumulated in fractions with a greater particle size.

The observations are in agreement with the results of laboratory studies with pure clays, where it has also been found that the amount of organics that can be bound to clay particles is limited (Pinck et al., 1954; Harter and Stotzky, 1971; Marshman and Marshall, 1981). In those studies, the amount of organics that could be bound per amount of dispersed, pure clay was generally higher than the amount of organics bound per amount of clay and silt in our study. This is logical, as the clustering of clay packets into stable microaggregates in the soil leads to a reduction in the specific surface area of the clays (Stotzky, 1986).

There neither was a difference between the amounts of C and N that can be associated by clay and silt particles for soils from temperate and tropical regions. In uncultivated systems in the tropics the rate of input can be so high that the equilibrium level of organic matter is high despite the fact that the decomposition rate is also high (Greenland et al., 1992). The amounts of total soil C and N, and C and N bound to clay and silt particles were lower for most Australian soils than for other soils. We assume that this is due to the fact that crop growth and, as a consequence, the amount of residue incorporated into the soil is limited due to the very low annual precipitation (often less than 400 mm yr^{-1}) in large areas of Australia (Amato et al., 1985) and/or the deficiency of phosphate (Russell, 1960). The input of organic C and N was obviously too low to reach an equilibrium and saturation level of the clay and silt particles similar to those in other uncultivated soils.

The correlation between the percentage soil particles < 20 μm and the amounts of C and N associated with this fraction was very close, but it might even be improved when other soil characteristics are determined and included in the analysis. It is known that soil pH and clay type affect the amount of humus that can be associated with clay particles (Varadachari et al., 1994).

In agreement with the hypothesis, there was no correlation between the amounts of C and N in the fraction > 20 μm and soil texture. The amounts of C and N in coarser size fractions are related to the amount of residue that is incorporated into the soil and not to soil characteristics (Bonde et al., 1992; Hassink, 1995; Hassink et al., 1995). Due to the wide variation in the amounts of C and N in the fraction > 20 μm, correlations between total soil

C and N and the percentage particles < 20 µm were not clear.

The observation that the decrease in clay- and silt-associated C and N upon cultivation of soils was generally less than the decrease in C and N in the particle size fraction > 20 µm confirms that clay and silt particles protect C against microbial degradation. It is also in line with the general observation that in arable soils most of the soil organic matter is found in the clay and silt size fraction, whereas in forest and grassland soils the contribution of sand size organic matter to total soil organic matter is greater (Adams, 1980; Christensen, 1992).

The assumption that the protective capacity of a soil is limited suggests that the degree of saturation of the protective capacity of a soil affects the decomposition rate and stabilization of freshly applied substrate. We hypothesize that less of the applied C is preserved in the soil when all protective sites are occupied than when sites are available to physically protect organic C and N against microbial decomposition.

ACKNOWLEDGEMENTS

I thank L. Brussaard, H.G. van Faassen, P.J. Kuikman, J.J. Neeteson, P.C. de Ruiter and K.B. Zwart for critically reading and improving the manuscript and for helpful discussions.

REFERENCES

Adams, T.McM. 1980. Macro organic matter content of some Northern Ireland soils. Rec. Agric. Res. 28:1-11.

Amato, M., and J.N. Ladd. 1992. Decomposition of ^{14}C-labelled glucose and legume materials in soil: properties influencing the accumulation of organic residue C and microbial biomass C. Soil Biol. Biochem. 24:455-464.

Amato, M., J.N. Ladd, A. Ellington, G. Ford, J.E. Mahoney, A.C. Taylor, and D. Walsgott. 1985. Decomposition of plant material in Australian soils. IV Decomposition in situ of ^{14}C- and ^{15}N-labelled legume and wheat materials in a range of Southern Australian Soils. Aust. J. Soil Res. 23:603-611.

Balesdent, J., G.H. Wagner, and A. Mariotti. 1988. Soil organic matter turnover in long-term field experiments as revealed by carbon-13 natural abundance. Soil Sci. Soc. Am. J. 52:118-124.

Bates, J.A.R. 1960. Studies of a Nigerian forest soil. The distribution of organic matter in the profile and in various soil fractions. J. Soil Sci. 11:246-257.

Bonde, T.A., B.T. Christensen, and C.C. Cerri. 1992. Dynamics of soil organic matter as reflected by natural ^{13}C abundance in particle size fractions of forested and cultivated oxisols. Soil Biol. Biochem. 24:275-277.

Christensen, B.T. 1992. Physical fractionation of soil and organic matter in primary particle size and density separates. Adv. Soil Sci. 20:1-89.

Dalal, R.C., and R.J. Mayer. 1986. Long-term trends in fertility of soils under continuous cultivation and cereal cropping in Southern Queensland. III Distribution and kinetics of soil organic carbon in particle-size fractions. Aust. J. Soil Res. 24:281-292.

Dalal, R.C., and R.J. Mayer. 1987. Long-term trends in fertility of soils under continuous cultivation and cereal cropping in Southern Queensland. VI Loss of total nitrogen from different particle-size and density fractions. Aust. J. Soil Res. 25:83-93.

Deys, W. B. 1961. The determination of total nitrogen in herbage samples (in Dutch). Inst. Biol. Scheik. Onderz. Landbouwgewassen Wageningen Jaarb. Med. 89:90.

Elliott, E.T., C.A. Cambardella, and C.V. Cole. 1993. Modification of ecosystem processes by management and the mediation of soil organic matter dynamics. p. 257-268. *In* K. Mulongoy and R. Merckx (ed.) Soil organic matter dynamics and sustainability of tropical agriculture. John Wiley & Sons, New York.

Elustundo, J., D.A. Angers, M.R. Laverdiere, and A. N'Dayegamiye. 1990. Étude comparative de l'agrégation et de la matière organique associée aux fractions granulométriques de sept soils sous culture de maïs ou en prairie. Can. J. Soil Sci. 70:395-202.

Feller, C., E. Fritsch, R. Poss, and C. Valentin. 1991. Effet de la texture sur le stockage et la dynamique des matières organiques dans quelques sols ferrugineux et ferrallitiques. Cah. O.R.S.T.O.M. Ser. Pedol. 26:25-36.

Garwood, E.A., C.R. Clement, and T.E. Williams. 1972. Leys and soil organic matter. III. The accumulation of macro-organic matter in the soil under different swards. J. agric. Sci. Camb. 78:333-341.

Genstat Manual. 1987. A General Statistical Program. Clarendon, Oxford.

Golchin, A., J.M. Oades, J.O. Skjemstad, and P. Clarke. 1994. Soil structure and carbon cycling. Aust. J. Soil Res. 32:1043-1068.

Greenland, D.J, A. Wild, and D. Adams. 1992. Organic matter dynamics in soils of the tropics-from myth to complex reality. p. 17-33. *In* R. Lal and P.A. Sanchez (ed.) Myths and science of soils of the tropics. Proceedings of an international symposium. Am. Soc. Agron. and Soil Sci. Soc. of America, Madison, WI.

Harter, R.D., and G. Stotzky. 1971. Formation of clay-protein complexes. Soil Sci. Soc. Am. J. 35:383-389.

Hassink, J. 1994. Effects of soil texture and grassland management on soil organic C and N and rates of C and N mineralization. Soil Biol. Biochem. 26:1221-1231.

Hassink, J. 1995. Density fractions of soil macroorganic matter and microbial biomass as predictors of C and N mineralization. Subm. to Soil Biol. Biochem.

Hassink, J., F.J. Matus, C. Chenu, and J.W. Dalenberg. 1995. Interactions between soil biota, soil organic matter and soil structure. Adv. Agroecol. (accepted)

Jenkinson, D.S. 1988. Soil organic matter and its dynamics. p. 564-607. *In* A. Wild (ed.) Russel's soil conditions and plant growth, 11th ed., Ch. 18. Longman, New York.

Leinweber, P., and G. Reuter. 1992. The influence of different fertilization practices on concentrations of organic carbon and total nitrogen in particle-size fractions during 34 years of a soil formation experiment in loamy marl. Biol. Fert. Soils 13:119-124.

Lugo, A.E., and S. Brown. 1993. Management of tropical soils as sinks or sources of atmospheric carbon. Plant Soil 149:27-41.

Marshman, N.A., and K.C. Marshall. 1981. Bacterial growth on proteins in the presence of clay minerals. Soil Biol. Biochem. 13:127-134.

Mebius, L.J. 1960. A rapid method for the determination of organic carbon in soil. Anal. Chim. Acta 22:120-124.

North, P.F. 1976. Towards an absolute measurement of soil structural stability using ultrasound. J. Soil Sci. 27:451-459.

Pinck, L.A., R.S. Dyal, and F.E. Allison. 1954. Protein-montmorillonite complexed, their preparation and the effect of soil microorganisms on their decomposition. Soil Sci. 78:109-118.

Russell, J.S. 1960. Soil fertility changes in the long-term experimental plots at Kybybolite, South Australia. Aust. J. Agric. Sci. 11:902-926.

Skjemstad, J.O., L.J. Janik, M.J. Head, and S.G. McClure. 1993. High energy ultraviolet photo-oxidation: a novel technique for studying physically protected organic matterin clay- and silt-sized aggregates. J. Soil Sci. 44:485-499.

Stevenson, F.J. 1982. Humus chemistry. John Wiley & Sons, New York.

Stotzky, G. 1986. Influence of soil mineral colloids on metabolic processes, growth, adhesion, and ecology of microbes and viruses. p. 305-428. *In* P.M. Huang and M. Schnitzer (ed.) Interactions of soil minerals with natural organics and microbes. SSSA Spec. Publ. No. 17. Madison, WI.

Tiessen, H., and J.W.B Stewart. 1983. Particle-size fractions and their use in studies of soil organic matter. II. Cultivation effects on organic matter composition in size. Soil Sci. Soc. Am. J. 47:509-514.

Tisdall, J. M., and J. M. Oades. 1982. Organic matter and water stable aggregates in soils. J. Soil Sci. 13:141-163.

Turchenek, L.W., and J.M. Oades. 1979. Fractionation of organo-mineral complexes by sedimentation and density techniques. Geoderma 21:311-343.

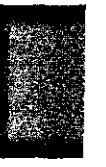
Varadachari, Ch., A.H. Mondal, D. C. Nayak, and K. Ghosh. 1994. Clay-humus complexation: effect of pH and the nature of bonding. Soil Biol. Biochem. 26:1145-1149.

Van Veen J.A., and P.J. Kuikman. 1990. Soil structural aspects of decomposition of organic matter by microorganisms. Biogeochemistry 11:213-233.

Zhang, J., M.L. Thompson, and J.A. Sandor. 1988. Compositional differences in organic matter among cultivated and uncultivated argiudolls and hapludalfs derived from loess. Soil Sci. Soc. Am. J. 52:216-222.

CHAPTER 5

THE DEGREE OF SATURATION OF THE PROTECTIVE CAPACITY OF A SOIL CONTROLS THE PRESERVATION OF ^{14}C LABELED RESIDUES



By: J. Hassink. Submitted to Soil Science Society of America Journal

THE DEGREE OF SATURATION OF THE PROTECTIVE CAPACITY OF A SOIL CONTROLS THE PRESERVATION OF ^{14}C-LABELED RESIDUES

ABSTRACT

The aim of the present study was to test the hypothesis that the decomposition of applied residue C in soil is not determined by soil texture *per se* but by the degree of saturation of the protective capacity of a soil. The protective capacity of a soil was defined as the maximum amount of clay- and silt-associated C (in grassland and uncultivated soils), which is closely correlated with the percentage particles < 20 µm. To test this hypothesis ^{14}C-labeled ryegrass was mixed through 11 soil samples differing in texture and in saturation deficit, i.e. the difference between the actual amount of clay- and silt-associated C and the maximum amount that can be associated with these particles. The percentage of applied ^{14}C that had respired after adding ^{14}C-labeled ryegrass was closely correlated (r = -0.85) with the saturation deficit after 3 days of incubation. After 53 days, differences in $^{14}CO_2$ production were small for sandy soils, but still closely correlated with the saturation deficit for loams and clays (r = -0.88). The correlation between $^{14}CO_2$ production and soil texture was much weaker. The amount of ^{14}C associated with the particle size fraction < 20 µm at the end of the incubation showed a significant correlation with the percentage particles < 20 µm and the saturation deficit. Our results confirm the hypothesis that the degree of saturation of the protective capacity of a soil predicts the decomposition rate of residue C much better than soil texture only.

INTRODUCTION

The preservation of ^{14}C-labeled organic residues in soil is generally positively correlated with its clay content (Ladd et al., 1981, 1985; Merckx et al., 1985; Van Veen et al., 1985; Jocteur Monrozier et al., 1991; Amato and Ladd, 1992). The correlation is assumed to result from the greater physical protection of soil organic matter in fine-textured soils than in coarse-textured soils (Jenkinson, 1988; Van Veen and Kuikman, 1990; Hassink et al., 1995). Soil organic matter is generally physically protected by adsorption on or coating by clay and silt particles in microaggregates (Tisdall and Oades, 1982; Skjemstad et al., 1993). Differences in the decomposition of ^{14}C labeled residues have often been established during the first weeks after application (Ladd et al., 1992; Van Veen et al., 1985). Amato and Ladd (1992) found that the differences in the amount of residual ^{14}C in the soil were entirely due to differences in the amount of ^{14}C present in the microbial biomass.

In spite of this general trend, the preservation of ^{14}C-labeled residues has not always been found to be higher in fine-textured soils than in coarse-textured soils (Sörensen, 1975;

Gregorich et al., 1991; Bremer and Kuikman, 1994).

Hassink (1995) hypothesized that the capacity of a soil to physically preserve organic matter is limited and that the maximum can be reached in soils that have been uncultivated or under grass for at least 30 years, i.e. assuming that in these types of soils the amounts of clay- and silt-associated C and N reached the maximum. The amounts of C and N that were associated with clay and silt particles in these soils were highly correlated with the proportion of clay and silt particles in the soil. From the observed relationship between C in the clay- and silt-size fraction and the percentage of soil particles < 20 μm in uncultivated and grassland soils, the protective capacity of a soil was defined as: C_{max} in fraction < 20 μm (g kg^{-1} soil) = 4.09 + 0.37 x % particles < 20 μm. Soils that contain the maximum amount of clay- and silt-associated C, as predicted by the equation, are assumed to be saturated; soils with lower amounts of clay- and silt-associated C are assumed not to be saturated.

The degree of saturation of the protective capacity of a soil might be an important factor in the preservation of freshly applied residues in the soil (Hassink, 1995). Less of the applied C should be preserved in the soil when all protective sites are occupied than when sites are available to physically protect organic C against microbial decomposition. When applied C is preserved by its association with clay and silt particles, it is expected that differences in the amounts of applied C that are preserved in the soil are due to differences in the amounts of applied C that are associated with clay and silt and not due to differences in the amounts of applied C that are preserved in the microbial biomass as was found by Amato and Ladd (1992).

Amato and Ladd (1992) and Gregorich et al. (1991) determined the decomposition of ^{14}C-labeled glucose in a wide range of Australian and Canadian soils, respectively. Amato and Ladd (1992) found that fine-textured soils preserved more ^{14}C than coarse-textured soils, while Gregorich et al. (1991) did not find a relationship with soil texture. Gregorich et al. (1991) observed a close correlation between the clay content of the soil and its organic C content. The linear relationship between clay content and total organic C suggests that the protective capacity of these Canadian soils was saturated to the same extent in all soils. The coarse-textured soils of Amato and Ladd (1992) had similar organic C contents as the coarse-textured soils studied by Gregorich et al. Their fine-textured soils, however, had considerably lower organic C contents than would have been expected assuming a linear relationship between clay content and protective capacity of a soil (Hassink et al., 1995). In all cited experiments where the preservation of ^{14}C-labeled material in soil was positively correlated with its clay content Australian soils were used (Ladd et al., 1981, 1985; Merckx et al., 1985; Van Veen et al., 1985; Jocteur Monrozier et al., 1991; Amato and Ladd, 1992), whereas in the cited experiments where soil texture did not affect the preservation of ^{14}C, soils from other continents were used (Sörensen, 1975; Gregorich et al., 1991; Bremer and Kuikman, 1994). In line with this Hassink (1995) observed that in fine-textured uncultivated Australian soils less C and N was associated with clay and silt particles than in corresponding soils from other continents, suggesting that, unlike other uncultivated soils, the protective capacity of the

Australian soils was not saturated. These results support the assumption that in the studies where no effect of soil texture on the preservation of residue C in soil was found the protective capacity of fine-textured soils was saturated, whereas in studies where the preservation of residue C in soil increased with the clay content of the soil the protective capacity of fine-textured soils was not saturated.

The general aim of the present study was to test the hypothesis that the degree of saturation of the protective capacity of a soil determines the decomposition rate of applied residue C. We also tested whether differences in the amounts of ^{14}C that are preserved in the soil are due to differences in the amounts of ^{14}C preserved in the microbial biomass (Amato and Ladd, 1992) or due to differences in the amounts of clay- and silt-associated ^{14}C. We determined the decomposition of ^{14}C-labeled ryegrass in the soil after mixing it through soil samples that differed in soil texture and amounts of clay- and silt-associated C.

MATERIALS AND METHODS

General experimental setup

Seventy-five mg dried uniformly labeled ryegrass with a specific activity of 546 Bq mg^{-1} C was cut into pieces of approximately 1 cm length (Hassink and Dalenberg, 1995) and was mixed through 75 g of each of 11 samples taken from fine- or coarse-textured soils that differed in organic C content. The differences in organic C contents between the samples were due to differences in history, organic matter input and sampling depth. Samples 1 to 4 were taken from the top 25 cm of a sandy and clay soil that had been kept bare for 30 years. Samples 1 and 3 had received no organic C, while samples 2 and 4 had received 10 t C per ha annually by the application of farmyard manure. The other samples were taken from the top 10 cm (5, 6, 8 and 10) and the 10-25 cm depth layer (7, 9 and 11) of grasslands. Samples 10 and 11 were taken from a grassland soil that was reclaimed from the sea in 1957 and was expected to have a lower organic C content than other grassland soils with the same texture (Hassink, 1994). Characteristics of the samples are given in Table 1.

Grass was mixed through samples (triplicate) and they were incubated at a water potential close to -10 kPa at 20 °C. The samples were incubated in 1.5-l airtight jars containing a vial of 10 ml 0.5 M NaOH. The trapped CO_2 was precipitated as carbonate with excess $BaCl_2$, the excess NaOH was titrated with 0.5M HCl and the soil samples were incubated with a new vial of NaOH at 3, 10, 17, 32 and 53 days after the start of the incubation (Hassink, 1994). For determination of $^{14}CO_2$, a 1 ml aliquot of the NaOH-$Na_2{}^{14}CO_3$ solution was diluted with 1 ml water in a glass scintillation vial with 10 ml of a scintillation cocktail (Insta-Gel, Packard Instruments Company). Samples were counted in a liquid scintillation counter Rackbeta II 1215, Wallac.

At the beginning of the incubation total organic C and microbial biomass C were

determined in unamended triplicate samples. Soil organic C was defined as dichromate-oxidizable C according to Kurmies (Mebius, 1960). The microbial biomass was determined by the fumigation extraction method (Vance et al., 1987) using a k_{EC} value of 0.45 (Wu et al., 1990).

At the end of the incubation the percentages of applied ^{14}C that were present in the microbial biomass and in the clay and silt fraction (< 20 μm) were determined. ^{14}C in microbial biomass was determined after mixing 0.4 ml of the fumigated and non-fumigated 0.5 M K_2SO_4 solutions with 1.6 ml water and 12 ml of the scintillation cocktail. The fraction < 20 μm was determined after treating the samples ultrasonically for 15 minutes with a probe-type ultrasound generating unit. The dispersed soil suspension was shaken end over end and Stoke's law was applied to calculate the correct settling time when particles < 20 μm were isolated by siphoning the suspension at the appropriate depth (Hassink, 1995); ^{14}C in the clay and silt fraction was determined after destruction with a mixture of bichromate and sulfuric acid at 160 °C and capturing the produced CO_2 in NaOH solution (Hassink and Dalenberg, 1995).

Table 1. Some characteristics of the soil samples that were used for incubation.

No.	Granular composition, % particles <			pH-KCl	C (%)	Microbial biomass (g kg^{-1} soil)	C in particle size fraction < 20 μm (g kg^{-1} soil)
	2 μm	20 μm	50 μm				
1	9	17	26	7.0	0.49	0.010	5.8
2	9	17	26	7.0	2.81	0.184	12.8
3	45	66	72	7.0	1.13	0.087	10.2
4	45	66	72	7.0	4.00	0.317	27.6
5	2	8	10	5.5	1.53	0.136	7.0
6	3	6	9	4.9	2.40	0.442	6.8
7	3	6	9	4.9	1.55	0.101	6.0
8	24	37	72	4.8	4.30	0.770	21.6
9	24	37	72	4.8	3.00	0.245	8.8
10	23	38	72	7.0	3.40	0.317	13.0
11	23	38	72	7.0	3.00	0.133	11.9

Equation to estimate the protective capacity of a soil

Hassink (1995) found that the capacity of a soil to physically preserve organic C by its association with clay and silt particles (protective capacity) is linearly related to the clay and silt content of a soil: protective capacity (g C kg^{-1} soil) = 4.09 + 0.37 x % particles < 20 μm.

The maximum amount of C that can be associated with clay and silt particles minus the

actual amount of clay- and silt-associated C is called saturation deficit. Figure 1 shows the saturation deficit of the different soil samples. Three samples contained more clay- and silt-associated C than the calculated amount for saturation conditions. These differences were within the standard error of the equation describing saturation conditions.

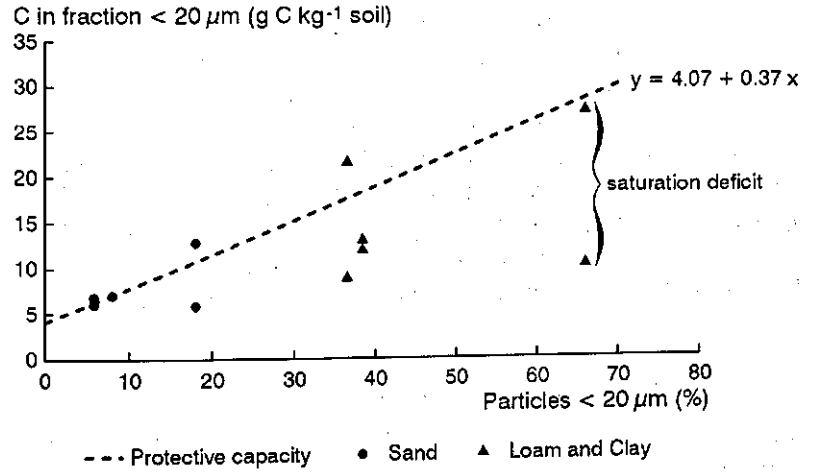


Fig. 1. The saturation deficit of the soils, i.e. the difference between the actual amount of C in the particle size fraction < 20 μm and the maximum amount that can be associated with this particle size fraction (protective capacity in g C kg^{-1} soil; see text). The protective capacity was determined with independent data (Hassink, 1995).

Statistical analysis

The relationships between $^{14}CO_2$ production and soil characteristics, and between soil characteristics and the distribution of ^{14}C in the soil were analyzed with correlation and stepwise multiple regression techniques (Genstat, 1987).

RESULTS

Correlation of $^{14}CO_2$ with soil characteristics

After 3 days of incubation significant differences in $^{14}CO_2$ production were found. The percentages of applied ^{14}C that were respired to $^{14}CO_2$ ranged from 15 to 27% (Fig. 2). We found a close negative correlation between produced $^{14}CO_2$ and the saturation deficit ($r = -0.85$; Fig. 2, Table 2). When the clay soil with the high saturation deficit was excluded from

the analysis, the correlation between saturation deficit and produced $^{14}CO_2$ was still close ($r = -0.76$). The correlation between $^{14}CO_2$ production and soil texture (percentage particles < 20 µm) was weak (Table 2). $^{14}CO_2$ was not significantly correlated with total soil C or microbial biomass.

The differences in $^{14}CO_2$ production between the soils that developed during the first 3 days remained roughly the same during the incubation. For the sandy soils the differences in $^{14}CO_2$ production during 53 days were small (respiration ranging from 68.4 to 73.5% of applied ^{14}C) and were not significantly correlated with the saturation deficit; for the loams and clays, however, differences in $^{14}CO_2$ production were considerable (respiration ranging from 56 to 68% of applied ^{14}C) and still showed a significant correlation with the saturation deficit (Table 2; Fig. 3). $^{14}CO_2$ production still had the closest correlation with the saturation deficit ($r = -0.84$; Fig. 3). When the clay soil with the high saturation deficit was excluded from the analysis, the correlation between saturation deficit and produced $^{14}CO_2$ was still close ($r = -0.74$).

The correlations between the saturation deficit and $^{14}CO_2$ were not significantly ($p < 0.05$) different when the saturation deficit was defined as a percentage instead of an absolute amount.

Table 2. Correlation coefficients between soil characteristics and the percentage ^{14}C respired after 3 and 53 days and the equations giving the best estimations of $^{14}CO_2$ production.

	0-3 days	0-53 days
Saturation deficit		
all soils	-0.85	-0.84
clay soils only	-0.87	-0.88
sandy soils only	-0.68	-0.57
Organic C	0.27	0.03
Microbial biomass	0.28	0.25
% clay + silt	-0.45	-0.71

% ^{14}C respired 0-3 d = 22.6 (0.64) - 0.4477 (0.0914) x saturation deficit (g C kg^{-1} soil)
% ^{14}C respired 0-53 d = 68.7 (1.05) - 0.701 (0.149) x saturation deficit (g C kg^{-1} soil)

() = standard error of difference

Distribution of ^{14}C in the soil at the end of the incubation

The percentage of applied ^{14}C found in the microbial biomass at the end of the incubation ranged from 8 to 13% and was not significantly correlated with soil texture, residual soil ^{14}C or the saturation deficit.

The percentage of applied ^{14}C that was recovered in the clay and silt fraction was closely correlated with residual soil ^{14}C after 53 days of incubation, the saturation deficit and the

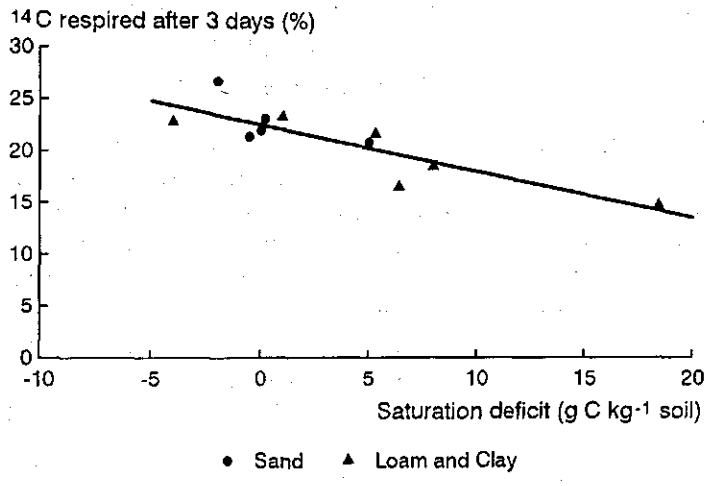


Fig. 2. Relationship between the percentage applied ^{14}C respired to $^{14}CO_2$ after 3 days of incubation and the saturation deficit of a soil (g C kg^{-1} soil). (% ^{14}C respired = 22.6 (0.64) - 0.4477 (0.0914) x saturation deficit; () = standard error of difference)

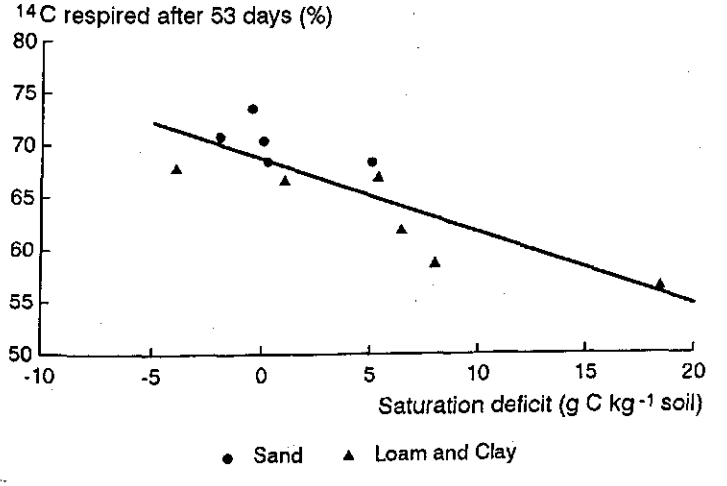


Fig. 3. Relationship between the percentage applied ^{14}C respired to $^{14}CO_2$ after 53 days of incubation and the saturation deficit of a soil (g C kg^{-1} soil). (% ^{14}C respired = 68.7 (1.05) - 0.701 (0.149) x saturation deficit; () = standard error of difference)

percentage soil particle < 20 µm (Table 3). The equation describing the best relationship between ^{14}C in the fraction < 20 µm and soil characteristics was: % of applied ^{14}C associated with the fraction < 20 um = 6.72 (2.1) + 0.23(0.07) x % of soil particles < 20 um + 0.67 (0.23) x the saturation deficit (explaining 81.3% of the variation).

Table 3. Correlation coefficients between soil characteristics and the percentage ^{14}C associated with the particle size fraction < 20 µm after 53 days, and the equation giving the best estimation of ^{14}C in the particle size fraction < 20 µm.

Residual ^{14}C in soil	0.80
Saturation deficit	0.80
Total C	0.25
Total microbial biomass	0.22
% clay + silt	0.84

% ^{14}C in fraction < 20 µm = 6.72 (2.1) + 0.23(0.07) * % < 20 µm + 0.67 (0.23) x saturation deficit (g C kg^{-1} soil)

() = standard error of difference

DISCUSSION

The aim of the present study was to test the hypothesis that the degree of saturation of the protective capacity of a soil determines the preservation of applied residue C in soil instead of soil texture *per se*. The hypothesis could generally be confirmed. During the first 3 days after the application of ^{14}C, considerable differences in $^{14}CO_2$ production between soils were found that were significantly correlated with the saturation deficit, while the correlation with soil texture was much weaker. The correlation between $^{14}CO_2$ production and the saturation deficit was weaker for the coarse-textured soils than for the fine-textured soils. This is not because coarse-textured soils are principally different from fine-textured soils, but probably because the range in saturation deficit was smaller for coarse-textured soils.

In agreement with other studies, differences in $^{14}CO_2$ evolution established during the first weeks after application of residues were maintained throughout the incubation period (Van Veen et al., 1985; Ladd et al., 1992). During later periods of incubation, the correlation of $^{14}CO_2$ production and soil texture increased, while the correlation with the saturation deficit remained the same. After 53 days, fine-textured soils tended to preserve more residue C in the soil than coarse-textured soils, but the availability of protective sites on clay and silt particles for the binding of C was still the dominant factor, leading to considerable differences in preserved ^{14}C between the fine-textured soils.

According to Amato and Ladd (1992) residual ^{14}C is highly correlated with soil clay

content (when excluding soils with a low pH) after the application of ^{14}C glucose and plant material. Analysis of their results indicated that residual soil ^{14}C was also closely correlated with the saturation deficit (Hassink et al., 1995). The results suggest that in experiments where no effect of soil texture on residual ^{14}C in soil was found the protective capacity of fine-textured soils was saturated, whereas in experiments where residual ^{14}C in the soil was positively correlated with its clay content, the protective capacity of fine-textured soils was not saturated.

Our results are also in agreement with laboratory studies where proteins were added to pure clays. In those studies it was found that the decomposition rate of protein depended on the protein-to-clay ratio: with increasing protein-to-clay ratio a lower percentage of the protein was physically protected against microbial decomposition and a higher percentage was decomposed (Pinck et al., 1954; Marshman and Marshall, 1981).

Amato and Ladd (1992) and Ladd et al. (1992) observed that differences in residual soil ^{14}C were entirely due to differences in the amounts of ^{14}C in the microbial biomass, suggesting that microbial biomass was stabilized rather than products of cell death and metabolism (Ladd et al., 1992). In the present study differences in residual soil ^{14}C were not correlated with microbial ^{14}C, but with ^{14}C in the clay- and silt-size fraction. Amato and Ladd (1992) determined microbial ^{14}C 44 and 66 weeks after the application of ^{14}C, while in the present study microbial and clay- and silt-associated ^{14}C were determined 53 days after the application of ^{14}C. The first weeks after the application of ^{14}C, most of it is soluble or in the sand size fraction and not associated with clay and silt particles (Nicolardot et al., 1992; Hassink and Dalenberg, 1995). Newly synthesized microbial biomass is mainly feeding on these labeled soluble and sand size fractions. After longer periods of incubation, most of the preserved ^{14}C and microbial ^{14}C becomes associated with clay and silt particles (Hassink and Dalenberg, 1995). After 53 days the microbial ^{14}C may still partly consist of newly synthesized, not yet stabilized microbial ^{14}C, and microbial ^{14}C that is associated with clay and silt particles. This might be the cause of the absence of a correlation between preserved ^{14}C and microbial ^{14}C. Amato and Ladd (1992) did not determine clay- and silt-associated ^{14}C. As after 44 and 66 weeks almost all ^{14}C is associated with clay and silt particles (Nicolardot et al., 1992) it may be assumed that microbial ^{14}C is also associated with clay and silt particles. So the observed differences in the amounts of microbial ^{14}C between soils in the experiment of Amato and Ladd (1992) are probably a reflection of differences in clay- and silt-associated ^{14}C.

The amount of ^{14}C associated with the clay- and silt-size fraction was positively correlated with both the clay and silt content and the saturation deficit of the soil. This is in line with observations that organic matter is generally protected by adsorption of organics on or coating by clay and silt particles in microaggregates (Tisdall and Oades, 1982; Skjemstad et al., 1993) and that the amount of residue C that can become associated with clay and silt particles is determined by the degree of saturation of the clay and silt particles with organic matter.

Conclusions and suggestions for further research

This experiment showed that the saturation deficit of a soil is an important factor in the decomposition rate of ryegrass ^{14}C. Further research, including microscopic observations and determination of the specific surface of clay and silt particles, should increase our understanding of the protective capacity of a soil. Further research on a wider range of soils and with different types of residues, including ^{15}N-labeled residues, will indicate whether the phenomenon that the saturation deficit determines the decomposition rate of applied residues in soil is generally valid and whether the saturation deficit also determines the release of N from residues. Research should also be directed to the development of organic matter models that include the protective capacity of soils.

ACKNOWLEDGEMENTS

I thank J.W. Dalenberg and A. Lahr for their skillful technical assistance and L. Brussaard, H.G. van Faassen, P.J. Kuikman, J.J. Neeteson, P.C. de Ruiter and K.B. Zwart for their useful suggestions to improve the manuscript.

REFERENCES

Amato, M., and J.N. Ladd. 1992. Decomposition of ^{14}C-labeled glucose and legume material in soils: properties influencing the accumulation of organic residue C and microbial biomass C. Soil Biol. Biochem. 24:455-464.

Bremer, E., and P. Kuikman. 1994. Microbial utilization of ^{14}C[U]glucose in soil is affected by the amount and timing of glucose additions. Soil Biol. Biochem. 26:511-517.

Genstat Manual. 1987. Genstat. A General Statistical Program. Clarendon, Oxford.

Gregorich, E.G., R.P. Voroney, and R.G. Kachanoski. 1991. Turnover of carbon through the microbial biomass in soils with different textures. Soil Biol. Biochem. 23:799-805.

Hassink, J. 1994. Effects of soil texture and grassland management on soil organic C and N and rates of C and N mineralization. Soil Biol. Biochem. 26:1221-1231.

Hassink, J. 1995. The capacity of soils to preserve organic C and N by their association with clay and silt particles. Subm. to Soil Sci. Soc. Am. J.

Hassink, J., and J.W. Dalenberg. 1995. Decomposition and transfer of plant residue ^{14}C between size and density fractions in soil. Subm. to Soil Biol. Biochem.

Hassink, J., F.J. Matus, C. Chenu, and J.W. Dalenberg. 1995. Interactions between soil biota, soil organic matter and soil structure. Accepted by Adv. Agroecology.

Jenkinson, D.S. 1988. Soil organic matter and its dynamics. p. 564-607. *In*: A. Wild (ed.)

Russel's soil conditions and plant growth, 11th ed. Longman, New York.

Jocteur Monrozier, L., J.N. Ladd, R.W. Fitzpatrich, R.C. Foster, and M. Raupach. 1991. Components and microbial biomass content of size fractions in soils of contrasting aggregation. Geoderma 49:37-62.

Ladd, J.N., J.M. Oades, and M. Amato. 1981. Microbial biomass formed from ^{14}C, ^{15}N-labeled plant material decomposing in soils in the field. Soil Biol. Biochem. 13:119-126.

Ladd, J.N., M. Amato., and J.M. Oades. 1985. Decomposition of plant materials in Australian soils III Residual organic and microbial biomass C and N from isotope-labeled plant material and soil organic matter decomposing under field conditions. Austr. J. Soil Res. 23:603-611.

Ladd, J.N., L. Jocteur-Monrozier, and M. Amato. 1992. Carbon turnover in [^{14}C]glucose/^{15}N ammonium amended soils of contrasting aggregate stabilities. Soil Biol. Biochem. 24:359-371.

Marshman, N.A., and K.C. Marshall. 1981. Bacterial growth on proteins in the presence of clay minerals. Soil Biol. Biochem. 13:127-134.

Mebius, L.J. 1960. A rapid method for the determination of organic carbon in soil. Anal. Chim. Acta 22:120-124.

Merckx, R., A. Den Hartog, and J.A. Van Veen. 1985. Turnover of root-derived material and related microbial biomass formation in soils of different texture. Soil Biol. Biochem. 17:565-569.

Nicolardot, B., D. Cheneby, and M.R. Allard. 1991. Availability of carbon and nitrogen contained in different soil particle-size fractions. p. 107-116. *In* J. Kubat (ed.) Humus, its structure and role in agriculture and environment. Elsevier, Amsterdam.

Pinck, L.A., R.S. Dyal, and F.E. Allison. 1954. Protein-montmorillonite complexes, their preparation and the effects of soil microorganisms on their decomposition. Soil Sci. 78:109-118.

Skjemstad, J.O., L.J. Janik, M.J. Head, and S.G. McClure. 1993. High energy ultraviolet photo-oxidation: a novel technique for studying physically protected organic matter in clay- and silt-sized aggregates. J. Soil Sci. 44:485-499.

Sörensen, L.H. 1975. The influence of clay on the rate of decay of amino acid metabolites synthesized in soils during decomposition of cellulose. Soil Biol. Biochem. 7:171-177.

Tisdall, J.M., and J.M. Oades. 1982. Organic matter and water-stable aggregates in soils. J. Soil Sci. 33:141-163.

Vance, E.D., P.C. Brookes, and D.S. Jenkinson. 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19: 703-707.

Van Veen, J.A., and P.J. Kuikman. 1990. Soil structural aspects of decomposition of organic matter by microorganisms. Biogeochemistry 11:213-233.

Van Veen, J.A., J.N. Ladd, and M. Amato. 1985. Turnover of carbon and nitrogen through the microbial biomass in a sandy loam and a clay soil incubated with [^{14}C(U)]glucose and [^{15}N]$(NH_4)_2SO_4$ under different moisture regimes. Soil Biol. Biochem. 17:257-274.

Wu, J., R.G. Joergensen, B. Pommerening, R. Chaussod, and P.C. Brookes. 1990. Measurement of soil microbial biomass C by fumigation-extraction-an automated procedure. Soil Biol. Biochem. 22:1167-1169.

CHAPTER 6

C AND N MINERALIZATION IN SANDY AND LOAMY GRASSLAND SOILS: THE ROLE OF MICROBES AND MICROFAUNA

By: J. Hassink, A. Neutel and P.C. de Ruiter. 1994. Soil Biology & Biochemistry 26, 1565-1571.

C AND N MINERALIZATION IN SANDY AND LOAMY GRASSLAND SOILS: THE ROLE OF MICROBES AND MICROFAUNA

ABSTRACT

A food web model was used to evaluate the role of soil microorganisms, protozoa and nematodes and their interactions on carbon (C) and nitrogen (N) mineralization in sandy and loamy grassland soils. The differences in C mineralization between the sites did not correspond with the differences in N mineralization. To analyze this discrepancy between the mineralization patterns, C and N mineralization rates were calculated using the observed densities of soil organisms. The C mineralization pattern could be explained from differences in the bacterial biomass estimated on each site. Food web calculations carried out to explain the discrepancy between C and N mineralization patterns indicated that faunal activity played a minor role but that the observed C and N mineralization rates could satisfactorily be calculated using different bacterial C:N ratios as determined for the soil types (8 for the sandy soils; 4.5 and 6 for the loams).

INTRODUCTION

It has been observed frequently that N mineralization is lower in fine-textured soils than in coarse-textured soils (Catroux *et al.*, 1987; Van Veen *et al.*, 1985; Hassink, 1994[a]), while for C mineralization these differences between fine- and coarse-textured soils were not found (Hassink, 1994[a]).

Pore size distribution seems to affect the biomass and functioning of bacteria and the soil fauna (Hassink *et al.*, 1993[a]). In coarse-textured soils the grazing pressure of bacterivorous nematodes and flagellates on bacteria was higher than in fine-textured soils. The higher grazing pressure per bacterium coincided with a higher rate of N mineralization per bacterium (Hassink *et al.*, 1993[a]), while no correlation between grazing pressure and CO_2 production was found.

Soil fauna may significantly increase N mineralization (Coleman *et al.*, 1978; Coleman *et al.*, 1983), since 1) the C:N ratios of the soil fauna are usually high compared to the C:N ratio of their food, whereas for microbes the opposite may be the case; excess N will be excreted by the grazers as NH_4^+ (Hunt *et al.*, 1987), and 2) the soil fauna may stimulate microbial growth rates through grazing (Clarholm, 1985; Coleman *et al.*, 1978; Woods *et al.*, 1982); this second mechanism would also stimulate C mineralization by microbes.

An important factor determining the rate of N mineralization is the C:N ratio of the microbes and that of their substrate. When bacteria decompose organic matter, more inorganic N is released from the organic matter when the bacteria have a higher C:N ratio, while C mineralization is not affected by the C:N ratio of the bacteria (Hunt *et al.*, 1987; De Ruiter

et al., 1993[a]). In a comparison of six grassland soils it was found that the C:N ratio of the microbial biomass was higher in sandy soils than in loams and clays and was positively correlated with the N mineralization rate per unit of microbial biomass N (Hassink *et al.*, 1993[b]).

Hassink (1994[a]) studied C and N mineralization rates in a series of sandy, loamy and clayey grassland soils. Some of the soils were analyzed with respect to the abundance of the soil microbes and soil fauna (Hassink *et al.*, 1993[a,b]). In the present study we evaluated whether differences in grazing by the soil fauna on microbes and the C:N ratio of the bacteria may explain the observed differences in C and N mineralization between two sandy soils (Heino and Tynaarlo) and two loams (Swifterbant and Burum).

MATERIALS AND METHODS

Field description and soil sampling

The top 10 cm of two sandy soils (Heino and Tynaarlo) and two loams (Swifterbant and Burum) were sampled in October 1990 and May 1991. The sites were intensively managed grasslands, grazed by dairy cows. They had been managed as grassland for at least eight years prior to sampling. Some characteristics of the soils are given in Table 1. Three mixed samples each consisting of 20 bulked cores were taken from each location at each sampling date. The samples were sieved through a 0.008-m mesh screen; roots and stubble were removed and the samples were analyzed separately.

Table 1. Soil characteristics of the grassland soils studied.

Location	Soil type	Org. C (%)	Org. N (%)	pH-K-Cl	Granular composition (% particles <)		
					2 µm	16 µm	50 µm
Heino	Sand	1.98	0.11	5.0	1.9	2.8	8.7
Tynaarlo	Sand	4.38	0.25	4.4	2.4	4.4	23.5
Swifterbant	Loam	1.77	0.15	7.0	23.0	38.4	72.2
Burum	Loam	5.37	0.55	4.8	24.1	36.5	71.7

Measurements

The soils were analyzed with respect to C and N mineralization rates (during incubation) and to the abundance of bacteria, fungi, protozoa and nematodes. A description of the methods is given by Hassink *et al.* (1993[a,b]). The organisms were aggregated into functional groups

(sensu Moore *et al.*, 1988): bacteria, fungi, flagellates, amoebae, and bacterivorous, predaceous, fungivorous and phytophagous nematodes, as to construct a food web diagram (Fig. 1). Microarthropods (collembola and mites) and annelids were neglected because their contribution to mineralization was assumed insignificant (De Ruiter *et al.*, 1993[a]).

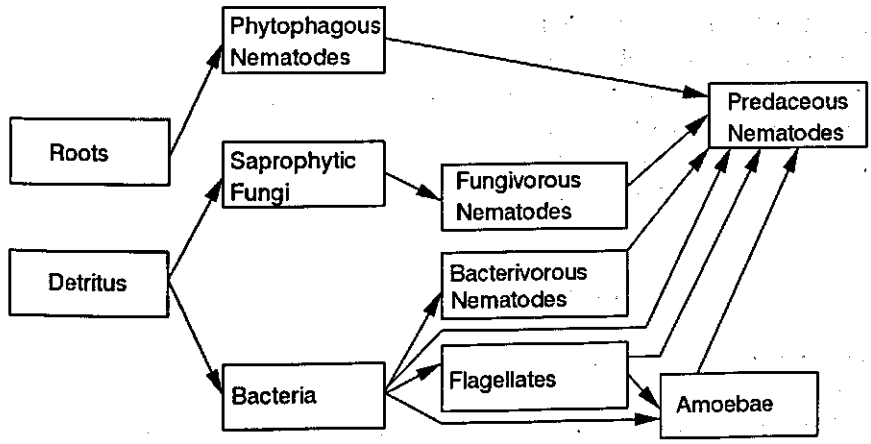


Fig. 1. Diagram of the soil food web of the grassland soils.

Calculation of C and N mineralization

Hunt *et al.* (1987) presented a model to derive N mineralization from the feeding rates of soil organisms. The model calculates feeding rates (C flows) among trophic groups based on the steady state assumption, i.e. production balances energy losses due to natural death and predation.

$$F = \frac{D_{nat}\, B + P}{e_{ass}\, e_{prod}} \qquad (1)$$

where F: feeding rate (kg C ha^{-1} $year^{-1}$); D_{nat}: specific natural death rate ($year^{-1}$); B: biomass (kg C ha^{-1}); P: death rate due to predation (kg C ha^{-1} $year^{-1}$); e_{ass}: assimilation efficiency; e_{prod}: production efficiency.

If a predator was considered to feed on more than one prey type, then both the preference of the predator for a given prey and the relative abundances of the prey types were taken into account:

$$F_i = \frac{w_i \; B_i}{\sum_{i=1}^{n} w_i \; B_i} \; F \qquad (2)$$

where F_i: feeding rate on prey i (kg C ha^{-1} year^{-1}); w_i: preference for prey i relative to other prey types and n: number of potential prey types.

The calculations started with the feeding rates of the predaceous nematodes, since in the present food web description only natural death was assumed to reduce the biomass of this group (Fig. 1). The predatory losses in the groups one trophic level down were calculated from the feeding rates of the top predators. These losses were added to the nonpredatory losses in order to calculate the feeding rates of the groups at this level. All feeding rates were subsequently calculated throughout the food web, working back to the primary consumers, i.e. microorganisms and saprotrophs (see De Ruiter *et al.* 1993[a]). Feeding rates per type of prey are needed to estimate P (eq. 1) at the lower trophic levels. C and N mineralization rates for each trophic interaction were calculated as follows:

$$CO_2 = e_{ass} \; (1 - e_{prod}) \; F \qquad (3)$$

$$N_{min} = e_{ass} \; \left(\frac{1}{r_{prey}} - \frac{e_{prod}}{r_{pred}}\right) F \qquad (4)$$

where CO_2: C mineralization rate (kg C ha^{-1} year^{-1}); N_{min}: N mineralization rate (kg N ha^{-1} year^{-1}); r_{prey}: C:N ratio of prey; r_{pred}: C:N ratio of predator.

For the calculations, the following parameters are required: biomass of the functional groups, specific natural death rates, food preference weighing factors, assimilation efficiencies, production efficiencies and C:N ratios (Table 2).

The biomass of the eight functional groups are given by Hassink *et al.* (1993[a]). The average biomass at the two sampling dates of each group is given in Table 2. All physiological parameter values were chosen equal to those by Hunt *et al.* (1987) except specific (natural) death rates of bacteria that were derived from the observed C mineralization rates and biomass. Specific natural death rates given by Hunt *et al.* (1987) refer to a temperature of 10°C, whereas the soil incubation was done at 20°C. Therefore, the mineralization rates were adjusted to 10°C, using a Q_{10} of 3.0 (Johnsson *et al.*, 1987).

First we calculated N mineralization rates using a bacterial C:N ratio of 4 at each site (Hunt *et al.*, 1987). In succeeding calculations the different C:N ratios for different soil types were used according to Hassink (1994[b]). The C:N ratio of the substrate (detritus) of bacteria and fungi was kept constant at 12.5 for each site (Scholefield *et al.*, 1991).

Table 2. Biomass (kg C ha^{-1}; average of October 1990 and May 1991) and physiological parameter values for each functional group in the top 10 cm of grassland soils.

	Biomass of functional groups				Physiological parameters			
	Heino	Tynaarlo	Swifter-bant	Burum	C:N	assimilation efficiency	production efficiency	natural death rate ($year^{-1}$)
Microbes								
Bacteria	144	201	216	357	4	1.00	0.30	*
Fungi	2.62	3.19	6.73	3.42	10	1.00	0.30	1.2
Protozoa								
Amoebae	3.75	5.64	5.75	5.19	5	0.95	0.40	6.0
Flagellates	0.78	0.81	0.62	0.72	5	0.95	0.40	6.0
Nematodes								
Phytophagous	0.16	0.17	0.28	0.09	5	0.25	0.37	1.08
Bacterivorous	0.65	0.51	0.10	0.30	5	0.60	0.37	2.68
Fungivorous	0.11	0.08	0.19	0.09	5	0.38	0.37	1.92
Predaceous	0.19	0.20	0.08	0.03	5	0.50	0.37	3.00

* = Calculated (see Materials and methods)

RESULTS

Biomass of soil biota and observed C and N mineralization

The total biomass (microbes and soil fauna) is presented in Fig. 2. At all sites virtually the total biomass consisted of bacteria. The biomass pattern closely resembled the pattern of observed C mineralization as shown in Fig. 3. This resemblance indicates that C mineralization can be directly related to biological feeding activity, especially of the bacteria, according to equations (1) and (3). The observed annual N mineralization rates of the four grassland soils are shown in Fig. 4. The differences between the sites in the observed average annual C mineralization did not resemble the pattern of differences in N mineralization.

Ignoring the C mineralization by other functional groups, a specific death rate of bacteria (including natural death and death due to predation) can be derived from equations (1) and

(3). This led to approximately similar death rates of the bacteria at all four sites: 6.3, 5.9, 6.1 and 7.5 $year^{-1}$ in Heino, Tynaarlo, Swifterbant and Burum, respectively. For further calculations we used an average death rate of 6 $year^{-1}$ for all sites.

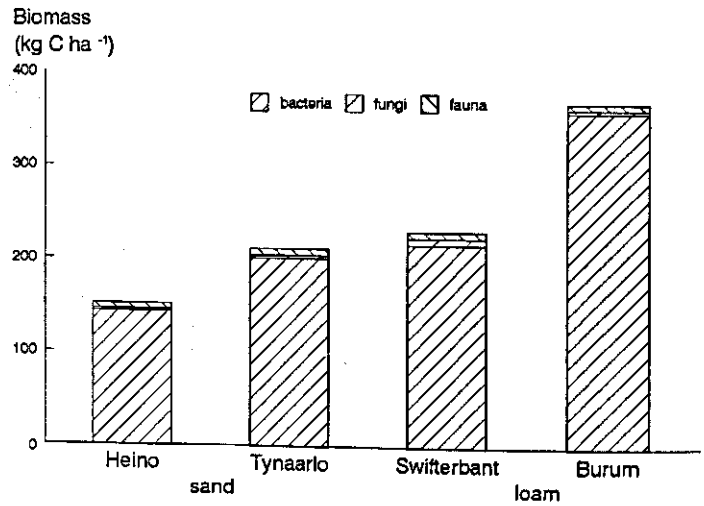


Fig. 2. Observed biomass of bacteria, fungi and fauna (average of two measurements in October 1990 and May 1991; kg C ha^{-1}).

Contribution of the fauna to mineralization

The fauna may contribute to C and N mineralization in two ways: directly through their own C and N mineralization and indirectly through their effect on the growth rate of the microbes (eq. 1). The contribution of the fauna to C mineralization as estimated by the model is shown in Fig. 5a. Faunal C mineralization rates ranged from 5% (Burum) to 13% (Heino) of the total C mineralization. The calculated pattern of faunal C mineralization did not resemble the observed overall C mineralization pattern (cf. Fig. 5a and Fig. 3). From the calculated faunal contribution to C mineralization it appeared that the death rate of bacteria due to predation was approximately 8% of the total death rate. The specific natural death rate of the bacteria and the specific death rate due to predation can therefore be estimated as approximately 5.5 $year^{-1}$ and 0.5 $year^{-1}$, respectively. Incorporating the influence of the fauna, the calculated overall C mineralization still gave a fairly good approximation of the observed C mineralization rates (Fig. 3) because the faunal influence did not substantially influence the pattern determined by bacterial feeding activity.

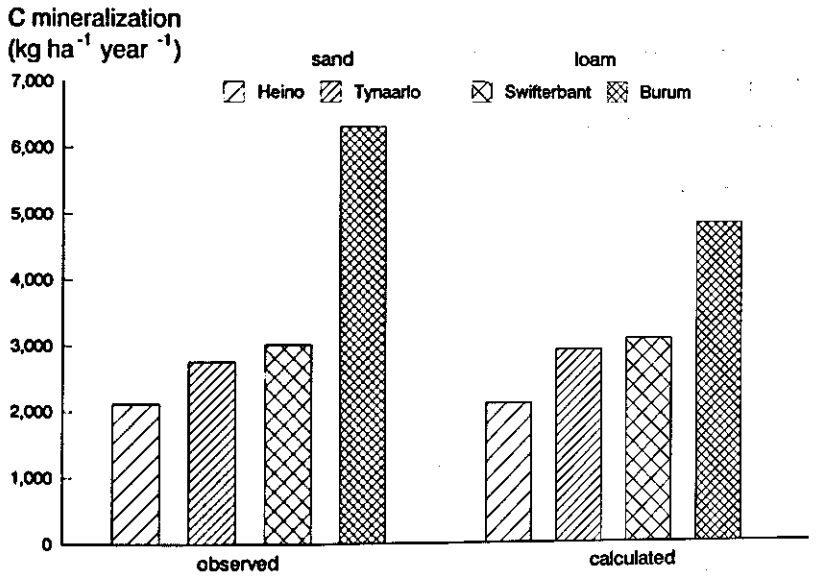


Fig. 3. Observed (left) and calculated (right) overall C mineralization (kg ha^{-1} $year^{-1}$) in the top 10 cm of four grassland soils at 10°C. Calculation with complete food web.

The faunal contribution to N mineralization was first calculated using a bacterial C:N ratio of 4 (Hunt *et al.*, 1987) for each site. The calculated contributions of the fauna to N mineralization (Fig. 5b) resembled the calculated contributions of the fauna to C mineralization (Fig. 5a). Using this model and parameter values, calculated N mineralization rates did not, however, resemble the observed rates (Fig. 4, calculation 1). The calculated rates were generally too low and, more importantly, the patterns of observed and calculated N mineralization did not match. Also, the faunal pattern did not resemble the observed overall N mineralization pattern (cf. Fig. 5b and Fig. 4, observed).

Effect of bacterial C:N ratios on N mineralization

In calculation 2 the soil fauna was neglected and a distinction was made between the bacterial C:N ratios in the sandy soils and in the loams. The value 8 was chosen for the sandy soils (sites Heino and Tynaarlo), the value 5 was chosen for the loams (sites Swifterbant and Burum). These values were the C:N ratios of an average sandy soil and loam, respectively (Hassink, 1994[b]). The use of these different C:N ratios led to N mineralization rates closer to the observed rates (Fig. 4, calculation 2). In calculation 3, a distinction in C:N ratio of the bacteria was made between the two loams as to improve the fit between predicted and observed mineralization rates: a C:N of 6 for Burum, and 4.5 for Swifterbant was found to mimic the observations more satisfactorily (Fig. 4, calculation 3). Incorporation of the fauna

in the calculations using the latter bacterial C:N ratios did not result in a better fit between calculated and observed mineralization rates.

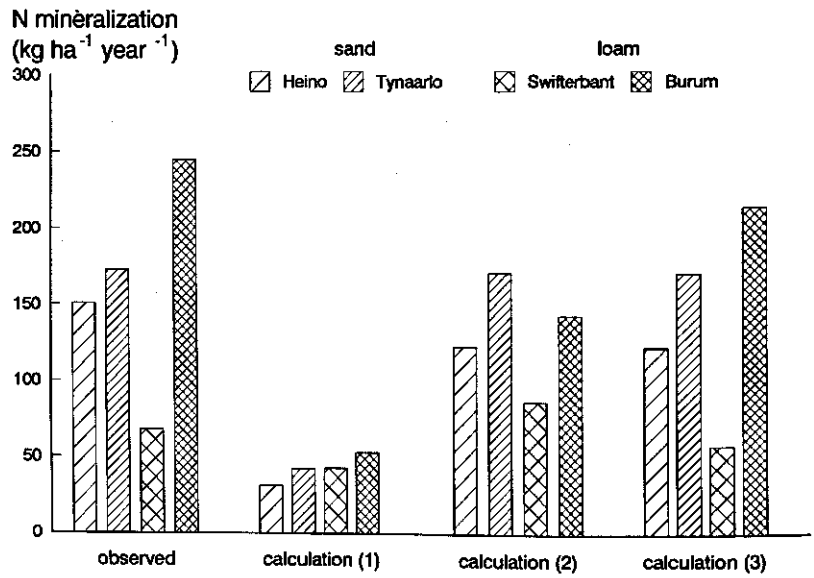


Fig 4. Observed (left) and calculated overall N mineralization (kg ha^{-1} $year^{-1}$) in the top 10 cm of four grassland soils at 10°C.
Calculation (1): calculation with complete food web; bacterial C:N ratio at all sites = 4
Calculation (2): calculation with bacteria only; bacterial C:N ratio = 8 at sites Heino and Tynaarlo, and 5 at sites Swifterbant and Burum
Calculation (3): calculation with bacteria only; bacterial C:N ratio = 8 at sites Heino and Tynaarlo, 4.5 at site Swifterbant, and 6 at site Burum.

DISCUSSION

C mineralization

Differences in C mineralization rates between the four grasslands could satisfactorily be explained by the differences in bacterial biomass. Bacterial specific death rates were derived from the observed C mineralization rates (neglecting the contribution of the fungi and the fauna) leading to approximately the same value (6) on all sites, indicating that there were no substantial differences in bacterial activity between the four sites. The contribution of the fauna to C mineralization was calculated using a food web model (Hunt *et al.*, 1987).

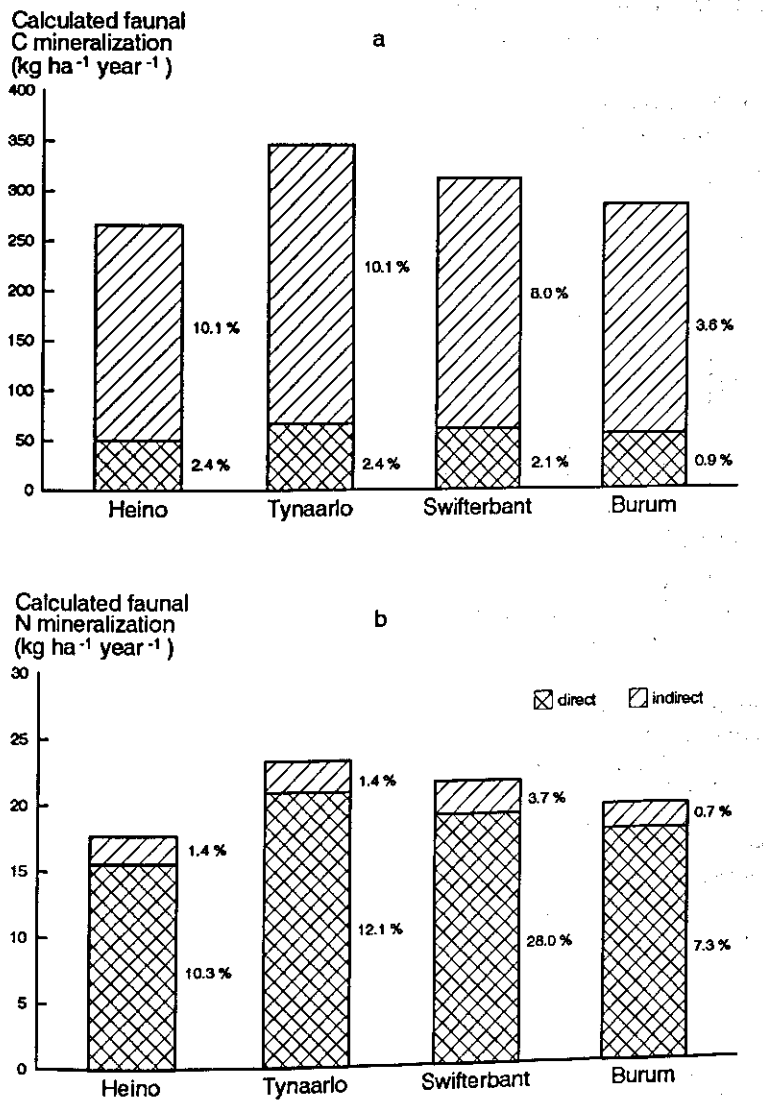


Fig. 5. Calculated direct and indirect contribution of the fauna to overall C (a) and N (b) mineralization (kg ha^{-1} $year^{-1}$). Percentages refer to faunal contribution (direct and indirect) to the observed C or N mineralization.

It appeared that in the present grassland soils the contribution of the fauna to C mineralization was small. Also the pattern of faunal contribution to C mineralization was different from the observed pattern of total C mineralization, indicating that activity of the soil fauna could not have contributed substantially to the differences in C mineralization observed.

N mineralization

In a previous study we found a positive correlation between the grazing pressure on bacteria by bacterivorous nematodes and N mineralization (Hassink *et al.*, 1993[a]), but the present study did not reveal evidence that faunal activity contributed to the observed differences in N mineralization between the four sites. First because N mineralization by the fauna was estimated to be small and second because the calculated pattern of N mineralization did not match the observed pattern of N mineralization. The direct contribution of the fauna to N mineralization ranged from 7.3% to 28.0%. These percentages are lower than those reported in other studies (Hunt *et al.*, 1987; Andrén *et al.*, 1990; De Ruiter *et al.*, 1993[a,b]) indicating direct contributions of the fauna to N mineralization of 30-50%. De Ruiter *et al.* (1993[a]) analyzed arable soil and found that the biomasses of bacteria, fungi and soil fauna were in the same range as found in the present study, but the observed N mineralization rates were much lower. De Ruiter *et al.* (1993[a]) calculated N mineralization rates using a specific bacterial death rate of 0.5 $year^{-1}$, which is much lower than the presently derived death rate. According to the present calculations, bacterial feeding activity is high in grasslands, reducing the relative importance of other groups. This might be related to the continuous high input of decaying root and leaf material in grassland soils, whereas the input of organic residues in the arable system is much lower and concentrated at harvest time. This high input seems not to lead to a high biomass of bacteria but to a high activity. This is in agreement with a Swedish study where it was observed that the microbial biomass was not different under arable cropping and a grass ley but that the activity of the microorganisms was considerably higher under a grass ley than under arable cropping (Andrén *et al.*, 1990).

C:N ratios of bacteria and their substrate

Earlier studies indicated that N mineralization is very sensitive to changes in the C:N ratio of microbes and their substrate (Hunt *et al.*, 1987; De Ruiter *et al.*, 1993[a]). The C:N ratio of the substrate was kept the same for all sites. The incorporation of fresh substrates into grassland soils may be as high as 10 t ha^{-1} $year^{-1}$ (Ryden, 1984); we therefore assumed that the C:N ratio of the substrate of the bacteria will largely be determined by the composition of the fresh organic material supplied each year. As all our sites are intensively managed grasslands, the quality of the incorporated organic material should be the same on all sites. Before incubating soil samples to measure mineralization, clearly visible roots and stubble were removed from the samples. So it could be assumed that bacteria used organic residues

that were partly decomposed. It was assumed that the C:N ratio of this material was 12.5: the C:N ratio of residual organic matter of dead plant material in grassland soils after 1 year of decomposition (Scholefield *et al.*, 1991).

Bacterial C:N ratios were set at 8 and 5 for the sandy soils and the loams, respectively. These values were found to be the average C:N ratios of the microbial biomass in sands and loams, respectively (Hassink, 1994[b]). The variation in C:N ratio of the microbial biomass between sampling dates and between sites within one soil type, however, was large (Hassink, 1994[b]; Hassink *et al.*, 1993[b]). In the present study the fit between the observed and calculated N mineralization rates could be further improved when a distinction was made between the C:N ratio of the bacteria in the two loams: a lower value (4.5) in the Swifterbant soil and a higher value (6.0) in the Burum soil. Within the group of loams, the polder soils (e.g. Swifterbant) often had lower microbial C:N ratios than the other loams. When the polder soils were excluded from the analysis, the average C:N ratio of the microbial biomass in loams and clays was 6. So, the C:N ratios giving the best fit were very close to the measured C:N ratios.

The cause of the higher C:N ratios of the microbes in the sandy soils is not clear. It is assumed that fungi have a higher C:N ratio than bacteria. The fraction of fungi in the microbial biomass, however, was very small in all soils. According to Tezuka (1990), the C:N ratio of the bacteria depends on the C:N ratio of their food. This would object against the assumption that the C:N ratio of the bacterial substrate was similar in all soils.

In conclusion, this study has shown that the C:N ratio of bacteria is an important factor in N mineralization but more research is needed to explain the observed relationship between soil texture and bacterial C:N ratio.

ACKNOWLEDGEMENTS

We gratefully acknowledge the skillful technical assistance of G. Hoenderboom, A. Lahr and A. Vos. We thank L.A. Bouwman, G. de Jong, G. Koopman, K.B. Zwart and L. Brussaard for their support and helpful discussions.

REFERENCES

Andrén O., Lindberg T., Boström U., Clarholm M., Hansson A.C., Johansson G., Lagerlöf J., Paustian K., Persson J., Pettersson R., Schnürer J., Sohlenius B. and Wivstad M. (1990) Ecology of arable land. Chapter 5. Organic carbon and nitrogen flows. *Ecological Bulletins* **40**, 85-126.

Catroux G., Chaussod R. and Nicolardot B. (1987) Assessment of nitrogen supply from the soil. *Comptes Rendues de l'Academie d'Agriculture de France* **3**, 71-79.

Clarholm M. (1985) Possible role for roots, bacteria, protozoa, and fungi in supplying nitrogen to plants. In *Ecological Interactions in Soil: Plants, Microbes and Animals* (A.

H. Fitter, D. Atkinson, D. J. Read and M. B. Usher, Eds.), pp. 355-365. British Ecological Society Special Publications, Oxford.

Coleman D. C., Anderson R. V., Cole C. V., Elliott E. T., Woods L. and Campion M. K. (1978) Trophic interactions in soils as they affect energy and nutrient dynamics. IV. Flows of metabolic and biomass carbon. *Microbial Ecology* **4,** 373-380.

Coleman D. C., Reid C. P. P. and Cole C. V. (1983) Biological strategies of nutrient cycling in soil ecosystems. *Advances in Ecological Research* **13,** 1-55.

De Ruiter P.C., Moore J.C., Zwart K.B., Bouwman L.A., Hassink, J., Bloem J., De Vos J.A., Marinissen J.C.Y., Didden W.A.M., Lebbink G. and Brussaard L. (1993[a]) Simulation of nitrogen mineralization in belowground food webs of two winter wheat fields. *Journal of Applied Ecology* **30,** 95-106.

De Ruiter P.C., Van Veen J.A., Moore J.C., Brussaard L and Hunt H.W. (1993[b]) Calculation of nitrogen mineralization in soil food webs. *Plant and Soil* **157,** 263-273.

Hassink J., Bouwman L.A., Zwart K.B. and Brussaard L. (1993[a]) Relationships between habitable pore space, biomass and activity of bacteria and fauna and mineralization rates in grassland soils. *Soil Biology & Biochemistry* **25,** 47-55.

Hassink J., Bouwman L.A., Zwart K.B., Bloem J. and Brussaard L. (1993[b]) Relationships between soil texture, physical protection of organic matter, soil biota, and C and N mineralization in grassland soils. *Geoderma* **57,** 105-128.

Hassink J. (1994[a]). Effects of soil texture and grassland management on soil organic C and N and rates of C and N mineralization. *Soil Biology & Biochemistry* **26,** 1221-1231.

Hassink J. (1994[b]). Effect of soil texture on the size of the microbial biomass and on the amount of C and N mineralized per of microbial biomass in Dutch grassland soils. *Soil Biology & Biochemistry* **26,** 1573-1581.

Hunt H. W., Coleman D. C., Ingham E. R., Ingham R. E., Elliott E. T., Moore J. C., Rose S. L., Reid C. P. P. and Morley C. R. (1987) The detrital food web in a shortgrass prairie. *Biology and Fertility of Soils* **3,** 57-68.

Johnsson H., Bergström B., Jansson P.E. and Paustian K. (1987) Simulated nitrogen dynamics and losses in a layered agricultural soil. *Agriculture Ecosystems and Environment* **18,** 333-356.

Moore J.C., Walter D.E. and Hunt H.W. (1988) Arthropod regulation of micro- and mesobiota in belowground food webs. *Annual Review of Entomology* **33,** 419-439.

Ryden J.C. (1984) The flow of nitrogen in grassland. *The Fertilizer Society,* Proceedings No 229, 44 pp.

Scholefeld D., Lockyer D.R., Whitehead D.C. and Tyson K.C. (1991) A model to predict transformations and losses of nitrogen in UK pastures grazed by beef cattle. *Plant and Soil* **132,** 165-177.

Tezuka Y. (1990) Bacterial regeneration of ammonium and phosphate as affected by the carbon:nitrogen:phosphorus ratio of organic substrates. *Microbial Ecology* **19,** 227-238.

Van Veen J.A., Ladd J.N. and Amato M. (1985) Turnover of carbon and nitrogen through the

microbial biomass in a sandy loam and a clay soil incubated with [^{14}C(U)]glucose and [^{15}N]$(NH_4)_2SO_4$ under different moisture regimes. *Soil Biology & Biochemistry* **17**, 747-756.

Woods L.E., Cole C.V., Elliott E.T., Anderson R.V. and Coleman D.C. (1982) Nitrogen transformations in soil as affected by bacterial-microfaunal interactions. *Soil Biology & Biochemistry* **14**, 93-98.

CHAPTER 7

DENSITY FRACTIONS OF MACRO-ORGANIC MATTER AND MICROBIAL BIOMASS AS PREDICTORS OF C AND N MINERALIZATION.

By: J. Hassink. 1995. Accepted by Soil Biology & Biochemistry.

DENSITY FRACTIONS OF MACROORGANIC MATTER AND MICROBIAL BIOMASS AS PREDICTORS OF C AND N MINERALIZATION

ABSTRACT

Macroorganic matter of arable soils which had received different inputs of organic residues for 25 years and grassland soils that had been under grass for at least 8 years was fractionated into light, intermediate and heavy fractions using a stable silica suspension as heavy liquid. For all residue treatments, the C-to-N ratios of organic matter decreased in the order light, intermediate, heavy macroorganic matter (fraction > 150 μm) and non-macroorganic matter (fraction < 150 μm). Residue application had a stronger effect on the amount and C-to-N ratio of macroorganic matter fractions than on the amount and C-to-N ratio non-macroorganic matter.

Textural effects were apparent with the proportions of soil N in the light and intermediate fractions being higher in coarse-textured grassland soils than in fine-textured grassland soils.

C and N mineralization were positively correlated with the amount of C and N in the light fraction and the active microbial biomass. The correlation with mineralization decreased with increasing stability of the organic matter fractions. C and N mineralization per unit of total microbial biomass were lower in fine-textured soils than in coarse-textured soils. This is ascribed to a greater physical protection of the organic matter in fine-textured soils than in coarse-textured soils.

INTRODUCTION

It is generally accepted that soil organic matter contains fractions with a rapid turnover rate and fractions with a slower turnover rate (Cambardella and Elliott, 1992). The fractions with a rapid turnover (active fractions) are assumed to play a dominant role in soil nutrient dynamics (Janzen *et al.*, 1992). One method of measuring active soil organic matter involves the use of densiometric techniques to isolate the light fraction (Greenland and Ford, 1964; Janzen *et al.*, 1992). The light fraction has been found to be more sensitive to differences in management and inputs of residues than total organic matter (Dalal and Mayer, 1987; Janzen *et al.*, 1992). Meijboom *et al.* (1995) have developed a new and simple density fractionation procedure using silica suspensions and recovered three density fractions: the light fraction consisting of recognizable plant residues, the intermediate fraction of partly humified material and the heavy fraction of amorphous organic material. The density fractionation was performed in the sand-size organic matter (macroorganic matter; > 150 μm) as it has been observed that organic C in this fraction is much more labile than organic C in the clay and silt size fractions (Tiessen and Stewart, 1983; Tiessen *et al.*, 1984; Dalal and Mayer, 1986).

My first objective was to test to what extent the amount and quality of density fractions respond to differences in inputs of residues. No data on the effects of different residues on the amount and quality of the light, intermediate and heavy macroorganic matter fractions are available. I hypothesized that the effect of residue input on the quality and quantity of soil organic matter fractions decreases in the order light, intermediate, heavy macroorganic matter and non-macroorganic matter. To test this hypothesis we fractionated organic matter in a sandy and a clay soil that had received a constant annual input of residues of varying quality (C-to-N ratio) for 25 years.

The decomposition and mineralization of organic matter fractions and microbial turnover is more rapid in coarse-textured soils than in fine-textured soils (Van Veen and Kuikman, 1990). This is caused by a greater physical protection of soil organic matter and the microbial biomass in fine-textured soils (Verberne *et al.*, 1990). Therefore active organic matter pools such as macroorganic matter and the microbial biomass (Jenkinson and Ladd, 1981) do not necessarily correlate with C and N mineralization when soils of different textures are compared (Hassink, 1993). It has been stated that the light fraction (which consists of mineral-free organic matter) and the active microbial biomass (determined by the substrate-induced respiration method) are not physically protected (Young and Spycher, 1979; Hassink, 1993). Therefore, I hypothesized that the light fraction and the active microbial biomass correlate more strongly with C and N mineralization than with any other organic matter fraction (such as total microbial biomass) when soils with different textures are included in the analysis. To test this hypothesis 20 old grassland soils which differed in soil texture were sampled.

MATERIALS AND METHODS

To test the first hypothesis samples were taken from the top 25 cm of sandy soils (17 % of particles are < 16 µm; pH-KCl 7.4) and clays (66 % of particles are < 16 µm; pH-KCl 7.0) which had been kept bare for 25 y and had received 1) no organic C and N, or 2) 10 tonnes C ha^{-1} y^{-1} by the application of either lucerne (2.5 % N; 43.6 % C); wheat chaff (0.8 % N; 42.5 % C); chaff + lucerne or; farmyard manure (2.9 % N; 42.7 % C; FYM). The residues were applied at the beginning of June and mixed through the top 25 cm of the soil.

To test the second hypothesis samples were collected from soils (Table 1) which had been under grass for at least 8 y. A mixed sample consisting of 20 bulked cores was taken from the 0-10 and 10-25 cm layers of each location.

Washing of the soil samples and size and density fractionation of organic matter

Dried soil samples (250 g) were rewetted and frozen for 1 week. After thawing, the samples were wet-sieved over a 250 µm and a 150 µm mesh sieve. The samples were placed on the

top sieve (250 μm) and washed with tap water. The macroaggregates were destroyed by pushing the soil through the top sieve during the washing procedure until the water passing the sieve became clear. The material present on both sieves was washed into a bucket. The material in the bucket was swirled and the organic material was separated from the mineral material by decantation. The organic material was poured into a small tray with a 150 μm mesh sieve at the bottom and sides of 10 cm heigth. The mineral material was retained at the bottom of the bucket. Swirling and decantation was repeated several times until there were no more visible organic particles in the mineral fraction. The mineral fraction was discarded.

The organic material was fractionated in Ludox TM. Ludox is an aqueous colloidal dispersion of silica particles produced by Du Pont. The tray containing the organic material was placed in Ludox with a density of 1.37 g cm^{-3}, and was stirred several times. The floating fraction was collected and placed in a similar tray that was placed in Ludox with a density of 1.13 g cm^{-3}. Mixing was repeated until the quantity of floating material became negligible. The organic material placed into the 1.13 g cm^{-3} Ludox was also separated into a floatable and a sinking fraction. Finally, three fractions were obtained: a light fraction with a density < 1.13 g cm^{-3}; an intermediate fraction with a density between 1.13 and 1.37 g cm^{-3}, and a heavy fraction with a density > 1.37 g cm^{-3}. The three fractions were washed with tap water and dried. A more extensive description of the procedure and the characteristics of the Ludox is given by Meijboom *et al.* (1995).

The N content of the density fractions was determined according to Deys (1961) after destruction with sulfuric acid and salicylic acid. Organic C was determined by treating the samples with dichromate-H_2SO_4 according to Kurmies (Mebius, 1960).

Potential mineralization rates

Three mixed samples, each consisting of 20 bulked cores, were taken from every residue treatment (sandy and clay soil) or location (grassland soils). The field-moist samples were sieved (8 mm) and kept at a moisture content close to 60% of the water holding capacity for 12 weeks at 20°C.

N mineralization was determined by measuring the increase in mineral N after incubation of soil samples in glass jars at 20°C for 12 weeks. Drying of the samples was prevented by covering the jars with an air-permeable, water-impermeable seal. Mineral N was measured colorimetrically after extraction with 1*N* KCl solution for 1 h using a soil-to-water ratio of 1:2.5.

C mineralization was measured by incubating the grassland soil samples in 1.5-l airtight jars containing a vial of 10 ml 0.5*M* NaOH. At the sampling dates, the trapped CO_2 was measured after precipitation of the carbonate with excess $BaCl_2$. The period between 10 and 20 days after the start of the incubation was used to calculate the C mineralization rate. A flush in CO_2 production was observed during the first 10 days, probably caused by the disturbance of the soil.

Table 1. Characteristics of the 0-10 and 10-25 cm layers of the grassland soils studied (pH and granular composition are only given for the top 10 cm because the differences between the two layers were very small)

Site No.	Location	Soil type	Exp. year	C (%)		C/N		pH (KCl)	Granular comp., % particles <		
				0-10	10-25	0-10	10-25	0-10	2μm	16μm	50μm
1	Heino 1	sand	1989	1.98	1.97	18.0	17.9	5.0	1.9	2.8	8.7
2	Jubbega	sand	1989	2.87	2.92	21.6	21.9	4.6	1.0	1.9	3.7
3	Tollebeek	sand	1989	0.95	0.92	10.0	10.5	7.0	4.4	9.2	28.9
4	Agterberg	sand	1989	3.09	2.26	17.5	17.4	4.9	3.0	5.8	9.0
5	Tynaarlo	sand	1989	4.38	3.48	17.8	21.8	4.4	2.4	4.4	23.5
6	Finsterwolde	sand	1989	5.37	2.13	10.9	9.9	5.0	8.4	13.3	23.2
7	Cranendonck	sand	1991	2.96	2.25	17.1	19.3	5.4	3.2	4.8	21.2
8	Dalfsen	sand	1991	3.88	5.49	20.8	40.5	5.1	1.9	3.1	9.8
9	Maarheeze	sand	1991	2.61	2.29	18.3	18.6	5.0	2.6	4.5	11.8
10	Tynaarlo 2	sand	1991	3.80	2.97	19.7	21.2	4.3	3.1	5.1	22.3
11	Swifterbant	loam	1989	1.77	1.87	11.6	10.8	7.0	23.0	38.4	72.2
12	Zeewolde	loam	1989	3.53	3.01	12.9	14.3	6.9	26.7	45.4	77.2
13	Slootdorp	loam	1989	3.84	3.38	14.6	14.7	6.9	17.0	26.0	38.8
14	Burum	loam	1989	5.37	2.13	9.8	9.1	4.8	24.1	36.5	71.7
15	Lelystad	loam	1991	3.07	1.68	11.1	12.1	7.1	21.6	35.6	56.4
16	Mijnsh.land.	loam	1991	2.20	1.40	9.6	9.1	7.2	20.1	33.5	79.9
17	Zaltbommel 2	clay	1989	6.07	5.23	9.2	8.9	5.4	51.1	76.0	86.4
18	Haskerdijk	clay	1991	5.60	4.04	11.1	11.1	4.9	54.0	77.2	88.5
19	Finsterwolde	clay	1991	3.23	1.80	10.1	9.3	7.1	45.8	65.8	85.1
20	Zaltbommel 3	clay	1991	3.98	3.28	9.5	8.9	5.4	51.0	74.3	90.6

Microbial biomass according to the FI and SIR method

The amount of N in the microbial biomass was determined in field-moist samples by the chloroform fumigation incubation (FI) technique (Jenkinson and Powlson, 1976). A k value of 0.4 was used to calculate the biomass N from the flush; the actual procedure used has been described by Hassink *et al.* (1991). Microbial biomass-N was used instead of microbial biomass-C because the fumigation incubation method yielded unrealistically high biomass-C values in the 10-25 cm layer of grassland soils (Hassink, 1993). The active microbial biomass was determined by the substrate induced respiration (SIR) method (Anderson and Domsch, 1978). The actual procedure used was described by Hassink (1993). The active microbial biomass was only determined in the grassland soils that were sampled in 1991.

Statistical analysis

The relationships between organic matter fractions and C and N mineralization or soil texture were analyzed with correlation and stepwise multiple regression techniques (Genstat, 1987). The fraction < 50 μm (clay + silt content) was taken as an index of soil texture (Hassink, 1994).

RESULTS

The amount of light, intermediate and heavy macroorganic matter in arable sandy and clay soils after 25 years of different annual inputs of organic residues

The annual addition of chaff, lucerne or a combination of both materials increased the total amount of soil organic C by a factor 2.3 in the sandy soil and 1.7 in the clay soil after 25 y in comparison with bare soil (Table 2). The largest increase in soil organic C was found in the farmyard manure treatment, which caused a 5.9-fold increase in the sandy soil and a 3.6-fold increase in the clay soil in comparison with bare soil.

For all amendments, the relative increase in the amount of light, intermediate and heavy fraction exceeded the relative increase in total organic C.

The annual addition of chaff led to a 7-10-fold increase of the light and intermediate fractions in both soils, while the heavy fraction was 5.5 and 3 times higher than in the bare treatment in the sandy soil and the clay, respectively (Table 2). The contribution of the three fractions to the total amount of organic C had increased from 4.8% to 12.3% in the sandy soil and from 3.3% to 7.6% in the clay (Table 2).

The annual addition of lucerne led to a 4.5-, 6.6- and 3.5-fold increase of the light, intermediate and heavy fraction in the sandy soil and to a 1.6-, 3.2- and 1.9-fold increase in the clay soil, respectively. The contribution of the three fractions to the total amount of organic C was 8.6% in the sandy soil and 4.0% in the clay (Table 2).

The annual addition of FYM gave the largest increase of the heavy fraction. The amount of heavy fraction was 24 and 12 times higher than in the bare treatment in the sandy and clay soil, respectively. The contribution of the heavy fraction to the total amount of organic C was 14.8% in the sandy soil and 8.3% in the clay. This contribution was considerably greater than in the chaff and lucerne treatments. In the sandy soil, the amounts of the light and intermediate fractions were 10-12 times higher than in the bare treatment, in the clay soil they were 4.6 and 13.6 times higher, respectively (Table 2).

C-to-N ratios of the macroorganic matter fractions after 25 y of different annual inputs of organic residues

The C-to-N ratio decreased in the order light, intermediate and heavy. The C-to-N ratios of these fractions were considerably wider than the C-to-N ratio of total organic matter (Table 3). The quality of the inputs had the strongest effect on the C-to-N ratio of the light fraction, but the effect on the intermediate and heavy fractions was also much greater than on the total soil organic matter pool. The C-to-N ratios of the macroorganic matter fractions were highest in the chaff treatment and least in the FYM treatment (Table 3).

Table 2. The amount of C in the light, intermediate and heavy macroorganic matter fraction and in the total soil, and the percentage total soil organic C in the fractions in a sandy and a clay soil after 25 y of different annual inputs of organic residues

Soil and treatments	Macroorganic matter fraction			
	Light mg kg^{-1} (%)	Intermediate mg kg^{-1} (%)	Heavy mg kg^{-1} (%)	Total mg kg^{-1}
Sandy soil				
Bare	18.7 (0.3)	43.9 (0.8)	198.9 (3.7)	5433
Chaff	182.2 (1.4)	290.9 (2.3)	1094.4 (8.6)	12767
Chaff + Lucerne	128.5 (1.0)	200.7 (1.6)	926.8 (7.5)	12400
Lucerne	84.6 (0.7)	291.6 (2.3)	698.3 (5.6)	12533
FYM*	180.8 (0.6)	508.4 (1.6)	4779.8 (14.8)	32267
Clay soil				
Bare	29.3 (0.3)	51.0 (0.5)	276.0 (2.5)	11150
Chaff	225.7 (1.1)	422.8 (2.2)	838.0 (4.3)	19650
Chaff + Lucerne	79.4 (0.4)	162.4 (0.9)	931.0 (5.0)	18500
Lucerne	47.3 (0.3)	163.2 (0.9)	534.2 (2.8)	19000
FYM*	134.0 (0.3)	692.8 (1.7)	3311.7 (8.3)	39950

* FYM = farmyard manure

Amounts of light, intermediate and heavy macroorganic matter in the grassland soils and their C-to-N ratio

The amounts of carbon in the light, intermediate and heavy fractions were considerably higher in the top 10 cm of the grassland soils than in the 10-25 cm layer (Table 4). The average C-to-N ratio decreased in the order light, intermediate, heavy, and was generally lower in the top 10 cm than in the 10-25 cm layer (Table 4). The contribution of the light, intermediate and heavy fractions to total soil organic C and N ranged from 0.1 to 2.6% for the light fraction, 0.3 to 23.8% for the intermediate fraction, and 2.2 to 34.0% for the heavy fraction

(Table 5). The contribution was generally higher in the top 10 cm than in the 10-25 cm layer (Table 5).

Table 3. C-to-N ratios of the light (L), intermediate (I) and heavy (H) macroorganic matter fractions and the bulk soil of a sandy and a clay soil after 25 y of different annual inputs of organic residues

Soil/treatment	C-to-N ratio of macroorganic matter fractions			
	L	I	H	Total
Sand				
Bare	nd*	16.4	13.9	9.4
Chaff	27.2	20.4	18.9	9.8
Chaff + Lucerne	20.5	19.9	17.5	8.3
Lucerne	20.5	18.5	15.2	7.1
FYM	17.0	15.9	11.1	8.1
Clay				
Bare	nd*	nd*	14.5	7.0
Chaff	35.9	26.0	20.9	8.2
Chaff + Lucerne	20.6	20.6	14.6	7.1
Lucerne	20.1	21.3	18.3	6.4
FYM	16.1	17.1	13.1	7.4

* nd = not determined; in these cases a C-to-N of 20 was used

There was no significant effect of soil texture on the amounts of the light, intermediate and heavy fractions and the proportions of soil organic C present in those fractions. There was, however, a significant negative correlation between soil texture and the proportions of soil organic N in the light and intermediate fraction for both the 0-10 cm and the 10-25 cm layers (r values -0.74 and -0.67 for the light fraction, and -0.63 and -0.53 for the intermediate fraction, respectively; Table 5). For the heavy fraction there was no such correlation.

Correlation of light, intermediate and heavy macroorganic matter with C and N mineralization rates

Grassland soils. The correlation with C mineralization was stronger for the light fraction than for the intermediate and heavy fractions and non-macroorganic C (Table 6). The correlations of C mineralization with microbial biomass N determined by the FI method and the microbial biomass determined by the SIR method (data of the soils of 1991 only) were weaker than the correlation between C mineralization and the light fraction, but higher than for the other organic matter fractions (Table 6).

Table 4. Amount of C in the light (L), intermediate (I) and heavy (H) macroorganic matter fractions of the top 10 cm and the 10-25 cm layer of grassland soils

Site No.	Location	Amount of C (g kg^{-1})					
		0-10 cm layer			10-25 cm layer		
		L	I	H	L	I	H
Sand							
1	Heino	0.38	0.68	2.88	0.24	0.68	3.43
2	Jubbega	nd*	nd*	nd*	0.33	2.26	7.08
3	Tollebeek	0.23	0.65	1.91	0.13	0.48	1.40
4	Agterberg	0.81	2.02	5.09	0.14	0.32	1.10
5	Tynaarlo	0.56	1.20	4.15	0.13	0.45	2.15
6	Finsterwolde	0.90	1.95	4.85	0.03	0.18	1.30
7	Cranendonck	0.21	1.39	2.57	0.13	0.73	2.30
8	Dalfsen	0.65	5.41	3.22	0.38	9.29	2.51
9	Maarheeze	0.44	2.07	2.36	0.20	1.60	1.64
10	Tynaarlo 2	0.42	2.03	2.39	0.23	0.52	1.05
Average							
C-to-N ratio		20	18	16	25	22	21
Loam							
11	Swifterbant	0.19	0.34	1.81	0.13	0.67	1.53
12	Zeewolde	0.58	1.47	3.58	0.09	0.58	2.49
13	Slootdorp	0.33	2.56	4.37	0.30	2.29	4.94
14	Burum	0.61	2.16	6.02	0.13	0.16	2.05
15	Lelystad	0.24	1.16	6.01	0.05	0.31	2.82
16	Mijnsh.land.	0.43	1.55	2.10	0.18	0.19	0.89
Average							
C-to-N ratio		20	18	14	25	18	15
Clay							
17	Zaltbommel 2	0.66	1.10	2.74	0.13	0.47	1.98
18	Haskerdijk	0.66	2.94	13.8	0.25	0.69	5.09
19	Finsterwolde	0.28	1.31	3.87	0.15	0.23	4.40
20	Zaltbommel 3	0.80	1.07	3.84	0.15	0.37	1.21
Average							
C-to-N ratio		20	18	14	25	18	15

* nd = not determined

The correlation with N mineralization was strongest for the light fraction and biomass-SIR (Table 6; Figs 1 and 2). The correlation with N mineralization decreased in the order light, intermediate, heavy and non-macroorganic N.

Table 5. Percentage total soil C and N in the light (L), intermediate (I) and heavy (H) macroorganic matter fractions of grassland soils

	Macroorganic matter fraction		
	L	I	H
C			
Sand			
0-10 cm	1.0-2.6 (1.8)*	2.7-17.5 (6.9)	7.4-17.4 (11.9)
10-25 cm	0.2-1.2 (0.8)	0.8-23.8 (5.6)	4.1-24.3 (10.1)
Loam			
0-10 cm	0.9-2.3 (1.4)	1.9- 8.4 (5.1)	10.2-23.1 (13.2)
10-25 cm	0.3-1.5 (0.7)	0.9- 6.8 (2.8)	8.2-14.6 (11.5)
Clay			
0-10 cm	1.0-2.3 (1.5)	1.8- 6.2 (4.0)	4.5-29.1 (14.8)
10-25 cm	0.5-1.0 (0.7)	0.9- 2.2 (1.5)	3.8-29.3 (14.3)
N			
Sand			
0-10 cm	0.7-2.2 (1.5)	2.7-16.5 (6.3)	10.2-17.7 (12.1)
10-25 cm	0.1-1.3 (0.7)	0.7-20.7 (5.3)	4.7-34.0 (11.2)
Loam			
0-10 cm	0.5-1.1 (0.7)	1.3- 5.5 (2.8)	5.8-15.2 (9.5)
10-25 cm	0.2-0.7 (0.4)	0.3- 5.5 (1.9)	3.7-13.4 (8.0)
Clay			
0-10 cm	0.2-1.0 (0.6)	0.4- 3.9 (2.0)	2.2-20.3 (9.3)
10-25 cm	0.2-0.8 (0.4)	0.5- 3.4 (1.5)	3.9-18.5 (12.5)

* () = average value

C and N mineralization per unit of microbial biomass FI-N were significantly ($p < 0.05$) higher in coarse-textured soils then in fine-textured soils (Figs 3 and 4). The calcareous soils are indicated separately in Fig. 3, as some of the produced CO_2 may have originated from the chalk. The relationships of the light, intermediate and heavy fractions and the microbial biomass-SIR with N mineralization were unaffected by soil texture (Figs 1 and 2).

For the complete set of soils sampled in 1989 and 1991, the best predictors of C and N mineralization were calculated by multiple regression analysis. The equation for the best predictor of C mineralization included significant ($p < 0.05$) contributions of the light fraction and the microbial biomass (FI-N). For N mineralization the light and intermediate fractions and the microbial biomass (FI-N) contributed significantly ($p < 0.05$) to the equation (Table 7).

Table 6. Correlation coefficients for the relationships between the amount of C or N in the light (L-C or L-N), in the intermediate (I-C or I-N) and in the heavy macroorganic matter fraction (H-C or H-N), in non-macroorganic matter (Non-MOM-C and Non-MOM-N), in the amount of N in the microbial biomass (Bio-N FI), in the amount of C in the active microbial biomass (Bio-SIR), and C and N mineralization. All correlations coefficients are significant ($p < 0.05$) unless stated otherwise.

	Mineralization	
	Grassland	Arable after 25 years of different annual inputs of organic residues
C		
L-C	0.79	
I-C	0.20*	
H-C	0.50	
Non-MOM-C	0.68	
Bio-N FI	0.70	
Bio-SIR†	0.73	
N		
L-N	0.77	0.94
I-N	0.59	0.75
H-N	0.56	0.83
Non-MOM-N	0.50	0.57
Bio-N FI	0.57	0.78
Bio-SIR†	0.77	

* = linear correlation is not significant at $P < 0.05$
† = only determined in the soils sampled in 1991

Arable soils after 25 y of different annual inputs of organic residues. The correlation with N mineralization was strongest for the light fraction. Again the correlation with N mineralization was less for non-macroorganic N than for N in the intermediate and heavy fraction (Table 6). In accordance with the results of the grassland soils, the relationship between N in the light fraction and N mineralization was not affected by soil texture (Fig. 5), whereas N mineralization per unit of microbial biomass FI-N was significantly ($p < 0.05$) higher in the sandy soil than in the clay (Fig. 6).

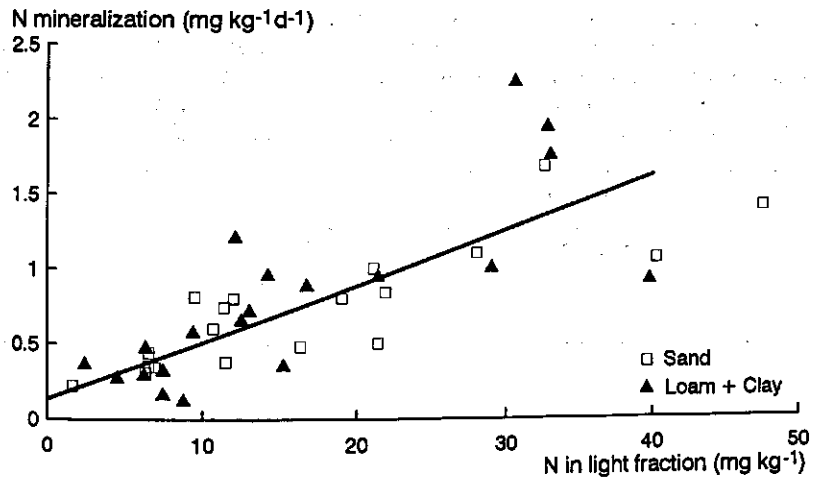


Fig. 1. Relationship between the amount of N in the light macroorganic matter fraction and N mineralization in the top 10 cm and the 10-25 cm layer of grassland soils. N mineralization = 0.20 (0.09) + 0.034 (0.005) x N in light fraction.
() = standard error of difference.

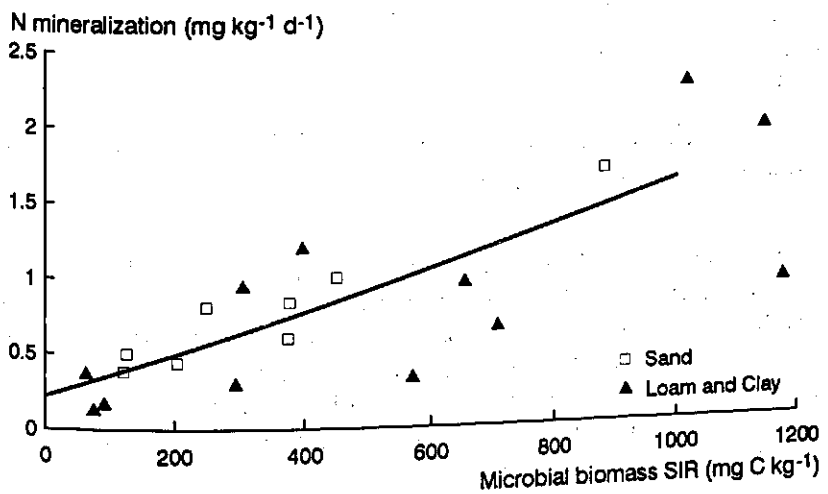


Fig. 2. Relationship between the amount of microbial biomass-SIR (mg C kg^{-1} soil) and N mineralization in the top 10 cm and the 10-25 cm layer of grassland soils. N mineralization = 0.23 (0.14) + 0.0013 (0.0002) x microbial biomass-SIR.
() = standard error of difference.

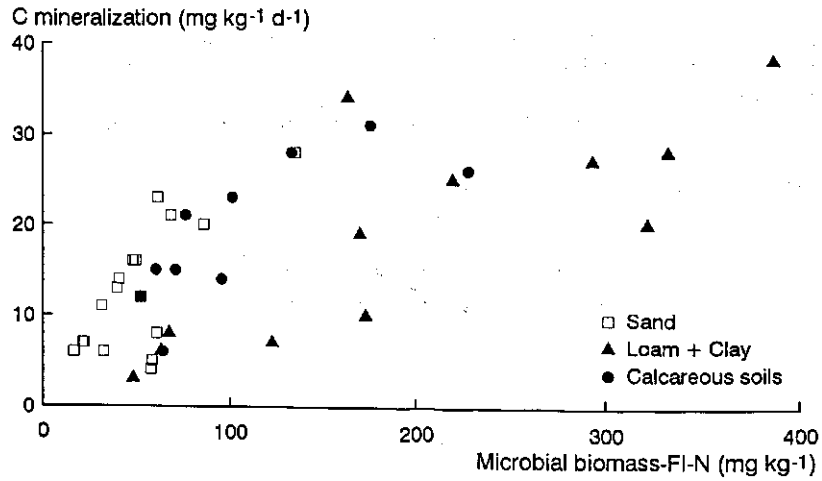


Fig. 3. Relationship between the amount of microbial biomass-FI-N (MB-N) and C mineralization (C min) in the top 10 cm and the layer 10-25 cm of grassland soils. C min:MB-N = 0.3055 (0.0241) - 0.00243 (0.00043) x % < 50 µm.
() = standard error of difference.

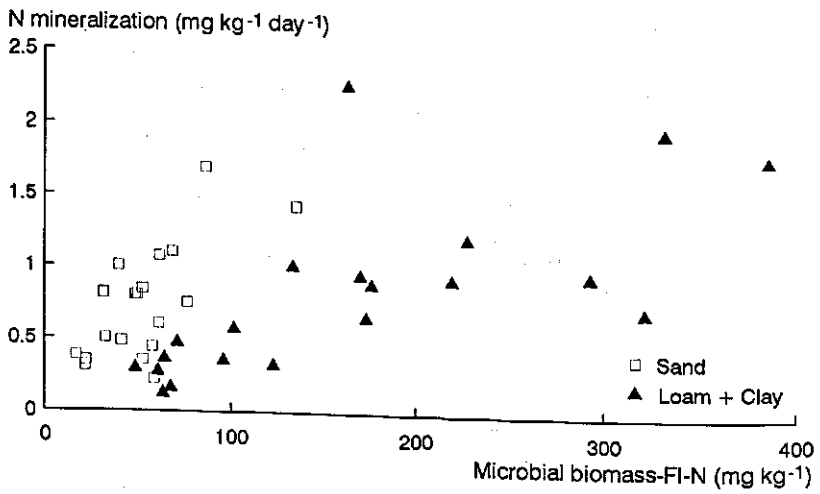


Fig. 4. Relationship between the amount of microbial biomass-FI-N (MB-N) and N mineralization (N min) in the top 10 cm and the 10-25 cm layer of grassland soils. N min:MB-N = 0.01692 (0.00133) - 0.000151 (0.000023) x % < 50 µm.
() = standard error of difference.

Table 7. Equations calculated with multiple regression analysis giving the best predictors for C and N mineralization (C min and N min in mg kg^{-1} d^{-1}, respectively) in grassland soils
L = The amount of light fraction in mg C or N kg^{-1} soil
MB-N = The amount of N in the microbial biomass determined by the fumigation incubation method in mg kg^{-1} soil
I = The amount of intermediate fraction in mg N kg^{-1} soil

C min = 0.0256 (0.0039) x L + 0.0451 (0.0091) x MB-N
N min = 0.021 (0.005) x L + 0.0018 (0.0005) x MB-N + 0.0025 (0.0007) x I

() = Standard error of difference.

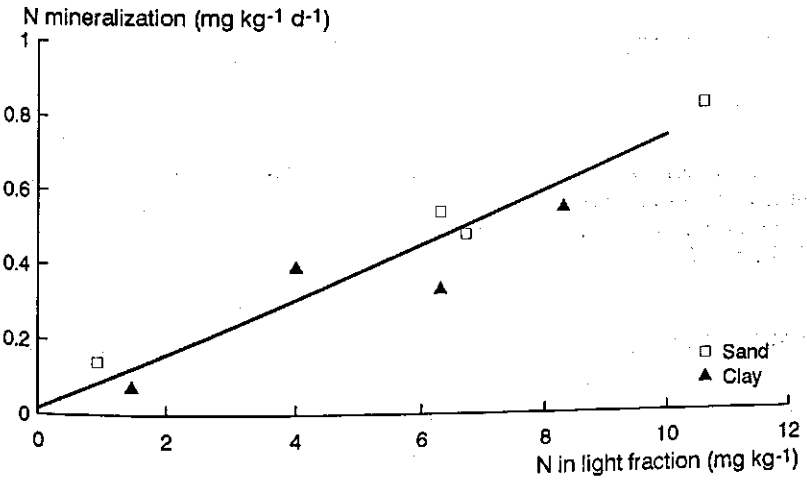


Fig. 5. Relationship between the amount of N in the light macroorganic matter fraction and N mineralization in the top 25 cm of the sandy soil and clay with different inputs of residues. N mineralization = 0.028 (0.063) + 0.0696 (0.0099) x N in light fraction.
() = standard error of difference.

DISCUSSION

Effects of residue application on the amount and C-to-N ratio of organic matter fractions

My first objective was to test to what extent the amount and quality of density fractions respond to differences in inputs of residues. The differences in residue application affected the amounts and the C-to-N ratios of the light, intermediate and heavy macroorganic matter to a much larger extent than total soil organic matter. This agrees with results of Shaymukhavetov *et al.*, 1985; Dalal and Mayer, 1987; Janzen *et al.*, 1992).

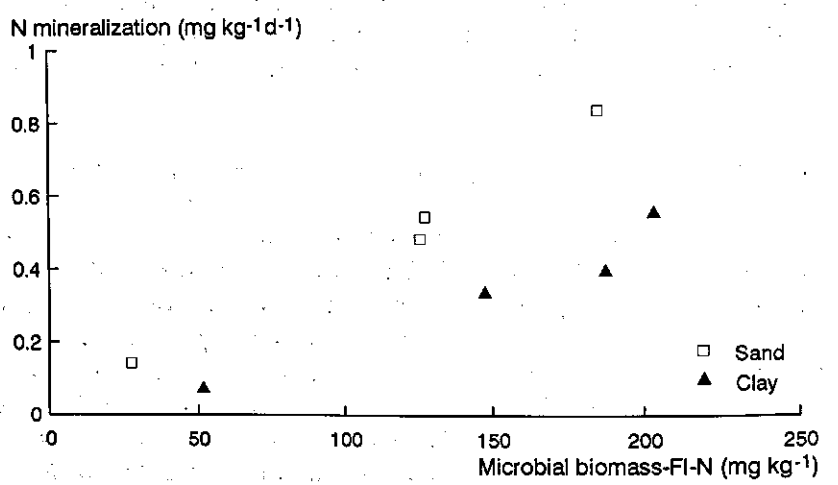


Fig. 6. Relationship between the amount of microbial biomass-FI-N (MB-N) and N mineralization (N min) in the top 25 cm of the sandy soil and clay with different inputs of residues. Sandy soil N min = 0.0043 (0.0005) x MB-N; Clay: N min = 0.0028 (0.0005) x MB-N.
() = standard error of difference.

The hypothesis that the effect of residue input on the quantity of soil organic matter fractions decreases in the order light, intermediate and heavy macroorganic matter was confirmed only for the chaff treatment. Lucerne application increased the intermediate fraction relatively more than the light and heavy fractions, whereas the application of FYM gave the strongest increase in the heavy fraction. I assume that the difference between chaff and lucerne was caused by lucerne being less resistent to decomposition than chaff so the light fraction of lucerne will be transferred to other (heavier) organic fractions more rapidly than that of chaff. The relatively high increase of the heavy fraction in the FYM treatment was due to the different composition of FYM. FYM consists of partly decomposed material; it contains not only light, but also intermediate and heavy organic matter.

In agreement with the hypothesis, the effect of residue input on C-to-N ratios was greater for the light fraction than for the intermediate and heavy fractions, whereas the C-to-N ratios of non-macroorganic matter was hardly affected by residue input. This agrees with the results of Adams (1982) and Christensen (1988) and with the conclusion that clay- and silt-bound organic matter are important in medium and long-term organic matter turnover (Christensen and Sörensen, 1985). It may be concluded that once organics become part of the more stabilized organic matter they have a constant C-to-N ratio, since 25 y of extreme differences in residue input did not affect its C-to-N ratio. The differences in C-to-N ratios between the different residue treatments were greatest for the light fraction, whereas residue application had the same effect on the C-to-N ratios of the intermediate and heavy fractions. This agrees

with the observation that the light fraction consisted mainly of partly decomposed plant residues, whereas the intermediate and heavy fractions contained more processed organomineral-complexed soil organic matter (Greenland and Ford, 1964; Meijboom *et al.*, 1995).

Amounts of light, intermediate and heavy macroorganic matter in grassland soils

The light fraction accounted for 0.9-2.6% of soil C in the top 10 cm of the grassland soils, the intermediate fraction for 1.8-9.4% and the heavy fraction for 4.5-19.4%. These values are considerably lower than those reported by Greenland and Ford (1964) who found that the light fraction accounted for 28.5-31.6% of soil C in two permanent pastures but considerably higher than the results of Warren and Whitehead (1988) who found that the macroorganic matter made up 0.6-3.8% of soil N in the top 15 cm of grassland soils. The difference with the data of Greenland and Ford may be attributed to the lower fractionation density we used: 1.13 instead of 2.0. The lower recovery of macroorganic matter in the study of Warren and Whitehead may be due to the larger mesh size they used (200 μm).

The amounts of the light, intermediate and heavy fractions of the top 10 cm were higher than those of the 10-25 cm layer. The proportion of soil C and N present in the light fraction was larger in the top 10 cm than in the 10-25 cm layer. The declining proportion of organic C and N in the light fraction with increasing depth has also been observed by Spycher *et al.* (1983) and Janzen *et al.* (1992). The proportion of organic C and N present in the intermediate and heavy fractions declined to a lesser extent with depth than in the light fraction. This is related to the concentration in light fraction input in the top layer of grassland soils.

I observed that the proportion of soil organic N present in the light and intermediate fractions decreased significantly with increasing clay + silt content whereas this was not observed for the heavy fraction. This agrees with the results of Greenland and Ford (1964), and it also confirms the conclusion that the light fraction is more important in sandy than in clay soils (Christensen, 1992).

Labile fractions as predictors of C and N mineralization

My second objective was to test the hypothesis that the light fraction and the active microbial biomass are better predictors of C and N mineralization than other (active) soil organic fractions when soils with different textures are compared. The results obtained confirmed this hypothesis.

Light, intermediate and heavy macroorganic matter. A good correlation was found between the amount of C and N in the light fraction and C and N mineralization in grassland soils and N mineralization in the soils from the long-term arable experiment with different inputs of

residues. The importance of the light fraction with respect to C mineralization has been recognized before (Dalal and Mayer, 1987; Janzen, 1987; Skjemstad and Dalal, 1987; Christensen, 1992). The light fraction consists of relatively labile constituents, such as carbohydrates (Skjemstad *et al.*, 1986). Plant structures were still recognizable in the light fraction and they stained intensely with acridine orange, indicating a low degree of decomposition (Meijboom *et al.*, 1995). The degree of decomposition increased in the order light, intermediate, heavy macroorganic matter and silt- and clay-bound organic matter. Organics associated with clay particles consisted of amorphous material that did not stain with acridine orange (Hassink *et al.*, 1993).

In agreement with Janzen (1987) I observed that N mineralization was strongly correlated with the N content of the light fraction. Adams (1980) and Sollins *et al.* (1984) did not find strong correlations between light fraction and N mineralization.

Besides chemical recalcitrance, the biological availability of organics in the soil is determined by the physical occlusion of the organic material (Cambardella and Elliott, 1993). The light fraction has a low ash content, indicating that it is not strongly associated with soil minerals (Meijboom *et al.*, 1995), and hence has a low degree of physical protection. This is supported by the observation that N mineralization per unit of N in light macroorganic matter was not affected by soil texture. I infer that during decomposition the degree of physical protection will increase since the heavy fraction includes the organomineral-complexed soil organic matter. Organics bound to clay and silt particles might be physically protected even more (Dalal and Mayer, 1986).

Although the correlation between the amount of C and N in the light fraction and C and N mineralization was high, the amount of light fraction was too small to account for total C and N mineralization. A substantial part of C and N mineralized must have originated from dying microbial biomass or more stabilized organic matter fractions. This is confirmed by observations that the equations giving the best predictors for C and N mineralization included the microbial biomass, and the microbial biomass and the intermediate fraction, respectively.

Microbial biomass. We observed that although the microbial biomass-FI can be considered as an active organic matter pool, it is not a good indicator of C and N mineralization when different soils are compared. C and N mineralization per unit of microbial biomass-N was significantly ($p < 0.05$) higher in coarse-textured soils than in fine-textured soils (Figs 3 and 4). This suggests that the microbes are more active in sandy soils than in loams and clays. It has been suggested that this is due to the fact that higher proportions of the microbes are physically protected in loams and clays than in sandy soils (Rutherford and Juma, 1992; Hassink *et al.*, 1993). This is in line with the observation that the relative contribution of light and intermediate macroorganic matter (the most labile organic matter pools with the lowest degree of physical protection) to total organic N was greater in coarse-textured soils than in fine-textured soils.

Unlike the total microbial biomass-FI, the correlation of microbial biomass determined by

the SIR method with C and N mineralization was not affected by soil texture. Biomass-SIR can be regarded as a measure of the active microbial biomass (Anderson and Domsch, 1978). We assume that the active microbial biomass is not physically protected in the soil and that the active microbial biomass feeds on labile organic matter fractions such as light and intermediate macroorganic matter, while the remainder of the microbial community, which is less active, feeds on more stabilized organic matter, such as silt- and clay-bound organics.

ACKNOWLEDGEMENTS

I thank L. Brussaard and J.J. Neeteson for their support and helpful discussions.

REFERENCES

Adams T.McM. (1982) The effects of agronomy on C and N distribution in soil organo-mineral fractions. *Journal of Agricultural Science Cambridge* **98**, 335-342.

Anderson J.P.E. and Domsch K.H. (1978) A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biology & Biochemistry* **10**, 215-221.

Cambardella C.A. and Elliott E.T. (1992) Particulate soil organic-matter changes across a grassland cultivation sequence. *Soil Science Society of America Journal* **56**, 777-783.

Cambardella C.A. and Elliott E.T. (1993) Methods for physical separation and characterization of soil organic matter fractions. *Geoderma* **56**, 449-457.

Christensen B.T. (1988) Effects of animal manure and mineral fertilizer on the total carbon and nitrogen contents of soil size fractions. *Biology & Fertility of Soils* **5**, 304-307.

Christensen B.T. (1992) Physical fractionation of soil and organic matter in primary particle size and density separates. *Advances in Soil Science* **20**, 1-89.

Christensen B.T. and Sørensen L.H. (1985) The distribution of native and labelled carbon between soil particle size fractions isolated from long-term incubation experiments. *Journal of Soil Science* **36**, 219-229.

Dalal R.C. and Mayer R.J. (1986) Long-term trends in fertility of soils under continuous cultivation and cereal cropping in Southern Queensland. III Distribution and kinetics of soil organic carbon in particle-size fractions. *Australian Journal of Soil Research* **24**, 293-300.

Dalal R.C. and Mayer R.J. (1987) Long-term trends in fertility of soils under continuous cultivation and cereal cropping in Southern Queensland. VI. Loss of total nitrogen from different particle-size and density fractions. *Australian Journal of Soil Research* **25**, 83-93.

Deys W.B. (1961) The determination of total nitrogen in herbage samples (in Dutch). Instituut voor Biologisch en Scheikundig onderzoek, Wageningen, Jaarboek, Mededelingen 89, 90.

Genstat Manual (1987) *Genstat. A General Statistical Program.* Clarendon, Oxford.

Greenland D.J. and Ford W. (1964) Separation of partially humified organic materials from

soil by ultrasonic dispersion. *Proceedings 8th International Congress of Soil Science*, Bucharest, Vol. III, 137-148.

Hassink J. (1993) Relationship between the amount and the activity of the microbial biomass in Dutch grassland soils: comparison of the fumigation-incubation method and the substrate-induced respiration method. *Soil Biology & Biochemistry* **25**, 533-538.

Hassink J. (1994) Effects of soil texture and grassland management on soil organic C and N and rates of C and N mineralization. *Soil Biology & Biochemistry 26*, 1221-1232.

Hassink J., Lebbink G. and Van Veen J.A. (1991) Microbial biomass and activity of a reclaimed-polder soil under a conventional or a reduced-input farming system. *Soil Biology & Biochemistry* **23**, 507-513.

Hassink J., Bouwman L.A., Zwart K.B., Bloem J. and Brussaard L. (1993) Relationships between soil texture, physical protection of organic matter, soil biota, and C and N mineralization in grassland soils. *Geoderma* **57**, 105-128.

Janzen H.H. (1987) Soil organic matter characteristics after long-term cropping to various spring wheat rotations. *Canadian Journal of Soil Science* **67**, 845-856.

Janzen H.H., Campbell C.A., Brandt S.A., Lafond G.P. and Townley-Smith L. (1992) Light-fraction organic matter in soils from long-term crop rotations. *Soil Science Society of America Journal* **56**, 1799-1806.

Jenkinson D.S. and Ladd J.N. (1981) Microbial biomass in soil: measurement and turnover. In *Soil Biochemistry* (E.A. Paul and J.N Ladd, Eds), pp 415-471. Marcel Dekker, New York.

Jenkinson D.S. and Powlson D.S. (1976) The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. *Soil Biology & Biochemistry* **8**, 209-213.

Mebius L.J. (1960) A rapid method for the determination of organic carbon in soil. *Analytica Chimica Acta* **22**, 120-124.

Meijboom F., Hassink J. and van Noordwijk M. (1995) Density fractionation of soil organic matter using silica suspensions. Accepted by *Soil Biology & Biochemistry*.

Rutherford P.M. and Juma N.G. (1992) Influence of texture on habitable pore space and bacterial-protozoan populations in soil. *Biology and Fertility of Soils* 12, 221-227.

Shaymukhavetov M.S., Titova N.A., Travnikova L.S. and Labenets Y.M. (1985) Use of physical fractionation methods to characterize soil organic matter. *Soviet Soil Science* **16**, 117-128.

Skjemstad J.O. and Dalal R.C. (1987) Spectroscopic and chemical differences inorganic matter of two Vertisols subjected to long periods of cultivation. *Australian Journal of Soil Research* **25**, 323-335.

Skjemstad J.O., Dalal R.C. and Barron P.F. (1986) Spectroscopic investigations of cultivation effects on organic matter of Vertisols. *Soil Science Society of America Journal* **50**, 354-359.

Sollins P., Spycher G. and Glassman C.A. (1984) Net nitrogen mineralization from light- and heavy-fraction forest soil organic matter. *Soil Biology & Biochemistry* **16**, 31-37.

Spycher G., Sollins P. and Rose S. (1983) Carbon and nitrogen in the light fraction of a forest soil: Vertical distribution and seasonal patterns. *Soil Science* **135**, 79-87.

Tiessen H. and Stewart J.W.B. (1983) Paricle-size fractions and their use in studies of soil organic matter: II. Cultivation effects on organic matter composition in size fractions. *Soil Science Society of America Journal* **47**, 509-514.

Tiessen H., Stewart J.W.B. and Hunt H.W. (1984) Concepts of soil organic matter transformations in relation to organo-mineral particle size fractions. *Plant and Soil* **76**, 287-295.

Van Veen J.A. and Kuikman P.J. (1990) Soil structural aspects of decomposition of organic matter by microorganisms. *Biogeochemistry* **11**, 213-233.

Verberne E.L.J., Hassink J., De Willigen P., Groot J.J.R. and Van Veen J.A. (1990) Modelling organic matter dynamics in different soils. *Netherlands Journal of Agricicultural Science* **38**, 221-238.

Warren G.P. and Whitehead D.C. (1988) Available soil nitrogen in relation to fractions of soil nitrogen and other soil properties. *Plant and Soil* **112**, 155-165.

Young J.L. and Spycher G. (1979) Water dispersible soil organic-mineral particles. I. Carbon and nitrogen distribution. *Soil Science Society of America Journal* **43**, 324-328.

CHAPTER 8

DECOMPOSITION RATE CONSTANTS OF SIZE AND DENSITY FRACTIONS OF SOIL ORGANIC MATTER

By: J. Hassink. Submitted to Soil Science Society of America Journal

DECOMPOSITION RATE CONSTANTS OF SIZE AND DENSITY FRACTIONS OF SOIL ORGANIC MATTER

ABSTRACT

One of the main drawbacks of models describing soil organic matter turnover is that most pools can not be determined directly. The aim of this study was to define meaningful soil organic matter fractions that can be determined directly and to determine their decomposition rate constants so that they can be incorporated into models. This would be a major step towards verification of models and the revision of inherent concepts. In the experiments soil organic matter was separated into size and density fractions using silica suspensions as heavy liquid. Two size and three density fractions were distinguished: C in microaggregates with diameters < 20 μm and 20-150 μm, and C in light (density < 1.13 g cm^{-3}), intermediate (density 1.13-1.37 g cm^{-3}) and heavy (density > 1.37 g cm^{-3}) fractions of the macroorganic matter pool (> 150 μm). The decomposition rate constants of the fractions decreased in the order light, intermediate and heavy macroorganic matter (23.9, 9.8 and 3.9 * 10^{-4} day^{-1}, respectively) and were lowest for C in the microaggregate fractions < 20 μm and 20-150 μm (0.5-0.7 * 10^{-4} day^{-1}). Since the rate constants of the fractions seemed not to depend on soil texture, they can probably be applied widely. We propose to use these fractions as the pools in future soil organic matter models.

INTRODUCTION

The first models describing soil organic matter dynamics have been constructed more than 15 years ago (Jenkinson and Rayner, 1977; Van Veen, 1977). Since then many mathematical and simulation models have been published, all being modifications of the first two, and no major advances seem to have been achieved since then. Generally, organic matter dynamics models include pools with a rapid turnover rate and pools with a slower turnover rate (Van Veen and Paul, 1981; Molina et al., 1983; Van Veen et al., 1984; Parton et al., 1987; Verberne et al., 1990). One of the main problems is that pools in organic matter models are mostly defined on a functional basis (degree of chemical or physical stabilization) and that, except for microbial biomass, they can not be determined directly by chemical or physical analytical fractionation procedures (Paustian et al., 1992). Successful development of techniques for direct measurement of pool sizes would represent a major step towards appropriate verification of models and the revision of inherent concepts (Bonde et al., 1992).

Chemical fractionation has not proven particularly useful in following the dynamics of organic material in soils (Duxbury et al., 1989). Physical fractionation of soil organic matter is considered less destructive, and the results obtained from physical soil fractions are anticipated to relate more directly to the structure and function of soil organic matter in situ

(Christensen, 1992). Macroorganic matter (> 150 μm) decomposes much faster than organic C in clay and silt size fractions (Dalal and Mayer, 1986; Tiessen and Stewart, 1983; Tiessen et al., 1984). Macroorganic matter contains a light and a heavy fraction. The light fraction consists of partly decomposed plant residues, which has a rapid turnover and is hardly associated with soil minerals and hence has a low degree of physical protection. The heavy fraction consists of relatively more processed material, and has a slower turnover rate (Greenland and Ford, 1964; Christensen, 1992) and a higher degree of physical protection (Hassink, 1995). Soil organic matter incorporated into microaggregates and adsorbed on or coated by clay particles has a high degree of physical protection against microbial degradation (Tisdall and Oades, 1982; Golchin et al., 1994). Microaggregates are considered to be the basic structural units in soils (Tisdall and Oades, 1982) containing a large part of the most stable organic matter in soil (Skjemstad et al., 1993). It is known that silt and clay particles occur together in microaggregates (Anderson and Paul, 1984).

Although it is accepted that physical fractionation yields functional soil organic matter fractions, models containing pools that are based on physical fractionation have not been developed so far. This might be due to the uncertainty of how the widely proposed techniques can be applied (Christensen, 1992) and to the lack of data estimating decomposition rates of the individual fractions.

Recently, a relatively simple size and density fractionation procedure, using stable silica to obtain solutions varying in density, has been proposed by Meijboom et al. (1995). In this procedure, soil is wet-sieved and macroorganic matter (> 150 μm) is separated into a light, an intermediate and a heavy fraction. Stable silica has advantages over the commonly used aqueous solutions of inorganic salts such as polytungstate; it does not penetrate the organic matter fractions, it is relatively cheap, not toxic and the density separation is less time-consuming (Meijboom et al., 1995; Hassink et al., 1995). The wet-sieving procedure includes a destruction of macroaggregates (> 250 μm), whereas microaggregates are left intact. This was postulated to be a sensible and reproducible way to treat soil samples, since according to Tisdall and Oades (1982) macroaggregates can be destroyed by agricultural practices, whereas microaggregates can not. Organics inside microaggregates would be largely protected against microbial degradation under normal agricultural practices. The proposed procedure is therefore assumed to prevent the release of organic matter that is incorporated into microaggregates (Gregorich et al., 1989).

In the present study soil organic matter was separated into five fractions: the light, intermediate and heavy fractions of the macroorganic matter pool (Meijboom et al., 1995), the microaggregate fractions with particle sizes between 20 and 150 μm and smaller than 20 μm. The study included fine-and coarse-textured soils. The first aim was to establish rate constants for the decomposition of these fractions. The second aim was to investigate whether soil texture affects the decay constants of the individual fractions.

MATERIALS AND METHODS

The decomposition rates of soil organic matter fractions were studied in two ways.

I. First it was tested whether the decomposition rate of size and density fractions were significantly different. To test this, samples were taken from the top 10 cm of a sandy (Tynaarlo), loamy (Burum) and clay (Finsterwolde) grassland soil, in March 1993, which had been under grass for at least 10 years; all sites were situated in the Netherlands. Characteristics of the soils are given in Table 1. Of each soil three coarsely sieved field-moist samples (500 g) each consisting of 20 bulked cores were used for fractionation. Rates of C mineralization of the isolated fractions and total soil were determined after incubating them at 20 °C and a water potential close to -10 kPa. C mineralization was measured by incubating soils and fractions in 1.5-l airtight jars containing a vial with 10 ml 0.5 M NaOH. The trapped CO_2 was precipitated as carbonate with excess $BaCl_2$ and the excess NaOH was titrated with 0.5 M HCl (Hassink, 1994). C mineralization was determined 7, 14, 21, 33, 45 and 67 days after the start of the incubation. The decomposition rates of the soil organic matter fractions were compared by expressing C mineralization as the percentage of C in each fraction which was mineralized to CO_2.

Table 1. Some characteristics of the top 10 cm of the grassland soils that were sampled in 1993.

	C (%)	N (%)	pH-KCl	Granular composition, % particles <	
				20 µm	50 µm
Tynaarlo	3.80	0.20	4.3	5.2	22.3
Burum	5.35	0.55	4.8	36.5	71.7
Finsterwolde	3.25	0.32	7.1	65.8	85.1

II. The rate constants of decomposition of the particle size and density fractions were determined by measuring the decrease of C in these fractions in a sandy and a clay soil that were kept bare for 15 years. In May 1961, 50 tonnes C per ha was mixed through the top 25 cm of a sandy and a clay soil by the application of lucerne. From then on the soils were kept bare till the last sampling in November 1976. Individual plots were 0.75 by 0.75 m, separated by concrete frames, sunk 25 cm into the soil. The plots were laid out in triplicate. Every spring the plots were emptied to a depth of 25 cm, the soil was mixed and the plots were refilled. In November 1961 and at later points in time till the last sampling in November 1976, samples were taken down to a depth of 20 cm. The samples were analyzed for total N and C. Stored samples that had been taken from November 1961 on were fractionated and the amount of C present in the fractions was determined. The amount of C present in each fraction at the different sampling times was expressed as the percentage of C initially present in the same fraction. November 1961 was taken as the starting point. The experiment was

located in Groningen, the Netherlands. Mean annual precipitation at the site is 780 mm and the mean annual temperature is 8.6 °C (KNMI, 1992). Characteristics of the soils at the start of the experiment in 1961 and the lucerne are presented in Table 2.

Table 2. Some characteristics of the top 25 cm of the sandy and clay soil at the beginning of the long-term experiment in 1961 and of the lucerne that was mixed through these soils in 1961.

	C (%)	N (%)	pH-KCl	Granular composition, % particles <	
				20 µm	50 µm
Sand	0.70	0.09	7.40	17.0	26.0
Clay	2.22	0.20	6.97	65.6	71.9
Lucerne	43.6	2.50			

Size and density fractionation of soil organic matter

Of the grassland soils field-moist samples were used for fractionation; the dried soil samples of the long-term experiment that started in 1961 were rewetted before fractionation. Samples of 250 g were washed on two sieves (top sieve: mesh size 250 µm; bottom sieve: 150 µm). The soil was pushed through the top sieve, till the water passing the sieve became clear. In this way all macroaggregates were destroyed. The mineral fraction was discarded by decantation, and after combining the organic fractions from both sieves, it was further fractionated in silica suspensions with a density of 1.13 and 1.37 g cm^{-3} as described by Meijboom et al. (1995). The organic matter recovered on both sieves (diameter > 150 µm) will be referred to as macroorganic matter. The macroorganic matter was separated into three fractions: a light fraction (density < 1.13 g cm^{-3}); an intermediate fraction (density between 1.13 and 1.37 g cm^{-3}) and a heavy fraction (density > 1.37 g cm^{-3}).

Samples of 50 g were washed on three sieves (top sieve: mesh size 250 µm; second sieve: 150 µm; bottom sieve 20 µm). The suspension passing the bottom sieve of 20 µm was collected in a bucket. The soil was pushed through the top sieve again (destroying the macroaggregates), till the water passing the sieve became clear. The material accumulating on the 20 µm sieve will be referred to as particle size fraction 20-150 µm. The suspension passing the 20 µm sieve was placed at 4 °C till it had sedimented (usually after 24 hours) and the clear solution was sucked of; this fraction will be referred to as particle size fraction < 20 µm. It was assumed that the particle size fractions 20-150 µm and < 20 µm primarily contained microaggregates as microaggregates are resistent to wet-sieving (Tisdall and Oades, 1982). All fractions were dried at 40 °C and analyzed for total C and N.

Chemical analysis

Total C in soil, macroorganic matter fractions and the particle size fraction 20-150 µm was defined as dichromate-oxidizable C according to Kurmies (Mebius, 1960). Total N in soil and those fractions was determined according to Deys (1961) by destruction with sulfuric acid and salycylic acid. Total C and N in the particle size fraction < 20 µm was determined with a CHN autoanalyzer (Carlo Erba NA 1500).

Calculation of first-order decomposition rate constants

The data of the long-term experiment with the bare sandy and clay soil were used to estimate decomposition rate constants. The amounts of C in the particle size and density fractions were assumed to decay exponentially, which is expressed by first-order decay rates (Paul and Clark, 1989). The exponential decay curves of the organic matter fractions were estimated by using Genstat (Genstat Manual, 1987). First, the sandy and clay soil were analyzed separately. When the rate constants were not significantly different ($p < 0.05$), the data of both soils were pooled before analysis.

Statistical analysis

Student's t-test was used to assess differences in C mineralization between soil organic matter fractions.

RESULTS

I. C mineralization of organic matter fractions of grassland soils

The rates of C mineralization were expressed as the cumulative percentage of C present in a fraction that was mineralized. The C mineralization rates for each fraction were not significantly different between the sandy, loam and clay grassland soils. Therefore, the average results of the three soils are presented.

C mineralization (% of C mineralized) differed significantly ($p < 0.05$) between the light, intermediate and heavy macroorganic matter fractions and the fractions < 150 µm during the total period of incubation, and decreased in the order light > intermediate > heavy macroorganic matter. Rates of C mineralization were lowest for the fractions 20-150 µm and < 20 µm and total soil C, and did not differ significantly from each other ($p < 0.05$; Fig. 1).

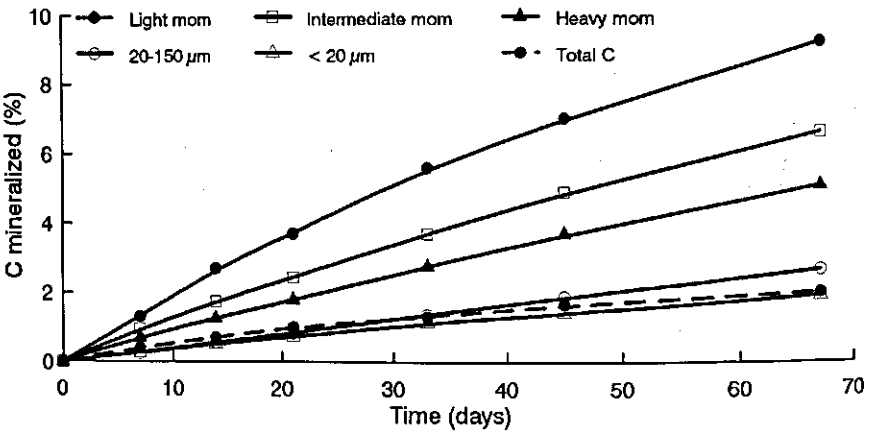


Fig. 1. Cumulative C mineralization of the light, intermediate and heavy macroorganic matter (mom) fractions, and the particle size fractions 20-150 µm and < 20 µm. Average value of a sandy, a loam and a clay soil. C mineralization is expressed as the percentage of C in the fraction mineralized to CO_2.

II. Decrease in the amounts of C in the organic matter fractions of the long-term field experiment and estimation of the decomposition rate constants

In agreement with the C mineralization rates of the fractions of the grassland soils, the decomposition rates of light, intermediate and heavy macroorganic C, and C in the fraction < 20 µm were not significantly ($p < 0.05$) different for the sandy and clay soil (Figs. 2, 3). For the sandy soil it was not possible to determine the decomposition rate of C in the fraction 20-150 µm, as the amount increased during the first years of the experiment. Hence, decomposition rate constants were calculated after combining the results of both soils, except for the fraction 20-150 µm where only the decomposition rates of the clay soil were used.

Also in agreement with the C mineralization rates of the fractions of the grassland soils, the rate constants of decomposition decreased in the order light (0.24 % day^{-1}) > intermediate (0.098 % day^{-1}) > heavy (0.039 % day^{-1}) macroorganic matter. The differences between the fractions were statistically signficicant ($p < 0.05$) (Fig. 2; Table 3). Decomposition rates of C in the fractions 20-150 µm and < 20 µm were significantly lower (0.005-0.007 % day^{-1}) than in the macroorganic matter fractions and did not differ significantly from each other ($p < 0.05$; Fig. 3; Table 3). The decomposition rates of total soil organic C did not differ significantly ($p < 0.05$) between the sandy soil and clay; they were similar to the C decomposition rates in the fractions 20-150 µm and < 20 µm (Table 3; Fig. 4).

For all fractions, the fitted first-order decomposition curves were close to the observed data (87 to 99 % of the variation in residual C was explained; Table 3).

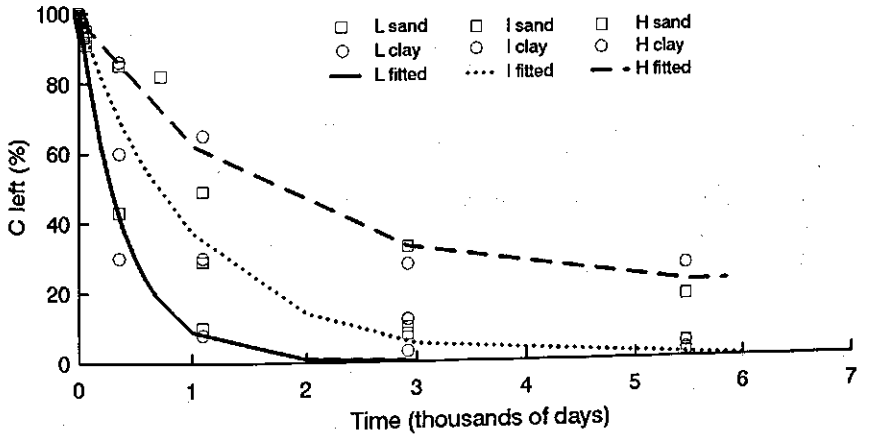


Fig. 2. Residual organic C (% of original amount) in the light (L), intermediate (I) and heavy (H) macroorganic matter fractions of the sandy and clay soil after keeping the soils bare.

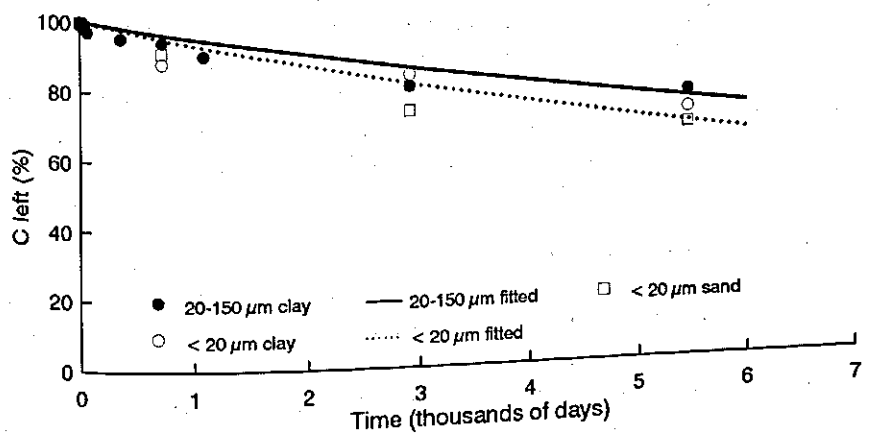


Fig. 3. Residual organic C (% of original amount) in the particle size fractions 20-150 μm and < 20 μm of the sandy and clay soil after keeping the soils bare.

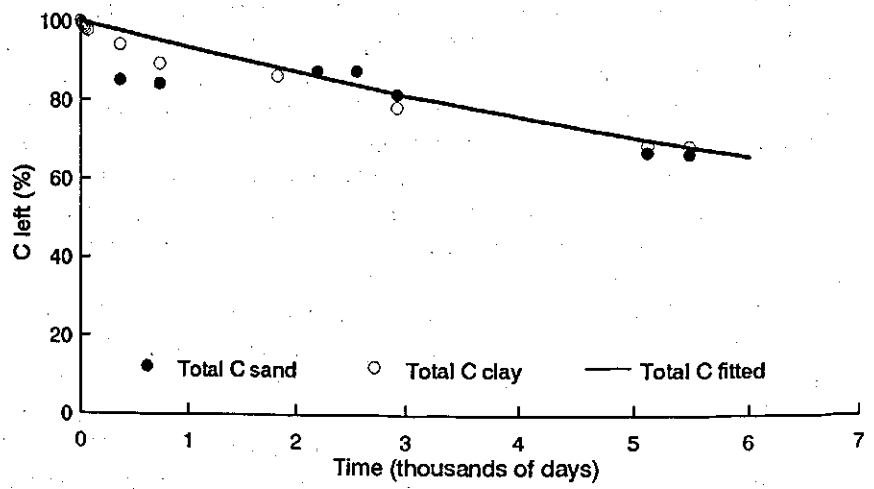


Fig. 4. Residual total amount of organic C (% of original amount) in the sandy and clay soil after keeping the soils bare.

Table 3. Decay rate constants of the soil organic matter fractions under field conditions. The results of the sandy soil and clay were combined.

Soil organic matter fraction	k value (day^{-1})		% of variance explained
Light mom†	0.00239	(0.0001863)	98.7
Intermediate mom	0.000984	(0.0000957)	97.8
Heavy mom	0.0003943	(0.0000397)	96.0
20-150 µm	0.000052	(0.0000045)	93.2
< 20 µm	0.0000707	(0.0000086)	86.6
Total soil C	0.0000713	(0.0000053)	90.2

() = Standard error
† mom = macroorganic matter (> 150 µm)

DISCUSSION

By measuring C mineralization rates of the size and density fractions that were isolated from grassland soils it was shown that the decomposition rates decreased in the order light > intermediate > heavy macroorganic matter and that decomposition rates of C in the particle size fractions 20-150 µm and < 20 µm (microaggregates) were lowest. The fractionation of

soil organic matter of the sandy and clay soil that were kept bare for a long time enabled us to estimate the decomposition rate constants of the particle size and density fractions. These constants also decreased in the order light > intermediate > heavy macroorganic matter > 20-150 μm and < 20 μm (microaggregates). In addition, it was found that the decomposition rate of the particle size and density fractions did not differ between the sandy and clay soil. This is in line with the assumptions of Van Veen et al. (1985) and Verberne et al. (1990). The fact that soil texture did not affect microbial availability of C in a certain fraction shows that the fractionation procedure can be widely applied. It may be concluded that the generally observed lower turnover of organic matter in fine-textured soils than in coarse-textured soils is due to a different distribution of soil organic C over fractions with a high and lower decomposition rate and not to differences in decomposition rates of the fractions.

The initial decrease in total organic C was somewhat higher in the sandy soil than the clay (Fig. 4), probably due to the higher percentage of soil C that was present in macroorganic matter fractions. During later periods, differences disappeared. In line with this it has been found that in fine-textured soils a greater part of soil C is present in fractions with a low decomposition rate (fractions that contain physically protected organic matter) than in coarse-textured soils (Hassink, 1995).

In many fractionation studies, the fate of soil C associated with primary particles (sand, silt and clay) was studied (Tiessen and Stewart, 1983; Balesdent et al., 1988; Bonde et al., 1992). There are conflicting data concerning the decomposition rates of clay-associated and silt-associated organic C. It is often found that silt-associated C is more stable than clay-associated C (Christensen, 1987); however, the opposite has also been observed (Gregorich et al., 1989; Bonde et al., 1992). The discrepancies probably relate to the use of soil sonication. To obtain primary particles, soils are usually dispersed ultrasonically and a variable percentage of the microaggregates is destroyed and a variable percentage of organic matter is redistributed among size fractions (Gregorich et al., 1988; Elliott and Cambardella, 1991).

The basic structural units in soils are considered to be microaggregates that are water-stable and not affected by agricultural practices (Edwards and Bremner, 1967; Tisdall and Oades, 1982). Microaggregates protect organic matter against microbial degradation (Tisdall and Oades, 1982). Destruction of microaggregates leads to a considerable increase in mineralization (Gregorich et al., 1989). By leaving these basic units intact, estimations of C mineralization rates are more realistic than estimations of C mineralization of primary particles. Silt-sized aggregates (2-20 μm) contain a major part of the most stable organic matter in soil (Skjemstad et al., 1993). It is likely that silt and coarse and fine clay occur together in microaggregates (Anderson and Paul, 1984). Aggregates of 20-250 μm diameter consist mainly of particles with diameters between 2-20 μm bonded together (Tisdall and Oades, 1982).

The decomposition rate constants that were estimated assuming first order decay are net decay rates. When part of the labile material (light > 150 μm) is transformed to more stable fractions during decomposition, gross decay rates of the more stable fractions are

underestimated. As the rate constants of the particle size fractions 20-150 µm and < 20 µm were, however, close to the rate constant of total soil organic C, any underestimations must have been small. A study of the fate of labelled material that is applied to the soil may reveal whether significant fractions of labile material are transformed to more stable fractions.

The percentage of soil C present in the light, intermediate and heavy macroorganic matter fractions in the top 10 cm of grassland soils were approximately 1.5, 5 and 13 %, respectively (Hassink, 1995). So approximately 80% of the total amount of soil C was present in microaggregates. The decay rate constants of the size and density fractions described in this study in some cases showed a very good correlation with the rate constants of non-measurable pools in organic matter models: the rate constant of the heavy macroorganic matter fraction was similar to the rate constant of the active physically protected organic matter pool in the model of Van Veen et al. (1985) and the chemically stabilized pool in the model of Jenkinson and Rayner (1977). The percentage of soil C present in the heavy macroorganic matter fraction (13%) was, however, considerably lower. The rate constants of organic C in the particle size fractions 20-150 µm and < 20 µm and the percentage soil C present in these fractions were close to the corresponding values of the stable soil organic matter pool in the NCSOIL model of Nicolardot and Molina (1994).

For the analysis of organic matter dynamics in soil we propose to distinguish four soil organic C fractions: C in light, intermediate and heavy macroorganic matter (> 150 µm) fractions and C incorporated in microaggregates (< 150 µm), and to use these units in organic matter dynamics models. Our study showed that the decomposition rate constants of the fractions were not affected by soil texture. Future research should be directed to the development of models based on physical fractionation and on the analysis of decay constants of those fractions in other soil ecosystems.

ACKNOWLEDGEMENTS

I thank L. Brussaard, J.J. Neeteson, P.C. de Ruiter and K.B. Zwart for critically reading the manuscript and helpful discussions.

REFERENCES

Anderson, D.W., and E.A. Paul. 1984. Organo-mineral complexes and their study by radiocarbon dating. Soil Sci. Soc. Am. J. 48:298-301.

Balesdent J., G.H. Wagner, and A. Mariotti. 1988. Soil organic matter turnover in long-term field experiments as revealed by carbon-13 natural abundance. Soil Sci. Soc. Am. J. 52:118-124.

Bonde T.A., B.T. Christensen, and C.C. Cerri. 1992. Dynamics of soil organic matter as reflected by natural ^{13}C abundance in particle size fractions of forested and cultivated

oxisols. Soil Biol. Biochem. 24:275-277.
Christensen, B.T. 1987. Decomposition of organic matter in particle size fractions from field soils with straw incorporations. Soil Biol. Biochem. 19:125-135.
Christensen, B.T. 1992. Physical fractionation of soil and organic matter in primary particle size and density separates. Adv. Soil Sci. 20:1-89.
Dalal, R.C., and R.J. Mayer. 1986. Long-term trends in fertility of soils under continuous cultivation and cereal cropping in Southern Queensland. III Distribution and kinetics of soil organic carbon in particle-size fractions. Austr. J. Soil Res. 24:293-300.
Deys, W.B. 1961 The determination of total nitrogen in herbage samples (in Dutch). Instituut voor Biologisch en Scheikundig onderzoek, Wageningen, Jaarboek, Mededelingen 89, 90.
Duxbury, J.M., M.S. Smith, and J.W. Doran. 1989. Soil organic matter as a source and a sink of plant nutrients. p. 33-67. *In* D.C. Coleman, J. M. Oades and G. Uehara (ed.) Dynamics of soil organic matter in tropical ecosystems. University of Hawaii Press, Honolulu.
Edwards, A.P., and J.M. Bremner. 1967. Microaggregates in soils. J. Soil Sci. 18:64-73.
Elliott, E.T., and C.A. Cambardella. 1991. Physical separation of soil organic matter. Agric. Ecosyst. Environ 34:407-419.
Genstat Manual. 1987. Genstat. A General Statistical Program. Clarendon, Oxford.
Gochin, A., J.M. Oades, J.O. Skjemstad, and P. Clarke. 1994. Soil structure and carbon cycling. Aust. J. Soil Res. 32:1043-1068.
Greenland, D.J., and W. Ford. 1964. Separation of partially humified organic materials from soil by ultrasonic dispersion. Vol. III, p. 137-148. Proc. 8th Int. Congr. Soil Sci., Bucharest.
Gregorich, E.G., R.G. Kachanoski, and R.P. Voroney. 1988. Ultrasonic dispersion of aggregates: distribution of organic matter in size fractions. Can. J. Soil Sci. 68:395-403.
Gregorich, E.G., R.G. Kachanoski, and R.P. Voroney. 1989. Carbon mineralization in soil size fractions after various amounts of aggregate disruption. J. Soil Sci. 40:649-659.
Hassink, J. 1994. Effects of soil texture and grassland management on soil organic C and N and rates of C and N mineralization. Soil Biol. Biochem. 26:1221-1231.
Hassink, J. 1995. Density fractions of soil macroorganic matter and microbial biomass as predictors of C and N mineralization. Accepted by Soil Biol. Biochem.
Hassink, J., J. Magid, and F.W. Meijboom. 1995. Density fractionation of macroorganic matter using silica suspensions and polytungstate. Submitted to Soil Biol. Biochem.
Jenkinson, D.S., and J.H. Rayner. 1977. The turnover of soil organic matter in some of the Rothamsted classical experiments. Soil Sci. 123:298-305.
KNMI. 1992. Climatological data of stations in the Netherlands for the period 1961-1990. Ministry of Transport, Public works and Water Management. De Bilt, the Netherlands. (In Dutch).
Mebius, L.J. 1960. A rapid method for the determination of organic carbon in soil. Anal. Chim. Acta 22:120-124.
Meijboom, F.W., J. Hassink, and M. van Noordwijk. 1995. Density fractionation of soil macroorganic matter using silica suspensions. Accepted by Soil Biol. Biochem.

Molina, J.A., C.E. Clapp, M.J. Shaffer, F.W. Chichester, and W.E. Larson. 1983. NCSOIL, A model of nitrogen and carbon transformations in soil: description, calibration, and behavior. Soil Sci. Soc. Am. J. 47:85-91.

Nicolardot, B., and J.E.A. Molina. 1994. C and N fluxes between pools of soil organic matter: Model calibration with long-term field experimental data. Soil Biol. Biochem. 26:245-251.

Parton, W.J., D.S. Schimel, C.V. Cole, and D.S. Ojima. 1987. Analysis of factors controlling soil organic matter levels in great plains grasslands. Soil Sci. Soc. Am. J. 51:1173-1179.

Paul, E.A., and F.E. Clark. 1989. Soil microbiology and biochemistry. Academic Press, London.

Paustian, K., W.J. Parton, and J. Persson. 1992. Modelling soil organic matter in organic-amended and nitrogen fertilized long-term plots. Soil Sci. Soc. Am. J. 56:476-488.

Skjemstad, J.O., L.J. Janik, M.J. Head, and S.G. McClure. 1993. High energy ultraviolet photo-oxidation: a novel technique for studying physically protected organic matter in clay- and silt-sized aggregates. J. Soil Sci. 44:485-499.

Tiessen, H., and J.W.B. Stewart. 1983. Particle-size fractions and their use in studies of soil organic matter: II. Cultivation effects on organic matter composition in size fractions. Soil Sci. Soc. Am. J. 47:509-514.

Tiessen, H., J.W.B. Stewart, and H.W. Hunt. 1984. Concepts of soil organic matter transformations in relation to organo-mineral particle size fractions. Plant and Soil 76:287-295.

Tisdall, J.M., and J.M. Oades. 1982. Organic matter and water stable aggregates in soils. J. Soil Sci. 33: 141-163.

Veen, J.A. van, 1977. The behavior of nitrogen in soil. A computer simulation model. Ph.D. Thesis, V.U. Amsterdam.

Veen, J.A. van, and E.A. Paul. 1981. Organic carbon dynamics in grassland soils. I. Background information and computer modelling. Can. J. Soil Sci. 61:185-201.

Veen, J.A. van, J.N. Ladd, and M.J. Frissel. 1984. Modelling C and N turnover through the microbial biomass in soil. Plant and Soil 76:257-274.

Veen, J.A. van, J.N. Ladd, and M. Amato. 1985. Turnover of carbon and nitrogen through the microbial biomass in a sandy loam and a clay soil incubated with [^{14}C(U)glucose and [^{15}N]$(NH_4)_2SO_4$ under different moisture regimes. Soil Biol. Biochem. 17:747-756.

Verberne, E.L.J., J. Hassink, P. De Willigen, J.J.R. Groot, and J.A. van Veen. 1990. Modelling organic matter dynamics in different soils. Neth. J. Agric. Sci. 38:221-238.

CHAPTER 9

DECOMPOSITION AND TRANSFER OF PLANT RESIDUE ^{14}C BETWEEN SIZE AND DENSITY FRACTIONS IN SOIL

By: J. Hassink and J.W. Dalenberg. Submitted to Soil Biology & Biochemistry

DECOMPOSITION AND TRANSFER OF PLANT RESIDUE ^{14}C BETWEEN SIZE AND DENSITY FRACTIONS IN SOIL

ABSTRACT

The aim of our study was to follow the transfer of ^{14}C-labeled ryegrass between size and density fractions of soil organic matter in a sandy and a loam soil. Our hypotheses were a) that the applied ^{14}C would be transferred from light and soluble fractions to intermediate and heavy macroorganic matter fractions and finally become stabilized in microaggregates, and b) that the physical protection of ^{14}C associated with microaggregates against decomposition would decrease with increasing saturation of the microaggregates with soil organic matter.

Generally, the hypotheses were confirmed. Immediately after application most of the label was present in the soluble and light macroorganic matter fractions. Newly synthesized microbial biomass fed on the labeled components of the fractions. The amounts of ^{14}C in the soluble and light macroorganic matter fractions decreased rapidly, while the amounts of ^{14}C in the intermediate and heavy macroorganic matter fractions and in microaggregates remained more or less stable. At the end of the incubation most of the residual soil ^{14}C was found in the microaggregates. In the sandy soil ^{14}C was concentrated in the 20-150 μm fraction, whereas in the loam a larger proportion was present in the < 20 μm fraction.

The mineralization rates of ^{14}C-labeled material were similar in the light, intermediate and heavy fractions of macroorganic matter and in the microaggregates 0 and 180 days after the application of ^{14}C-labeled ryegrass. In all fractions, ^{14}C mineralized more rapidly than total C. The results indicate that considerable amounts of ^{14}C must have transferred from the soluble and light macroorganic matter fractions and newly synthesized microbial biomass to the intermediate and heavy macroorganic matter fractions and the microaggregates, and that ^{14}C was not yet physically protected against microbial degradation during the whole incubation period. The degree of physical protection of ^{14}C against decomposition in the microaggregate fraction < 20 μm was negatively correlated with the degree of saturation of this particle size fraction with soil organic matter.

INTRODUCTION

The fate of plant residues incorporated into the soil has been studied extensively. In most studies the aim was to quantify the effects of resource quality, environmental factors and soil texture on the decomposition and stabilization of residue C (e.g. Jenny et al., 1949; Van Cleve, 1974; Amato and Ladd, 1992). The transformations of residue C in the soil, however, have received little attention. It has been observed that plant material that enters the soil as particulate organic matter is colonized by the microbial community, adsorbed by mineral

particles and reduced in particle size by abiotic factors or by the feeding activity of decomposer organisms (Swift et al., 1979; Golchin et al., 1994). Soluble matter can be adsorbed on mineral surfaces, immobilized by the microbial biomass or leached (Swift et al., 1979; Tiessen et al., 1984). During the last decades it has been recognized that soil structure exerts a dominant control over the stabilization of organic matter in soil (Van Veen and Kuikman, 1990) and it is generally accepted that most soil organic matter is finally protected by their association with clay and silt particles and by their location in microaggregates (Tisdall and Oades, 1982; Skjemstad et al., 1993; Golchin et al., 1994).

In most models that describe organic matter dynamics it is assumed that all plant residues (except lignin-like components) that enter the soil must pass the microbial biomass that partly mineralizes them, partly converts the residues into new products (Van Veen et al., 1984) and that residue C remaining in the soil is gradually transferred from labile pools to more stabilized pools.

Although the combination of size and density fractionation, measurements of the soil microbial biomass and isotope techniques would be a major step towards verification of concepts behind organic matter models (Bonde et al., 1992), the combination of these techniques has not been used yet in experiments studying the fate of plant residues.

Recently, a relatively simple size and density fractionation procedure using stable silica to produce solutions of different densities has been proposed to distinguish soil organic matter fractions (Meijboom et al., 1995; Hassink, 1995[a,b]). This technique separates macroorganic matter (> 150 μm) into a light fraction containing partly decomposed plant residues that are not yet associated with soil minerals, an intermediate fraction containing organic matter of which the plant structure is less clear, and a heavy fraction consisting of more processed decomposition products in which plant structure can no longer visually be recognized (Greenland and Ford, 1964; Meijboom et al., 1995). Microaggregates of 0-20 μm and 20-150 μm size were found to contain the most stable organic matter fractions (Hassink, 1995[b]). The application of labeled residues enables us to determine to what extent organic C is transferred from one soil organic matter pool to another.

In the present study we applied ^{14}C-labeled grass to a sandy and a loam soil and at various times after application we determined C and ^{14}C in seven soil organic C fractions: microbial biomass, soluble fraction, light, intermediate and heavy macroorganic matter and microaggregates (< 20 μm and 20-150 μm). The first objective of this study was to determine whether and to what extent residue C was transferred from one pool to another in soils of contrasting texture.

We hypothesized that irrespective of soil texture most ^{14}C would be found in the light macroorganic matter and soluble fraction immediately after application of plant residues, before gradually transferring from these labile fractions to intermediate and heavy macroorganic matter and becoming more and more protected in microaggregates as decomposition proceeeds. In the long term we expected most of the applied ^{14}C to be associated with clay and silt particles in the microaggregate fractions.

Changes in the amounts of ^{14}C in a fraction are the result of decay of ^{14}C in this fraction,

and the supply of ^{14}C to this fraction from more labile fractions. This means that the transfer of ^{14}C between fractions can be considerably larger than the observed net changes of ^{14}C in a fraction. To estimate the gross decay rates of ^{14}C in the different fractions, the isolated fractions of the sandy soil at day 0 and day 180 were incubated for determination of the mineralization rates.

Soil organic matter can be preserved by its association with clay and silt particles and by its location in microaggregates. Organic matter associated with clay and silt particles and microaggregates can be a heterogeneous pool of soil organic matter (Elliott and Cambardella, 1991; Matus, 1995). Studies with pure clays showed that adsorption of organics to clays decreases as the coverage of the clay surface with soil organic matter increases (Stotzky, 1986). It has been suggested that organics can form different layers around clay and silt particles and aggregates. Organics in external layers are less protected against decomposition than organics in internal layers (Skjemstad et al., 1993; Buyanovski et al., 1994). In a previous study the maximum amount of organic matter that can be associated with clay and silt particles in the soil was determined (Hassink, 1995[c]).

The second objective of this study was to test whether the degree of saturation of the clay and silt particles (< 20 μm) with soil organic matter affects the decomposition rate of ^{14}C that is associated with microaggregates with diameters < 20 μm. To test this, ^{14}C-labeled grass was applied to soils that differed in the degree of saturation of the clay and silt particles with organic matter (Hassink, 1995[d]). The microaggregate fractions < 20 μm were isolated 53 days after the application of ^{14}C, they were reincubated and the mineralization rates of C and ^{14}C in these fractions were determined.

MATERIALS AND METHODS

Experiment on residue C transfer between fractions and decomposition of residue C in these fractions

Dried, uniformely ^{14}C-labeled *Lolium perenne* shoot material was cut into pieces of approximately 1 cm length and mixed through sieved (0.008 m mesh size) soil, 150 mg per 100 g, taken from the top 10 cm of a sandy and a loam soil with a water potential close to -10 kPa. The *Lolium perenne* plants had been grown in a phytotron (Gorissen et al., 1995). Some characteristics of the soils and the grass are given in Tables 1 and 2.

After mixing the grass through the soils, samples were incubated at 20°C. ^{14}C mineralization (at 20°C), distribution of ^{14}C over density and size fractions, ^{14}C in microbial biomass and ^{14}C in the soluble fraction were determined in separate triplicate samples.

Cumulative ^{14}C mineralization was determined in 50-g soil samples 2, 6, 10, 20, 30 and 60 days after the start of the incubation by trapping $^{14}CO_2$ in excess NaOH (Hassink, 1994). ^{14}C in microbial biomass was determined by the fumigation extraction method (Vance et

al., 1987) using a k_{EC} value of 0.45 (Wu et al., 1990) in soil samples of 20 g (separate fumigated and non-fumigated sample) 0.5 h (0 days), 3, 7, 21, 60, and 180 days after the start of the incubation. ^{14}C in non-fumigated samples will be referred to as the soluble fraction.

^{14}C in size and density fractions was determined on the same days as microbial biomass according to Hassink (1995[b]) in 75-g soil samples. Five size and density (d) fractions were distinguished: light (d < 1.13 g cm^{-3}), intermediate (d 1.13-1.37 g cm^{-3}) and heavy (d > 1.37 g cm^{-3}) macroorganic matter (> 150 µm), microaggregates with diameters between 20 and 150 µm and diameters < 20 µm (Hassink, 1995[b]). Unlike an earlier experiment (Hassink, 1995[a]), the mineral fraction was not discarded by decantation, but was included in the macroorganic matter fractions in order to recover all applied ^{14}C. The distribution of C over the different fractions was determined in the unamended soils at the start of the experiment (Table 1).

Soil C was determined using a wet oxidation method (dichromate-oxidizable C; Mebius, 1960). CO_2 was trapped in 0.5 M NaOH. Soluble C and ^{14}C in the grass were determined by shaking 100 mg grass with 100 ml 0.01 M $CaCl_2$ for 1 h. Soluble C in grass was measured using a dry oxidation method; 30 µl of the $CaCl_2$ solution was injected in an analyzer for total organic C (TOC-500 Shimadzu) equipped with a furnace to reach 680°C and an infra-red CO_2 analyzer.

^{14}C resulting from mineralization and ^{14}C in the size and density fractions were determined after mixing 1 ml aliquots of the NaOH solutions diluted with 1 ml of water in a glass scintillation vial with 10 ml of a scintillation cocktail (Insta-Gel, Packard Instruments Company). Soluble ^{14}C in grass was determined in the $CaCl_2$ solution in the same way. Soluble ^{14}C in the soil samples and ^{14}C in microbial biomass were determined after mixing 0.4 ml of the 0.5 M K_2SO_4 solutions diluted with 1.6 ml water with 12 ml of the scintillation cocktail. Samples were counted in a liquid scintillation counter Rackbeta II 1215, Wallac.

To estimate the gross decay of ^{14}C in the different fractions, the fractions of the sandy soil isolated on day 0 and 180 were mixed through the sandy soil and the percentages of ^{14}C in each fraction that were mineralized to $^{14}CO_2$ were determined 3 (only at day 0) and 21 days after the start of incubation at 20°C and at a water potential of -10 kPa. The samples were incubated in triplicate. C mineralization was only determined in the samples of day 180. C mineralization of the incubated fraction was calculated as the difference between C mineralization in soil samples to which a fraction was added minus C mineralization in soil samples to which no fraction was added.

Table 1. Some characteristics of the top 10 cm of the sandy and the loam soil

Characteristics	Sand	Loam
C (%)	4.38	5.37
Distribution of C over fractions (%)		
soluble fraction	0.2	0.2
light macroorganic matter	3.3	1.2
intermediate macroorganic matter	14.5	3.6
heavy macroorganic matter	16.6	4.7
microaggregates 20-150 µm	41.5	22.3
microaggregates < 20 µm	23.9	68.0
pH (KCl)	4.4	4.8
Granular composition, % particles <		
2 µm	2.4	24.1
16 µm	4.4	36.5
50 µm	23.5	71.7

Table 2. Soluble C and specific activity of labeled ryegrass C

C soluble in 0.01 M $CaCl_2$ (%)	Specific activity (Bq mg^{-1} C)	
	grass	soluble fraction
22.5	546	576

Experiment on residue C decomposition in aggregates < 20 µm, as related to the degree of saturation of these aggregates

Seventy-five mg of the cut ryegrass described in the first experiment was mixed through 75 g of each of 11 soil samples that differed in the maximum amount of C that can be associated with clay and silt particles minus the actual amount of clay- and silt-associated C (saturation deficit; Hassink, 1995[c]). Grass was mixed through triplicate samples and they were incubated at a water potential close to - 10 kPa at 20°C. The setup of the experiment and the characteristics of the samples have been described by Hassink (1995[d]). After 53 days the fraction < 20 µm was collected from one of the triplicates of each sample by washing the samples on three sieves (top sieve: mesh size 250 µm; second sieve: 150 µm; bottom sieve 20 µm). The samples were pushed through the top sieve to destroy all macroaggregates. The

suspension passing the bottom sieve of 20 µm was collected in a bucket and placed at 4°C until it had sedimented (Hassink, 1995[b]). The < 20 µm fraction was dried at 30°C; ^{14}C in this fraction was determined as described above. The isolated < 20 µm fractions of seven samples that differed considerably in saturation deficit (samples 1, 2, 3, 4, 8, 10 and 11; Table 3; Hassink, 1995[d]) were rewetted by application of a soil suspension (sandy soil of experiment 1 mixed with demineralized water at a ratio of 1:10). The fractions were incubated at 20°C and at a water potential of -10 kPa. Cumulative C and ^{14}C mineralization was determined after 14 days of incubation.

Statistical analyses

Student's t test was used to assess differences in distribution of ^{14}C and specific activity between soils and dates (Genstat, 1987). The relationship between ^{14}C mineralization of the fraction < 20 µm and saturation deficit was analyzed with correlation and linear regression analysis (Genstat, 1987).

Table 3. Some characteristics and the saturation deficit of the soil samples that were used in the second experiment (Hassink, 1995[d])

Soil no.	Granular composition, % particles <			pH (KCl)	C (g kg^{-1})	C in particle size fraction < 20 µm (g kg^{-1} soil)	Saturation deficit* (g kg^{-1} soil)
	2 µm	20 µm	50 µm				
1	9	17	26	7.0	4.9	5.8	5.0
2	9	17	26	7.0	28.1	12.8	-2.0
3	45	66	72	7.0	11.3	10.2	18.4
4	45	66	72	7.0	40.0	27.6	1.0
8	24	37	72	4.8	43.0	21.6	-4.0
10	23	38	72	7.0	34.0	13.0	5.3
11	23	38	72	7.0	30.0	11.9	6.4

* Derived from the equation: Protective capacity (g C kg^{-1} soil) = 4.09 + 0.37 x % particles < 20 µm (Hassink, 1995[d])

RESULTS

Experiment on residue C transfer between fractions and decomposition of residue C in these fractions

Recoveries of ^{14}C

The recovery of ^{14}C, as $^{14}CO_2$ or as ^{14}C, in the soluble and size and density fractions was over 85% at day 0, but only 75% at day 3. Applied ^{14}C that was not recovered in these fractions is indicated as 'not recovered'(NR, Figs 1 and 2). Between 3 and 180 days, the recovery increased to 100% (Figs 1 and 2).

Distribution of ^{14}C immediately after the application of grass

Immediately after the application of grass, most of the applied ^{14}C was recovered in the soluble fraction (26-28%) and the light macroorganic matter fraction (31-32%; Figs. 1 and 2). The percentages of applied ^{14}C present in the intermediate and heavy macroorganic matter fractions were less than 8%. For all these fractions there were no differences between the sandy and loam soils. Substantial amounts of ^{14}C were found in the microaggregate fractions (0-20 and 20-150 μm). The sandy soil contained relatively more ^{14}C in the 20-150 μm size class than the loam (16 vs 11%), whereas for the < 20 μm size class the opposite was true (5 vs 19%; Figs. 1 and 2).

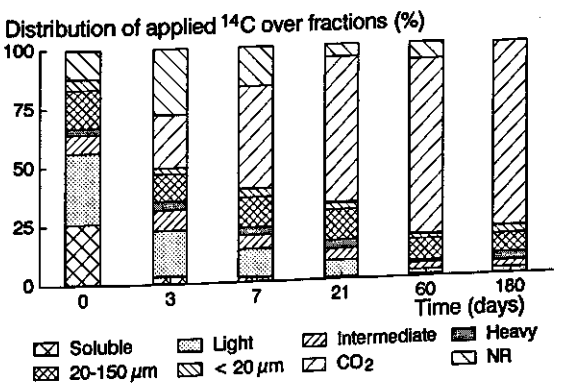


Fig. 1. Distribution of applied ^{14}C (%) over soluble organic matter, light, intermediate and heavy macroorganic matter, 20-150 μm and < 20 μm microaggregates, and mineralized ^{14}C (CO_2) in the sandy soil 0, 3, 7, 21, 60 and 180 days after the application of ^{14}C-labeled ryegrass. ^{14}C not recovered in any of those fractions is known as NR.

Changes in the distribution of ^{14}C in time

Rates of ^{14}C mineralization were very high initially and decreased during incubation. ^{14}C mineralization was not significantly ($P < 0.05$) different between the sandy and loam soils. After 7 days 36-43% of the applied ^{14}C was mineralized and after 60 days 67-75% (Figs. 1 and 2).

High initial ^{14}C mineralization rates coincided with a rapid decrease in soluble and light macroorganic matter ^{14}C. After 3 days the percentages of ^{14}C in the soluble and light macroorganic matter fractions had fallen to 3-4% and 17-20%, respectively. The amounts of ^{14}C in the other size and density fractions changed little (Figs. 1 and 2).

Microbial ^{14}C amounted to approximately 40% of the applied ^{14}C after 3 days in both soils and decreased rapidly thereafter (Fig. 3).

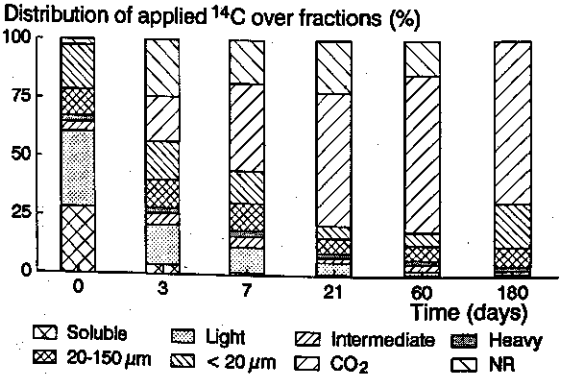


Fig. 2. Distribution of applied ^{14}C (%) over soluble organic matter, light, intermediate and heavy macroorganic matter, 20-150 µm and < 20 µm microaggregates, and mineralized ^{14}C (CO_2) in the loamy soil 0, 3, 7, 21, 60 and 180 days after the application of ^{14}C-labeled ryegrass. ^{14}C not recovered in any of those fractions is shown as NR.

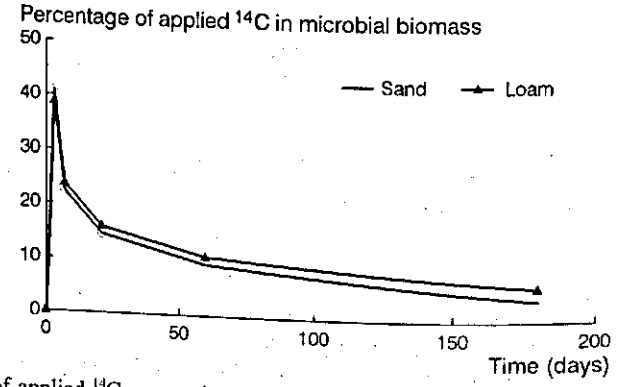


Fig. 3. Percentages of applied ^{14}C present in the microbial biomass 0, 3, 7, 21, 60 and 180 days after the application of ^{14}C-labeled ryegrass.

The distribution of ^{14}C in the soil over the different fractions changed considerably during incubation. The contribution of the soluble fraction to total soil ^{14}C decreased from more than 25% to less than 5% in 7 days (Figs. 4 and 5). The contribution of the light macroorganic matter fraction had fallen to less than 13% after 60 and 180 days (Figs. 4 and 5). The relative contribution of ^{14}C in the intermediate and heavy macroorganic matter fractions to total soil ^{14}C increased significantly ($P < 0.05$) during the first week of incubation and did not change significantly ($P < 0.05$) afterwards (Figs. 4 and 5). The percentage of soil ^{14}C present in the microaggregates increased significantly ($P < 0.05$) during incubation; in the sandy soil their contribution increased to approximately 60% and in the loam soil to approximately 85%. In the sandy soil ^{14}C was concentrated in the 20-150 μm size class, and in the loam in both the 20-150 and < 20 μm size classes after 60 days and primarily in the < 20 μm size class after 180 days (Figs. 4 and 5). At the beginning of the incubation the distribution of soil ^{14}C over the different pools was very different from that of total soil C, but as time proceeded the distributions became similar (cf. Table 1 with Figs. 4 and 5).

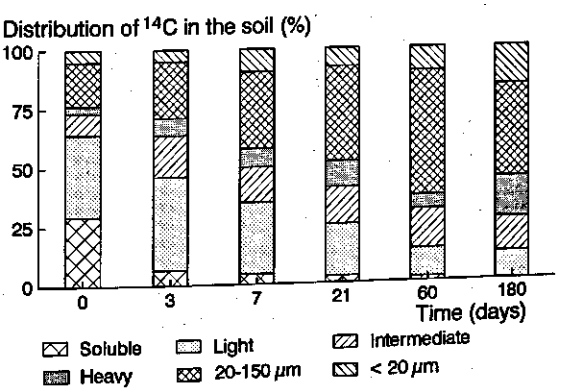


Fig. 4. Distribution of residual soil ^{14}C (%) over soluble organic matter, light, intermediate and heavy macroorganic matter, 20-150 μm and < 20 μm microaggregates in the sandy soil 0, 3, 7, 21, 60 and 180 days after the application of ^{14}C-labeled ryegrass.

Comparison of ^{14}C mineralization rates of reincubated fractions of the sandy soil isolated after 0 days and net changes in the amounts of ^{14}C in the fractions in the sandy soil during incubation

During the first 3 days, mineralization rates were not different for ^{14}C in the macroorganic matter fractions and the microaggregates (Table 4). Between 3 and 21 days, ^{14}C in the macroorganic matter fractions mineralized slightly faster than ^{14}C in the microaggregates, but the differences were only significant ($P < 0.05$) for the light fraction. Between 0 and 3, and between 3 and 21 days, the amount of ^{14}C in the light macroorganic matter fraction in the

sandy soil decreased significantly ($P < 0.05$) faster than the amount of ^{14}C in the incubated light macroorganic matter fraction. For ^{14}C in the microaggregates, this was the case for the period 0-3 days.

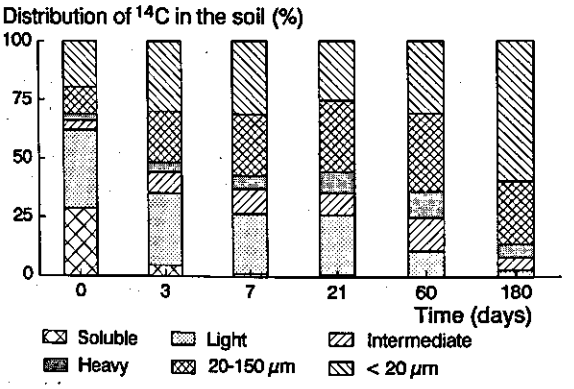


Fig. 5. Distribution of residual soil ^{14}C (%) over soluble organic matter, light, intermediate and heavy macroorganic matter, 20-150 µm and < 20 µm microaggregates in the loamy soil 0, 3, 7, 21, 60 and 180 days after the application of ^{14}C-labeled ryegrass.

Table 4. Amounts of ^{14}C present in size and density fractions isolated from the sandy soil 3 and 21 days after the application of ^{14}C (Soil); in size and density fractions isolated from the sandy soil after 0 days and reincubated for a period of 3 and 21 days (Isolated day 0), and amounts of C and ^{14}C in size and density fractions isolated from the sandy soil after 180 days and reincubated for a period of 21 days (Isolated day 180). Results are expressed as % of the amounts present in the fractions at day 0 (Soil) or at the beginning of the incubation period (Isolated).

	Soil, ^{14}C		Isolated day 0, ^{14}C		Isolated day 180	
					^{14}C	C
	3	21	3	21	21	21
Macroorganic matter						
Light	64	24	94	72	n.d.	n.d.
Intermediate	109	64	94	76	88	96
Heavy	132	129	92	73	88	98
Microaggregates						
20-150 µm	72	80	92	78	88	99
< 20 µm	56	58	93	80	90	99

n.d. = not determined

C and ^{14}C mineralization rates of reincubated fractions of the sandy soil isolated after 180 days

There was no difference between the mineralization rate of ^{14}C in the macroorganic matter fractions and the microaggregates (Table 4). ^{14}C mineralization rates of the fractions isolated after 180 days were significantly ($P < 0.05$) lower than ^{14}C mineralization rates of the fractions isolated on day 0 (Table 4). C in the microaggregates mineralized significantly ($P < 0.05$) slower than C in the intermediate and heavy macroaggregate fractions. C mineralized significantly slower ($P < 0.05$) than ^{14}C (Table 4).

Specific activity of the grass, mineralized C, microbial biomass and soil organic matter fractions

To compare the decomposition rate of freshly applied ^{14}C with that of native soil C in a different way, we established the specific activity (s.a.) of CO_2 produced during mineralization. We present average values of the soils because the s.a. of mineralized C and soil fractions between both soils did not differ significantly ($P < 0.05$). At the start of the incubation the s.a. of mineralized C did not differ from the s.a. of the grass and its soluble fraction (cf. Table 2 and Fig. 6). The s.a. of mineralized C decreased rapidly during incubation. During the first weeks of the incubation, the s.a. of the microbial biomass was lower than the s.a. of mineralized C. However, after 3 weeks the difference had disappeared (Fig. 6).

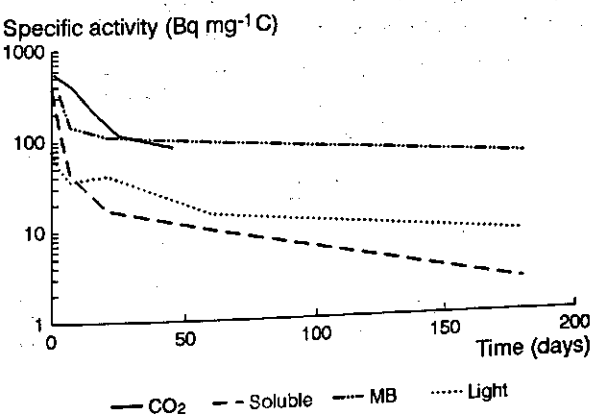


Fig. 6. Specific activities (Bq mg^{-1} C) of mineralized C (CO_2), soluble C (Soluble), C in the microbial biomass (MB), and C in the light macroorganic matter fraction (Light) 0, 3, 7, 21, 60 and 180 days after the application of ^{14}C-labeled ryegrass. Average values for the sandy soil and loam.

The s.a. of the soluble fraction was lower than the s.a. of the microbial biomass and decreased rapidly during incubation (Fig. 6).
The s.a. of the light macroorganic matter fraction decreased considerably during the incubation and was approximately 7 times lower than the s.a. of mineralized C (Fig. 6).
The s.a. of the intermediate and heavy macroorganic matter fractions and the microaggregates 20-150 µm and < 20 µm were very low (< 10 Bq mg^{-1} C) during the whole incubation and remained almost constant (Fig. 7).

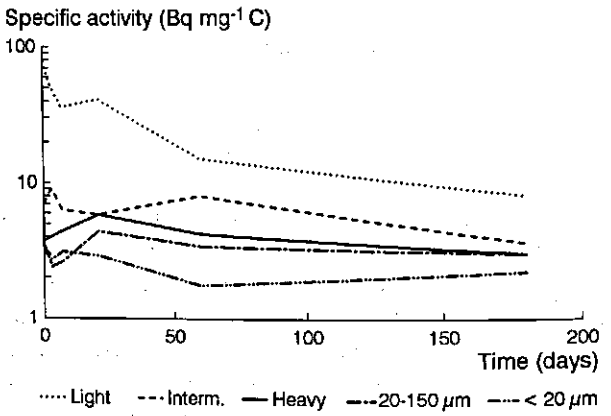


Fig. 7. Specific activities (Bq mg^{-1} C) of the light, intermediate and heavy macroorganic matter, and the 20-150 µm and < 20 µm microaggregate fractions 0, 3, 7, 21, 60 and 180 days after the application of ^{14}C-labeled ryegrass. Average values for the sandy soil and loam.

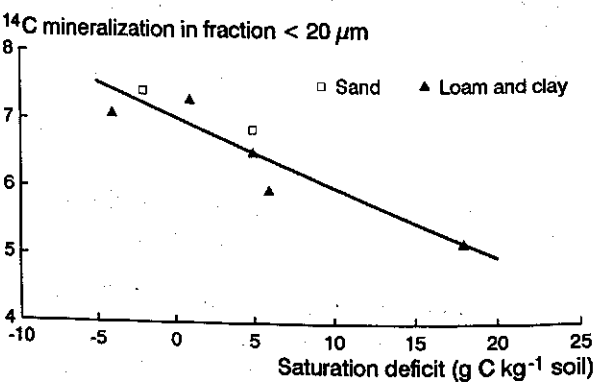


Fig. 8. Relationship between the percentage ^{14}C respired to $^{14}CO_2$ in the fraction < 20 µm after 14 days of incubation and the saturation deficit of a soil (g C kg^{-1} soil). (% ^{14}C respired = 7.03 (0.16) - 0.10 (0.02) x saturation deficit; () = standard error of difference.)

Experiment on the decomposition of residue C in aggregates < 20 μm, as related to the degree of saturation of these aggregates

The percentage of ^{14}C in the isolated fraction < 20 μm which mineralized after incubation of this fraction for 14 days ranged from 5.2 to 7.4%, and was negatively correlated (r = -0.9) with the saturation deficit of the fraction < 20 μm with organic matter (Fig. 8).

DISCUSSION

Transfer of ^{14}C between soil organic matter fractions

The first aim of this study was to determine whether plant C that is mixed through the soil is transferred from light macroorganic C and soluble C to intermediate and heavy macroorganic C fractions and accumulates in microaggregates in soils of contrasting texture.

We observed that 30% of the applied ryegrass ^{14}C was recovered in the light fraction of macroorganic matter and 25% in the soluble fraction immediately after application in both soils. The amount of ^{14}C recovered in the soluble fraction equalled the amount of grass ^{14}C that was extractable with 0.01 M $CaCl_2$. In contrast with our expectations, considerable amounts of ^{14}C were already present in other fractions: 20-30% in both microaggregate fractions and 7-11% in the intermediate and heavy macroorganic matter fractions. This suggests that the binding of organics to mineral particles (leading to the formation of heavy material) can take place very quickly (Strickland et al., 1992). The fact that 20-30% of the applied ^{14}C was recovered in the microaggregate fractions (< 150 μm) suggests also that the grass sample contained particles that were too small to be recovered in the macroorganic matter fractions.

The amounts of ^{14}C in the light macroorganic matter fraction and the soluble fraction decreased very rapidly, whereas the amounts of ^{14}C in the intermediate and heavy macroorganic matter fractions and the microaggregate fractions remained almost constant during incubation. From this observation it was not directly clear whether there was a transfer of ^{14}C from the light macroorganic matter fraction to the intermediate and heavy macroorganic matter fractions and finally to the microaggregate fractions. The relative constancy of the amounts of ^{14}C in the intermediate and heavy macroorganic matter fractions and microaggregates may be due to the fact that ^{14}C in these fractions had a very low decomposition rate, or that the decay of ^{14}C in a fraction was matched by the supply of ^{14}C from more labile fractions to this fraction. The reincubation of the isolated fractions after 0 and 180 days of the sandy soil showed that mineralization rates of ^{14}C were not significantly different for light, intermediate and heavy macroorganic matter fractions and microaggregates. The similar mineralization rates of the reincubated light macroorganic matter and the

microaggregate fractions corresponded with the decreases of ^{14}C in these fractions in the soil between 0 and 3 days (Table 4). Unlike the fast decrease of ^{14}C in the light macroorganic matter fraction, the amount of ^{14}C in the intermediate fraction of macroorganic matter increased in the sandy soil during the first 3 days and of the heavy macroorganic matter fraction during the first 21 days (Table 4). As ^{14}C had similar mineralization rates after 0 and 180 days in all fractions, there must have been a transfer of ^{14}C from the most labile fractions (^{14}C in the light macroorganic matter fraction, soluble ^{14}C and microbial ^{14}C) to the intermediate macroorganic matter fraction during the first few days after ^{14}C application and to the heavy macroorganic matter fraction during the first weeks after this application. The results suggest that transfer of ^{14}C to the microaggregate fractions was not important between 0 and 3 days but became significant between 3 and 21 days as the amount of ^{14}C recovered in the microaggregates did not decrease during that period.

It is not clear why the ^{14}C mineralization rates of the isolated, incubated light macroorganic matter fraction and the microaggregate fractions were smaller than the decreases in the amounts of ^{14}C present in the same fractions in the soil. The microbes decomposing the fractions might have been washed away during the isolation of the fractions, which might have caused a reduction in the mineralization rate of the fractions.

At the start of the incubation the distribution of ^{14}C over the fractions was very different from the distribution of total C, but after 60 days they became similar. After 60 days, most of the residual soil ^{14}C was found in the microaggregates; in the sandy soil in the 20-150 μm size fraction and in the loam in the 20-150 and < 20 μm size fractions. Unlike our results, Ladd et al. (1977) and Nicolardot et al. (1992) found that, independent of soil texture, most of the applied labeled C and N ended up in the silt and clay fraction. The discrepancy with our study is caused by the fact that they used sonication to obtain primary particles. Sonication destroys most of the microaggregates in the 20-150 μm size class and releases the clay and silt particles present in these aggregates (Tisdall and Oades, 1982) with the result that most of the label is found in the clay and silt size fraction. With the wet sieving procedure performed in our study, only the macroaggregates were destroyed and the microaggregates (which are not destroyed by agricultural practices) remained intact (Hassink, 1995[b]). The observation that most of the ^{14}C ended up in microaggregates is in agreement with previous observations that microaggregates contain a large proportion of the most stable organic matter in soil and protect organic matter (Skjemstad et al., 1990; Skjemstad et al., 1993; Golchin et al., 1994). It is also in agreement with the observation that the decomposition rate of organic C incorporated in microaggregates is much lower than that of other parts of the soil organic C pool (Hassink, 1995[b]).

After 3 days approximately 25% of the applied ^{14}C was not recovered in one of the fractions (Figs. 1 and 2, not recovered). This amount decreased during incubation. It is not clear where this ^{14}C was located.

It may be concluded that residue C was transferred from the light macroorganic matter and soluble organic matter fractions to the intermediate and heavy macroorganic matter fractions and accumulated in microaggregates and that newly synthesized microbial biomass fed on this

labile material. The transformations of ^{14}C were identical in the sandy soil and loam, except that there was a greater accumulation of ^{14}C in 20-150 μm microaggregates in the sandy soil; in the loam the accumulation was highest in the 0-20 μm microaggregates.

Heterogeneity of the isolated fractions

Several of our observations showed that the isolated fractions consisted of soil-derived C with a relatively low mineralization rate and applied ^{14}C with a higher mineralization rate.

The decrease in the s.a. of the soluble and light macroorganic matter fraction indicates that material derived from the grass decomposed much faster than soil- derived C recovered in the same fractions.

The observation that the s.a. of mineralized C was greater than that of soluble C, C in the light macroorganic matter fraction and microbial C (during the first weeks after ^{14}C application) also shows that ^{14}C compounds in those fractions mineralized faster than non-labeled (soil-derived) C in the same fractions. In agreement with a previous study (Hassink, 1995[b]) we found that less than 5% of the amount of C present in isolated size and density fractions was mineralized when the fractions were incubated at 20°C for 21 days. These values are considerably lower than the decomposition rates for ^{14}C that were determined in this study when isolated fractions of day 0 and 180 were incubated. It is generally found that applied C remaining in the soil decomposes faster than the native soil C (Christensen and Sörensen, 1985). In addition, the results of the present study show that 180 days after the application of plant C, plant-derived C in a recovered fraction still decomposed faster than soil-derived C.

In addition, it was found that - contrary to soil-derived C - the decomposition rates of ^{14}C were not significantly different between the isolated fractions, both 0 and 180 days after application of ^{14}C. This suggests that even ^{14}C that was recovered in the microaggregate fractions was not yet physically protected in the soil. Apparently more time is needed before applied C becomes physically protected against microbial attack. This is in agreement with the results of Matus (1995) and the observation that materials on the outside of microaggregates are made up of more labile material, while materials inside aggregates are much more stable (Skjemstad et al., 1993) and with the postulation that applied ^{14}C is associated with external layers of organic matter coatings on clay and silt particles, while the internal layers contain the physically protected organic matter (Buyanovsky et al., 1994).

The second experiment showed that the preservation of applied ^{14}C by microaggregates < 20 μm is negatively correlated with the degree of saturation of the particle size fraction < 20 μm with soil organic matter. This is in line with the above- mentioned observations and postulations. Apparently, plant-derived C is gradually reduced in size by abiotic and biotic factors and considerable amounts of residual plant C are recovered in the microaggregate size fractions. The degree of physical protection of this material against microbial decay probably increases in time and depends on the amount of protective sites that are still available. The

high decomposition rates of ^{14}C in the microaggregate size fractions of the soils in the first experiment are in line with the observation that the particle size fractions < 20 μm of the soils used in the first experiment were saturated with soil organic matter (Hassink, 1995c).

The observation that all fractions contain relatively young plant-derived C with a high decomposition rate that is independent of the size and density of the fraction and older soil-derived C with a lower decomposition rate that is different for each isolated fraction, and the observation that the decomposition rate of plant-derived C associated with the microaggregate fraction < 20 μm is affected by the degree of saturation of the protective sites with soil organic matter, complicates the simulation of the fate of recently applied C.

ACKNOWLEDGEMENTS

We thank D. Blaauw, H. van Beek and A. Lahr for performing the fractionations and ^{14}C analyses, A. Gorissen for supplying the labeled plant material, and L. Brussaard, A. Gorissen, J.J. Neeteson and K.B. Zwart for critically reading and improving the manuscript.

REFERENCES

Amato M. and Ladd J. N. (1992) Decomposition of ^{14}C-labelled glucose and legume materials in soil: properties influencing the accumulation of organic residue C and microbial biomass C. *Soil Biology & Biochemistry* **24**, 455-464.

Bonde T. A., Christensen B. T. and Cerri C. C. (1992) Dynamics of soil organic matter as reflected by natural ^{13}C abundance in particle size fractions of forested and cultivated oxisols. *Soil Biology & Biochemistry* **24**, 275-277.

Buyanovsky G. A., Aslam M. and Wagner G. H. (1994) Carbon turnover in soil physical fractions. *Soil Science Society of America Journal* **58**, 1167-1173.

Christensen B. T. and Sörensen L. H. (1985) The distribution of native and labelled carbon between soil particle size fractions isolated from long-term incubation experiments. *Journal of Soil Science* **36**, 219-229.

Elliott E. T. and Cambardella C. A. (1991) Physical separation of soil organic matter. *Agriculture Ecosystems and Environment* **34**, 407-419.

Genstat Manual (1987) *Genstat. A General Statistical Program.* Clarendon, Oxford.

Golchin A., Oades J. M., Skjemstad J. O. and Clarke P. (1994) Soil structure and carbon cycling. *Australian Journal of Soil Research* **32**, 1043-1068.

Gorissen A., Ginkel J. H. van, Keurentjes J. J. B. and Veen J. A. van (1995) Grass root decomposition is retarded when grass has been grown under elevated CO_2. *Soil Biology & Biochemistry* **27**, 117-120.

Greenland D. J. and Ford W. (1964) Separation of partially humified organic materials from

soil by ultrasonic dispersion. Proceedings 8th International Congress of Soil Science, Bucharest, Vol. III, pp. 137-148.

Hassink J. (1994) Effects of soil texture and grassland management on soil organic C and N and rates of C and N mineralization. *Soil Biology & Biochemistry* **26**, 1221-1231.

Hassink J. (1995[a]) Density fractions of soil macroorganic matter and microbial biomass as predictors of C and N mineralization. Accepted by *Soil Biology & Biochemistry*.

Hassink J. (1995[b]) Decomposition rate constants of size and density fractions of soil organic matter. Submitted to *Soil Science Society of America Journal*.

Hassink J. (1995[c]) The capacity of soils to preserve organic C and N by their association with clay and silt particles. Submitted to *Soil Science Society of America Journal*.

Hassink J. (1995[d]) The degree of saturation of the protective capacity of a soil controls the preservation of ^{14}C-labeled plant residues. Submitted to *Soil Science Society of America Journal*.

Jenny H., Gessel S. P. and Bingham F. T. (1949) Comparative study of decomposition rates of organic matter in temperate and tropical regions. *Soil Science* **68**, 419-432.

Ladd J. N., Parsons J. W. and Amato M. (1977) Studies of nitrogen immobilization and mineralization in calcareous soils-I. Distribution of immobilized nitrogen amongst soil fractions of different particle size and density. *Soil Biology & Biochemistry* **9**, 309-318.

Matus F. J. (1995) The distribution of soil organic matter of various aggregate size classes in arable soils. II. Residual organic ^{14}C, ^{15}N, microbial biomass ^{14}C and ^{14}C and ^{15}N mineralization rates in a sand and a clay soil. Submitted to *Soil Biology & Biochemistry*.

Mebius L. J. (1960) A rapid method for the determination of organic carbon in soil. *Analytica Chimica Acta* **22**, 120-124.

Meijboom F. W., Hassink J. and Noordwijk M. van (1995) Density fractionation of soil macroorganic matter using silica suspensions. Accepted by *Soil Biology & Biochemistry*.

Nicolardot B., Cheneby D. and Allard M. R. (1992) Availability of carbon and nitrogen contained in different soil particle-size fractions. In *Humus, its structure in agriculture and environment* (J. Kubat Ed.), pp. 107-116. Proceedings 15th Symposium Humus et Planta. Elsevier Publishers, Amsterdam.

Skjemstad J. O., Le Feuvre R. P and Prebble R. E. (1990) Turnover of soil organic matter under pasture as determined by ^{13}C natural abundance. *Australian Journal of Soil Research* **28**, 267-276.

Skjemstad J. O., Janik L. J., Head M. J. and McClure S. G. (1993) High energy ultraviolet photo-oxidation: a novel technique for studying physically protected organic matter in clay- and silt-sized aggregates. *Journal of Soil Science* **44**, 485-499.

Stotzky G. (1986) Influence of soil mineral colloids on metabolic processes, growth, adhesion, and ecology of microbes and viruses. In *Interactions of soil minerals with natural organics and microbes* (P. M. Huang and M. Schnitzer, Eds), pp. 305-428. Soil Sci. Soc. Am. Madison, Wisconsin.

Strickland T., Sollins P., Rudd N. and Schimel D. (1992) Rapid stabilization and

immobilization of ^{15}N in forest and range soils. *Soil Biology & Biochemistry* **24**, 849-855.

Swift M J., Heal O. W. and Anderson J. M. (1979) Decomposition in terrestrial exosystems. *Studies in Ecology*, Vol 5. Blackwell Scientific Publications, Oxford.

Tiessen J., Stewart J. W. B. and Hunt H. W. (1984) Concepts of soil organic matter transformations in relation to organo-mineral particle size fractions. *Plant and Soil* **76**, 187-295.

Tisdall J. M. and Oades J. M. (1982) Organic matter and water stable aggregates in soils. *Journal of Soil Science* **33**, 141-163.

Vance E. D., Brookes P. C. and Jenkinson D. S. (1987) An extraction method for measuring soil microbial biomass. *Soil Biology & Biochemistry* **19**, 703-707.

Van Cleve K. (1974) Organic matter quality in relation to decomposition. In *Soil organisms and decomposition in Tundra* (A. J. Holding, O. W. Heal, S. F. Maclean and P. W. Flanagan, Eds), pp. 311-324. Tundra Biome Steering Committee, Stockholm.

Van Veen J. A. and Kuikman P. J. (1990) Soil structural aspects of decomposition of organic matter. *Biogeochemistry* **11**, 213-233.

Van Veen J. A., Ladd J. N. and Frissel M. J. (1984) Modelling C and N turnover through the microbial biomass in soil. *Plant and Soil* **76**, 257-274.

Wu J., Joergensen R. G., Pommerening B., Chaussod R. and Brookes P. C. (1990) Measurement of soil microbial biomass C by fumigation-extraction-an automated procedure. *Soil Biology & Biochemistry* **22**, 1167-1169.

CHAPTER 10

A MODEL OF THE PHYSICAL PROTECTION OF ORGANIC MATTER IN SOILS

By: J. Hassink and A.P.M. Whitmore. Submitted to Soil Science Society of America Journal

A MODEL OF THE PHYSICAL PROTECTION OF ORGANIC MATTER IN SOILS

ABSTRACT

A computer simulation model describing the turnover of organic matter in soil in relation to physical protection is presented. The essential innovation of the model in comparison with others is that it describes physical protection explicitly as a function of the adsorption of organic matter to clay and silt particles. The net rate of decomposition of organic matter depends not simply on soil texture therefore, but on the degree to which the protective capacity of the soil is occupied. The rate at which organic matter becomes protected depends on both the amount of free organic matter and the degree to which the protective capacity is filled (or covered). The rate at which organic matter is released depends only on this degree of cover of the clay and silt particles with organic matter.

With this new model we were better able to predict the build-up and decline in amounts of soil organic matter in soils of different textures and initial organic matter contents, than with conventional, implicit descriptions of protection. The model closely followed the build-up and decline of organic matter in 10 soils to which grass residues were added each year for 10 years and then left without addition for a further 10 years. An estimate made with the model of the maximum capacity of each of these soils to protect organic matter was closely correlated with the clay plus silt fractions in the soils.

INTRODUCTION

The rate of decay of organic materials in soil is generally thought to be reduced by the presence of clay. Clay and organic matter interact in soil to form complexes and microaggregates which render organic substances less susceptible to biodegradation (Jenkinson, 1977; Ladd et al., 1981, 1985; Tisdall and Oades, 1982; Amato and Ladd, 1992; Skjemstad et al., 1993). As a result of this stabilizing effect, fine-textured soils usually contain more organic matter than coarse-textured soils that have received the same input of organic material (Kortleven, 1963; Jenkinson, 1988; Spain, 1990; Feller et al., 1991). In many computer models describing the turnover of carbon and nitrogen in soil, physical protection of organic matter has been treated empirically, whether explicitly or implicitly (Van Veen and Paul, 1981; Van Veen et al., 1985; Parton et al., 1987; Jenkinson, 1990; Verberne et al., 1990). In these models it was proposed that (i) clay soils have a greater capacity to preserve or protect microbial biomass and provide an environment for closer interaction between microorganisms and the products of decay which results in a larger proportion of carbon and nitrogen from decomposing microbial biomass becoming protected physically (Van Veen et

al., 1985; Verberne et al., 1990), or (ii) clay soils promote a higher efficiency of use of metabolic products by the soil biota (Van Veen et al., 1985; Parton et al., 1987; Jenkinson, 1990).

The mechanisms whereby organic compounds are bound to clay surfaces are seldom known. Organic matter can be bound to clay particles by cation and water bridging, anion and ligand exchange, hydrogen bonding and van der Waals forces (Theng, 1979). The binding of organics to clay surfaces has been described by adsorption-desorption kinetics (Burchill et al., 1981). Equilibrium adsorption is attained when the rate of adsorption equals the rate of desorption. The equilibrium adsorption of organics to clays can properly be described by isotherms (Harter and Stotzky, 1971). McLaren and Peterson (1965) gave equations to describe the adsorption and desorption of organic molecules to clay which depend on both the fraction of the free space on the surface of the clay and the fraction of the organic polymer bonded.

Hassink (1995[a]) suggested that the physical capacity of a soil to preserve organic matter is limited. The protective capacity was defined as the maximum amount of C that can be associated with clay and silt particles in the soil: C_{max} in the fraction < 20 μm (g kg^{-1} soil) = 4.09 + 0.37 x % particles < 20 μm (Hassink, 1995[a]). It was suggested that the degree of saturation of the protective capacity of a soil with organic matter would affect the preservation of applied carbon in residues in the soil. Less of the applied C should be preserved in the soil when all protective sites are occupied than when sites are available to stabilize organic C. Hassink (1995[b]) provided additional evidence of this by observing that the amount of carbon in residues decomposed in soil correlated better with the degree of saturation of the protective capacity of a soil than with its clay content. These ideas are also in line with the observation that adsorption decreases as the coverage of the adsorbing surface increases (Stotzky, 1986) and the observation that the amount of organics that can be bound to dispersed clay particles is limited (Pinck et al., 1954; Harter and Stotzky, 1971; Marshman and Marshall, 1981).

The aim of this paper is to test whether the long-term dynamics of soil organic matter can be simulated with a simple simulation model that is based on the assumptions that i) the preservation of applied C is controlled by the degree of saturation of the clay and silt size fraction with soil organic matter (instead of soil texture *per se*) and ii) that the protection of soil organic matter can be described kinetically in the same way as adsorption and desorption. We simulated both the increase in organic C in soils with different textures and different initial organic C contents receiving grass residues each spring as well as the subsequent decrease in soil organic C after the additions were stopped. We compared the results of this model with the predictions of two alternative descriptions of organic matter turnover and protection in which the stabilization of residues is controlled by the clay content of the soil only.

MATERIALS AND METHODS

Incubation experiment

In March 1966 soil from the top 20 cm of nine arable fields and one grassland field was taken, roots and stubble removed and sieved through a 0.008 m mesh screen. Four plastic containers were filled with approximately 6 l of each soil. The containers were placed outdoors under a roof during the initial four years. Subsequently they were kept in the greenhouse at ambient air temperature. The soils were kept bare during the whole incubation and the moisture content of the soils was kept between 50 and 70% of the water holding capacity by regularly watering. In two replicates of the containers of each soil 100 g of milled ryegrass was carefully mixed through the samples each spring (starting in 1966). After 10 years (from 1976) the application of ryegrass was stopped but the soils continued to be mixed each spring, and the containers were incubated for 10 more years. The samples in the two other containers of each soil were also mixed carefully each year, but did not receive ryegrass during the whole period.

Each year, before the addition of ryegrass, but after mixing, soil samples were taken and analyzed for their organic C content. Organic C is defined here as dichromate-oxidizable C according to Kurmies (Mebius, 1960). The pH (KCl) of the samples was determined in 1966 and 1976. Some characteristics of the soils are given in Table 1. As the containers were not leached during the incubation, concentrations of mineral N in the soils were expected to increase, especially in the treatment where grass was added. Mineral N (ammonium and nitrate) was measured colorimetrically after extraction with 1N KCl solution for 1h using a soil:water ratio of 1:2.5 in 1976 (Table 2). The soils Joh. Kerkhovenpolder 1-5 are located in a polder in the northeastern part of the Netherlands which was reclaimed from the sea approximately 150 years ago. The soils have been used as arable fields since then. The five locations were chosen to obtain soils with a range in texture but with a similar history. The other soils are located in other parts of the Netherlands and their history is not known. For Meppen and Heemskerk, the C:N ratio of the soil organic matter was considerably higher than 10. It was observed that as in many other sandy soils, they contain charcoal, probably due to the burning of the vegetation (Hassink, 1994). For the calculations, the C contents were adjusted to obtain a C:N ratio of 10.

Description of the model

We have borrowed ideas from the kinetics of adsorption and desorption in order to derive this model but in fact the description should be quite general to all mechanisms of physical or physico-chemical protection. Chemical protection, that is the resistance of organic matter to attack purely as a result of its chemical properties, will not be treated here because it is largely independent of the physical characteristics of soil. We will present the arguments in

Table 1. Some characteristics of the soils used in the long-term study.

	Granular composition % particles <			pH KCl		C (%)	N (%)
	2 μm	20 μm	50 μm	1966	1976	1966	1966
Joh. Kerkhovenpolder 1	9.9	14.0	38.4	7.2	6.6	1.21	0.12
Joh. Kerkhovenpolder 2	11.2	15.9	42.1	7.3	6.6	1.46	0.14
Joh. Kerkhovenpolder 3	20.3	29.4	77.2	7.3	7.0	1.57	0.15
Joh. Kerkhovenpolder 4	25.4	37.5	83.7	7.2	7.2	1.97	0.20
Joh. Kerkhovenpolder 5	44.4	64.9	95.1	7.1	7.1	2.31	0.21
NOP	3.3	4.3	69.5	7.3	6.3	0.77	0.09
Tzum	15.8	25.8	71.0	7.1	4.5	0.97	0.11
Heemskerk	2.3	4.0	9.4	6.8	4.4	1.00	0.06
Burum	27.1	39.9	74.1	4.4	4.4	2.60	0.28
Meppen	n.d.	n.d.	n.d.	5.4	4.4	3.03	0.18

Table 2. Ammonium (NH_4^+) and nitrate (NO_3^-) concentration in the soils (mg N kg^{-1} soil) in 1976 in the treatments without and with annual addition of grass residues.

	Without grass		With grass	
	NH_4^+	NO_3^-	NH_4^+	NO_3^-
Joh. Kerkhovenpolder 1	2	296	7	1826
Joh. Kerkhovenpolder 2	2	390	8	2546
Joh. Kerkhovenpolder 3	2	409	7	2318
Joh. Kerkhovenpolder 4	2	466	4	1500
Joh. Kerkhovenpolder 5	2	500	5	2463
NOP	0	265	21	2849
Tzum	1	304	141	1768
Heemskerk	0	213	32	1752
Burum	24	768	43	1553
Meppen	6	317	13	1322

general terms.

Suppose the soil has a fixed capacity X to protect organic matter physically in soil. We assume that organic matter is mainly protected through physico-chemical adsorption on surfaces of clay and silt particles. If θ is the fraction of this capacity currently holding organic matter, the rate at which more organic matter becomes protected is proportional to both the amount of free organic matter and to the fraction of the capacity (space or volume) available

(McLaren and Peterson, 1965; Burchill et al., 1981). Then the amount which becomes protected is:

$$k_a(1-\theta)N_{OM} \quad (1)$$

where N_{OM} is the amount of non-protected organic matter and k_a is the rate at which organic matter becomes adsorbed (protected). The release of physically protected organic matter, P_{OM}, is treated differently, however. The rate of desorption is independent of the amount of P_{OM} (zero order) and depends only on θ, the fraction of the protective capacity X, that is occupied. The probability of a molecule desorbing is then independent of whether an adjacent site is occupied or not (McLaren and Peterson, 1965; Burchill et al., 1981) or:

$$k_d\theta \quad (2)$$

where k_d is the rate of desorption (release of protected organic matter). Note, that although derived from the treatment of the desorption of gas molecules, equation (2) differs in relating the rate of release to the fraction of space occupied and not the amount of the capacity. Likewise adsorption in (1) is not related to the available capacity (θX) but to θ alone. This expresses the concept that large insoluble organic molecules do not move as freely over the adsorbing surface as small energetic gas molecules.

Simple expressions of the rates of adsorption and desorption underpin the derivation of the Langmuir isotherm that is used, among other things, in describing the binding of cations to soil particles. The capacity as we envisage it here may, however, well be three-dimensional, rather than two-dimensional, comprising of small spaces between particles or microaggregates. Nonetheless we assume a maximum capacity which can not be exceeded.

Comparison of our model with conventional models describing physical protection of organic matter in soils

Our model of physical protection of organic matter was compared with the ideas underlying two others. Fig. 1 (a, b and c) show schematically the flows of organic matter within the models. Mechanisms expressed in model (b) were used by Van Veen et al. (1985) and Verberne et al. (1990); those in model (c) by Jenkinson (1990). Note that the results given here are not derived from these author's own models but our own versions of their model based on their published descriptions. In each model there are separate pools of organic matter; in models (b) and (c) these pools turn over according to first-order kinetics. Residues are decomposed by the microbial biomass and microbial products may then become physically protected.

In our model, (a), protected organic matter P_{OM} is unable to decompose; it must become free before it can be attacked by microorganisms or by extracellular enzymes. We expect the

capacity X to be related to the clay or clay plus silt content of the soil.

In model (b) microbial products are distributed between non-protected (N_{OM}) and protected organic matter (P_{OM}); the actual distribution is governed by a specific parameter α_p that we expect to be related to the clay content of the soil. With increasing clay content, a greater proportion of microbial products becomes physically protected. Physically protected organic matter is able to decompose, but it decomposes at a slower rate than non-protected organic matter.

In model (c) there is a single pool of humified organic matter in soil but physical protection is modeled implicitly during the conversion of inputs. The higher the Cation Exchange Capacity (CEC) or clay content of a soil, the higher the efficiency (E) of utilization of metabolic products by the soil biota.

The essential innovation of model (a) is that, unlike the other models, the rate at which organic matter may become protected is not directly related to soil texture, but to θ. It allows all soils to protect or release organic matter in the same way regardless of organic matter content or soil texture if θ is currently the same.

The models (a) to (c) were programmed within MOTOR (Whitmore, 1995). In this way we ensured that the turnover of organic matter differed only in the ways explained above and not through any peripheral differences in differently programmed models. Each model (a) to (c) was coupled to the FSEOPT system (Stol et al., 1992) which seeks the least residual sum of the squares of the difference between measurements and simulations using the downhill simplex approach (Press et al., 1986). The best values of the parameters E (efficiency), K_R (rate of decomposition of residues), K_B (rate of decomposition of microbial biomass), K_N (rate of decomposition of N_{OM}), K_a (rate of adsorption) and K_d (rate of desorption) were sought; these were the same for all ten data-sets, but X varied from soil to soil. Combinations of parameters from this system were also used during the sensitivity analysis. Initial values of the non-protected organic matter pools were set at 25% of the total because initial simulations with the model suggested that this pool would be at about this level with regular annual inputs of organic matter. Long-term monthly weather data from the nearby weather station at Eelde airport were used to run the model for the first four years. During later years when the containers were kept inside the temperatures were rather higher. Measurements of the temperature in the greenhouse during 1993-94 allowed us to estimate how much.

Nitrate concentrations in the soils had increased considerably during the incubation, espcecially in the soils where grass was added. Ammonium concentrations remained low, however (Table 2). We assumed that the accumulation of nitrate in the soils did not affect the rates of organic matter decomposition.

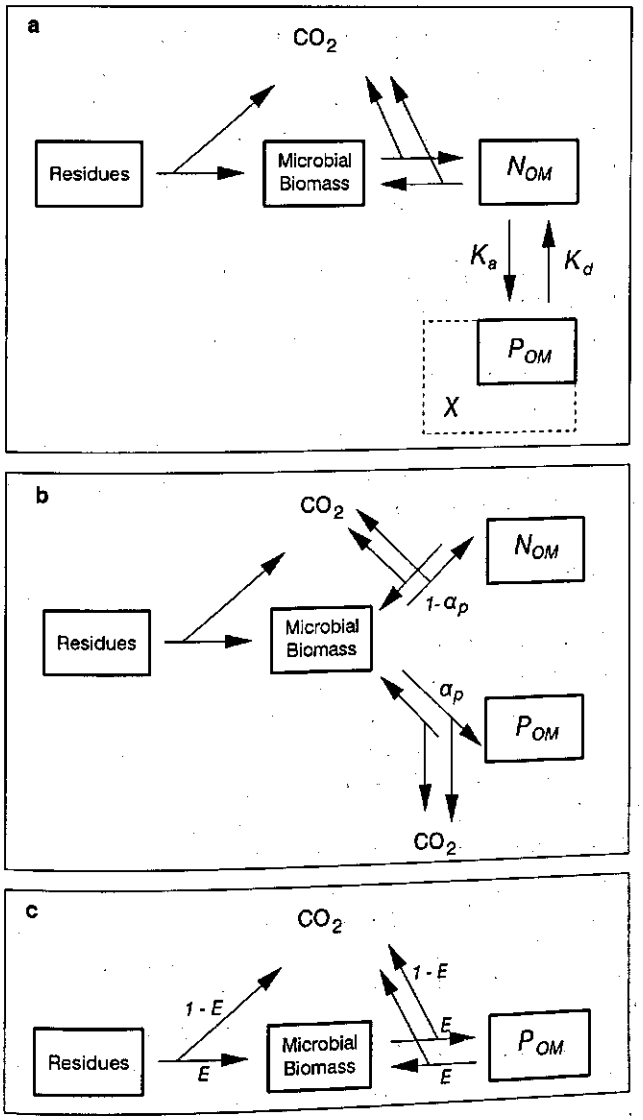


Fig. 1. Flow-chart of carbon in each of the three models showing only the essential differences between the models (a) where protection is explicitly modeled by adsorption-desorption kinetics, (b) where microbial products are distributed between protected and non-protected organic matter, and protected organic matter has a slower turnover rate than non-protected organic matter, (c) where soils which protect more organic matter release a smaller proportion of CO_2 during decomposition (higher efficiency).
N_{OM} = non-protected organic matter, P_{OM} = protected organic matter, X = capacity of a soil to protect organic matter (mg C kg^{-1} soil), α_p = distribution factor between P_{OM} and N_{OM} in model (b), E = efficiency of utilization of metabolic products in model (c).

RESULTS

By systematically varying the rate constants and efficiencies, all three models could be tuned to produce good fits of the data. The values of the derived rate constants and efficiencies of model (a) at best fit are given in Table 3 and the residual sums of squares found with each model are given in Table 4.

Table 3. Values of the most important parameters in our model (a) of protection of organic matter.

Description	Symbol	Value
Efficiency of use of organic matter	E	0.587
Rate of decomposition of fresh organic matter (residues)	K_R	0.494[1]
Rate of decomposition of biomass (2nd order)	K_B	0.487[2]
Rate of decomposition of N_{OM}	K_N	0.0225[1]
Rate of protection (adsorption)	K_a	0.0025[1]
Rate of release (desorption) of P_{OM}	K_d	0.0354[1]

N_{OM} = Non protected organic matter, P_{OM} = Protected organic matter.
[1] month^{-1}
[2] g μg^{-1} month^{-1}

Table 4. Statistics indicating the success of each model.

Model	Residual sum of squares	Mean deviation (% C)
(a)	2.856	0.0919
(b)	3.1125	0.1001
(c)	3.510	0.1129

Fig. 2 shows the data and the fit attained with model (a) to the measurements made on the soils taken from the Joh. Kerkhovenpolder. It is quite clear that the model is able to reproduce the build-up and decline of organic matter in soil very well (Fig. 2a). The organic matter build-up and decline was simulated almost equally well in soils made up of very different amounts of clay and silt. The decrease in soil C in the soils without additions was also simulated very well (Fig. 2b). Fig. 3 a and b show that the model fitted the data from the other five soils equally well. Judged by the statistics in Table 4 our model (a) is clearly superior to the others; judged by eye, our model remains better (diagrams for models b and c not shown), but all the models were within both experimental and a sensible error of about 5% to which organic matter in soil cannot be more precisely measured. Model (a) remained superior for a different reason, however. Because adsorption of organic matter onto the surfaces of clay and silt particles is the mechanism of physical protection in our model, there

should be a good relationship between the clay and silt content of soils and the simulated protective capacity (X). In the other models (b) and (c) the mechanism of physical protection is not stated explicitly and clay content is normally used to explain the differences in decomposition rates between soils. So there should be a good relationship between clay content and simulated values of α_p in model (b) and clay content and simulated efficiencies (E) in model (c).

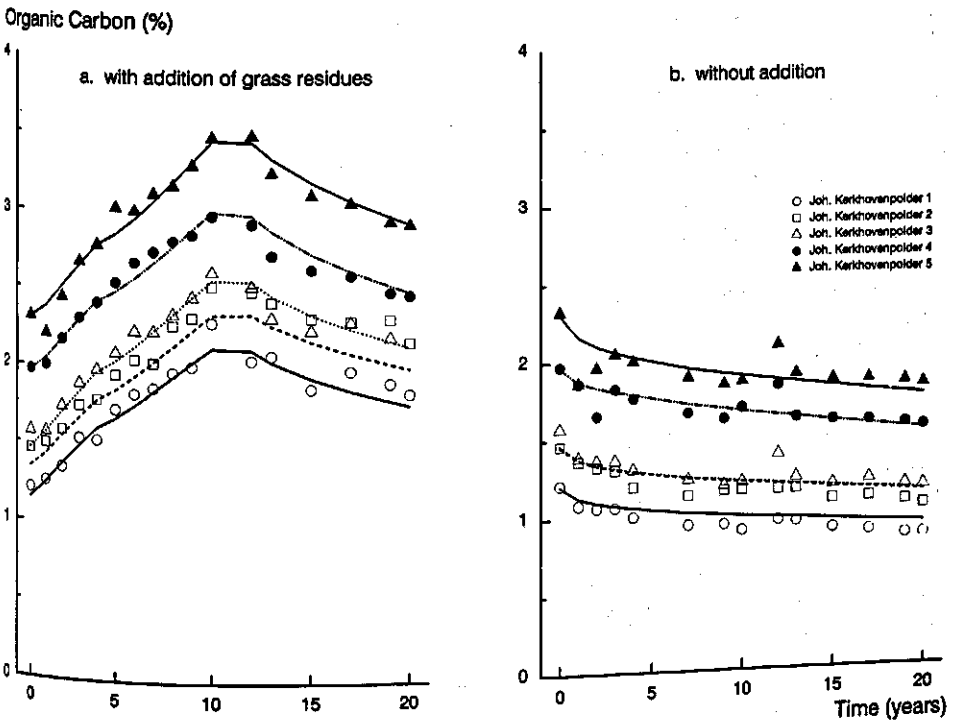


Fig. 2. The build-up and decline of organic carbon in soils number 1 to 5 in Table 1 (a) with the addition of grass residues every year for 10 years and no addition during a subsequent 10 years; (b) no addition throughout the 20 year period.

We found a linear relationship between the clay and clay + silt content of the soils (< 50 µm) and the protective capacity, X, in each soil. If an outlier (Burum; soil with a very low pH) is excluded the relationship between clay plus silt content and X becomes very good indeed (Fig. 4; Table 5). The relationships between the clay and clay + silt content of the soils and values of α_p in model (b) and E in model (c), were not significant. Table 5 gives the best relationships between particles and X in model (a) and α_p in model (b) and E in model (c). As can be seen from the size of the intercept in the first relationship of model (a),

a part of the protection of organic matter in soils was not related to the clay and silt content of the soil. A multiple regression of X on clay and silt content (giving separate weight to the two components) accounted for rather more of the variance in X but the relationship still possessed a large intercept (second relationship model (a); Table 5). The clay content accounts for about three times as much of the variation as the silt content.

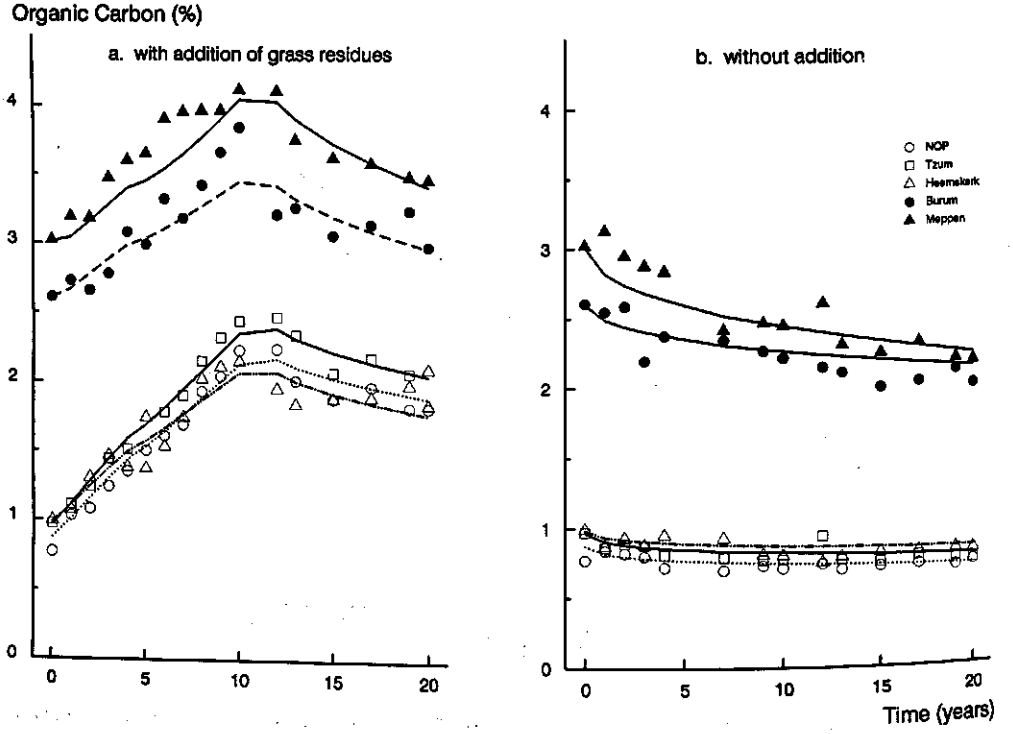


Fig. 3. The build-up and decline of organic carbon in soils number 6 to 10 in Table 1 (a) with the addition of grass residues every year for 10 years and no addition during a subsequent 10 years; (b) no addition throughout the 20 year period.

The amount of soil initially added to the containers was relatively small and so a correction was made in the model for the amount of soil removed and not replaced during sampling. The measured changes of the masses of the soils to which grass was added during the incubation, agreed quite well with the calculated changes of the masses of the soils. This shows that the correction is sound. Surprisingly, the correction is far less good for the soils receiving no organic matter addition. Soils appear to be losing mass at a greater rate than the sampling record suggests and no explanation can be offered for this observation. Accordingly the results and simulations in Figs. 2(b) and 3(b) should be given less weight than the results and simulations in Figs. 2(a) and 3(a).

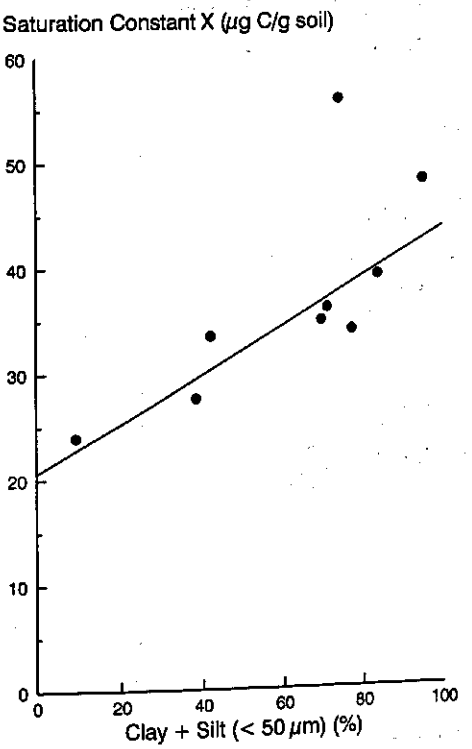


Fig. 4. The relationship found between the capacity of each soil to protect organic matter (X) and the amount of clay + silt (particles < 50 µm) in the soils in Table 1 (excluding Meppen).

Table 5. Relationships between the fraction < 50 µm or the fractions < 2 µm and 2-50 µm and the protective capacity (X; mg C kg^{-1} soil) of soils in model (a); between the fraction < 2 µm and the distribution factor α_p in model (b), and between the fraction < 2 µm and the efficiency of use of organic matter (E) in model (c). Burum and Meppen were excluded from the analyses.

Model (a): X = 20.6 + 0.232 x % < 50 µm. (77.9% of the variance is accounted for)
Model (a): X = 22.0 + 0.356 x %< 2µm + 0.142 x % (2-50 µm). (85.9% of the variance is accounted for)
Model (b): α_p = 0.553 + 0.00576 x % < 2µm (47.6% of the variance is accounted for)
Model (c): E = 0.469 - 0.00903 x % < 2µm (38.7% of the variance is accounted for)

Sensitivity Analysis

The rate constants derived from the ideas in models (b) and (c) have already been subjected to a sensitivity analysis in order to check that no one model parameter is responsible for explaining most of the variation in the simulated results (Burgers and Verberne, 1991, Bradbury et al., 1993). Model (a), however, contains novel ideas: saturation X, a rate of protection (K_a) and a rate of release (K_d). We examined the effects of changes of up to 40% in these parameter values on one of the soils in Table 1, Joh. Kerkhovenpolder (4). We estimated the effect of changes in each parameter of up to 40% on the organic carbon found in the soil after 10 years of addition of grass when we expected the changes to be at maximum. For comparison we also examined the sensitivity of the model in simulating measurements made on the same soil without addition of grass. The results are shown in Figs. 5 (a) and (b). We have also tested the sensitivity of the model to K_N, the rate of decomposition of non-protected organic matter; although this has been done by the other authors it seemed wise to check that our new concepts did not make the model over-sensitive to changes in this parameter. The gradient and rate of change of gradient of each of the lines relates how each parameter affects the retention of organic matter. The model is most sensitive to the value of X but this parameter has more effects if it underestimated than when overestimated. The model is least sensitive (in terms of percentage change in the parameter value) to the rate of release of organic matter from protected sites, K_d.

The effect on organic C retained in soil of a change in K_N of 40% was about 10%. This is very much in line with what Bradbury et al. (1993) found for the decomposition of their humus pool in a model roughly similar in structure to model (c) and in line with what Burgers and Verberne (1991) observed with a pool of organic matter turning over more slowly than the others such as in (b). This suggests that our addition of a mechanism for protection has not made the description of the decomposition of non-protected organic matter unsound. There is no great difference between the sensitivity of the model to each of these parameters whether or not grass residues were added to soil.

Table 6 shows the results of correlations between parameter values found during the final stages of the iteration. Five rate constants, the 10 different values of X (for each soil type) and the efficiency factor E were estimated during this procedure. Correlations between parameters often indicate that a model is over-parameterized, that is to say one parameter could easily serve the purpose of two or more if the model were to be phrased differently. A classic example of correlation in models of organic matter turnover is the size of an organic matter pool and its rate constant. Where these must both be estimated from data they are almost always highly correlated. Model (a) introduces more parameters into the calculation of organic matter turnover than models (b) or (c) and it is therefore particularly important to show that these are not redundant but do describe an important mechanism. Table 6 shows that none of the quantities was highly correlated with another in particular, the parameters introduced in model (a) X, K_d and K_a, were indeed independent of one another.

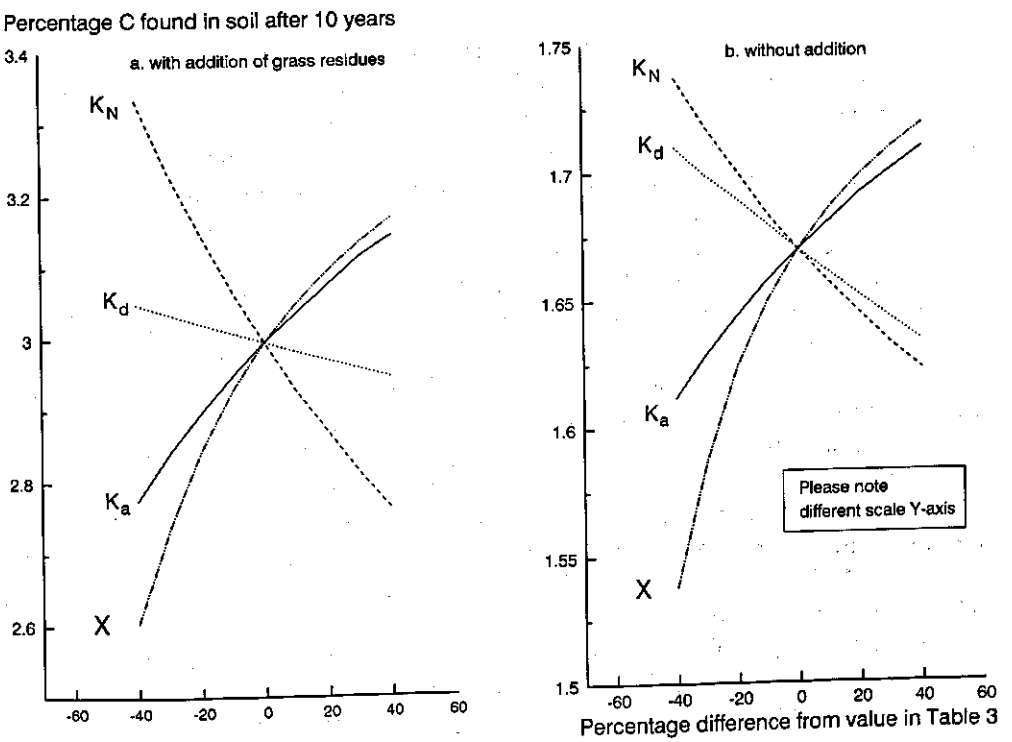


Fig. 5. The sensitivity of simulations made with model (a) of the amount of organic carbon found in soil 4 (Table 1) after 10 years to changes in each of four of the parameters listed in Table 3: (a) with addition of grass residues (b) without.

Table 6. Correlations between parameters in model (a).

Coefficients of correlation					
E	1				
K_N	-0.490	1			
K_a	-0.551	0.414	1		
K_d	-0.398	0.429	0.326	1	
X	0.620	0.493	0.553	0.265	1

E = Efficiency, K_N = rate of decomposition non-protected organic matter, K_a = rate of protection (adsorption), K_d = rate of release (desorption), X = Capacity of a soil to protect organic matter.

DISCUSSION

The fact that we observed a good relationship between the simulated protective capacities and clay contents of soils suggests that the degree of saturation of the protective capacity controls the build-up and decline of organic matter and that the adsorption-desorption mechanism provides a good explanation for the processes actually occurring in the soil. This observation is in line with the results found in laboratory studies with pure clays where it became increasingly difficult for adsorption to proceed as the coverage of the adsorbent surface increased (Stotzky, 1986).

The observation that the simulated protective capacity was closely correlated with soil texture, while α_p and E were not, suggests that the build-up and decline of soil organic matter is not regulated by soil texture *per se*. This is in line with previous findings (Hassink, 1995[b]) and it explains why clay soils with a low organic matter content preserve more applied C in soil than sandy soils (Amato and Ladd, 1992; Hassink et al., 1995), while the preservation of residue C in clay soils with a higher organic matter content is not different from that in sandy soils (Gregorich et al., 1991; Hassink et al., 1995).

In our model (a) we assumed that the mechanism of physical protection is adsorption of organic C to clay and silt particles. Considerable published evidence indicates that one of the principal factors responsible for enhanced retention of organic matter in soils is its ability to associate with clay and silt particles (Allison et al., 1949; Pinck et al., 1954; Theng, 1979; Marshman and Marshall, 1981; Martin and Haider, 1986; Hassink, 1995[a]). The assumption expressed in model (b) that with increasing clay content a greater proportion of the newly formed microbial products becomes and remains physically protected (Van Veen et al., 1985; Verberne et al., 1990) contradicts the results of Gregrorich et al., 1991 and Hassink, 1995[b], while the assumption in model (c), that the efficiency of utilization of metabolic products by soil biota increases with the clay content of the soil (Jenkinson, 1990), is greatly questioned (Van Veen and Kuikman, 1990).

In most cases a decrease in pH favors increased bonding of organics (Weber, 1970; Varadachari et al., 1994), although this is not always the case (Harter and Stotzky, 1971). If organics are held at clay surfaces through cation bridging, adsorption should increase as the pH decreases, because the protonation of amino and carboxyl groups will give rise to an increase in positive charges and correspondingly more attraction to anionic clays (Huang, 1990). If adsorption proceeds through van der Waals forces, however, this does not have to be the case (Hamzehi and Pflug, 1981). At low pH values, adsorption of organics can occur in the interlamellar spaces (Schnitzer and Kahn, 1972). The low pH of the Burum soil might have increased the strength of the binding of organic matter explaining why we estimated its protective capacity to be greater than that of soils with similar silt and clay contents.

The size of the intercept in Fig. 4 shows that a part of the protection being afforded to organic matter in soils was not related to the clay or silt content of the soil. About one third of the total protection of organic matter in clay rich soils appears not to be related to the clay content. This additional protection may occur through stabilization of organic matter by

organic or ferritic soil colloids (Burchill et al., 1981) or chemical stabilization of organic matter. Alternatively it is possible, even likely, that at low clay contents the relationship shown in Fig. 4 is no longer linear. Dilute clay particles in sandy soils associate far more organic matter per unit of clay than the clay particles in clay soils (Hassink et al., 1993; Matus, 1995; Hassink et al., 1995).

Elliot and Cambardella (1991) pointed out that clay- and silt-associated organic matter can be a heterogeneous pool of soil organic matter. It has been observed that in addition to adsorption on the surface of clay and silt particles, organics can also be incorporated into the interlamellar spaces (Theng et al., 1986; Boyd and Mortland, 1990). At loadings that exceed monolayer exterior surface coverage, it seems that organics are held by secondary surface adsorption forces and the organics appear to retain high activity (Hughes and Simpson, 1978). It has also been suggested that organics can form different layers around clay and silt particles and aggregates. Materials associated with external layers of organic matter coatings on clay and silt particles are younger and less protected than organics associated with internal layers (Skjemstad et al., 1993; Buyanovski et al., 1994). All these observations suggest that there is probably a continuum from non-protected to completely protected organic matter. If organic matter can itself bind further organic molecules then the Langmuir type description underpinning equations (1) and (2) is no longer valid. A Freundlich type approach which allows coverage to a depth greater than a mono-layer might be more appropriate but we have preferred the mechanistic Langmuir approach expressed as it is in terms of tangible properties of soil. McLaren and Peterson (1965) suggested that the adsorption of large molecules would depend not just on the probability of finding an available space but also on the probability of finding ν empty spaces where ν is the number of binding groups in the organic molecule. Likewise desorption would depend on ν too. It is, however, virtually inpossible to characeristize ν of the diverse molecules of the soil humus. If, however, protection is as much as entering or escaping from narrow pore necks or 'bottlenecks' the number of adjacent sites may be less important. In spite of our simplified assumptions that only a mono-layer of organic matter can be built up and that there is only free and completely protected organic matter, the model appeared to predict the build-up and decline of soil organic matter in different soils remarkably well.

CONCLUSION

A model is presented that provides an explanation for the apparently contradictory observations that soils receiving identical amounts of fresh residues as input but differing in texture have sometimes been reported to build up soil organic matter to the same extent (Gregorich et al., 1991) but on other occasions to different extents (Amato and Ladd, 1992). The net rate of accumulation of organic matter depends not on the protective capacity of a soil *per se*, but on the extent to which this capacity is filled with organic matter. The binding

and release of organic matter to these protective sites can be described well by simple adsorption-desorption kinetics.

ACKNOWLEDGEMENTS

We thank L. Brussaard, J.J. Neeteson, P. de Willigen and K.B. Zwart for critically reading the manuscript. We thank S. de Haan of the Institute for Soil Fertility Research at Haren for performing the long-term incubation experiment.

REFERENCES

Allison, F.E., M.S. Sherman, and L.A. Pinck. 1949. Maintenance of soil organic matter: I. Soil Sci. 68:463-478.

Amato, M., and J.N. Ladd. 1992. Decomposition of ^{14}C-labelled glucose and legume material in soils: properties influencing the accumulation of organic residue C and microbial biomass C. Soil Biol. Biochem. 24:455-464.

Burgers, S., and E.L.J. Verberne. 1991. Sensitivity analysis via different techniques, applied to a simulation model for organic matter dynamics (in Dutch). Instituut Bodemvruchtbaarheid Haren (Gr.), NOTA 246.

Boyd, S.A., and M.M. Mortland. 1990. Enzyme interactions with clays and clay-organic matter complexes. p. 1-28. *In* J.M. Bollag and G. Stotzky (ed.) Soil Biochemistry Vol. 6. Marcel Dekker, Inc., New York and Basel.

Bradbury, N.J., A.P. Whitmore, P.B.S. Hart, and D.S. Jenkinson. 1993. Modelling the fate of nitrogen in crop and soil in the years following application of ^{15}N-labelled fertilizer to winter wheat. J. Agric. Sci., Camb. 121:363-379.

Burchill, S., M.H.B. Hayes, and D.J. Greenland. 1981. Adsorption. p. 221-400. *In* D.J. Greenland and M.H.B. Hayes (ed.) The chemistry of soil processes. John Wiley & Sons, New York.

Buyanovsky, G.A., M. Aslam, and G.H. Wagner. 1994. Carbon turnover in soil physical fractions. Soil Sci. Soc. Am. J. 58:1167-1173.

Elliott, E.T., and C.A. Cambardella. 1991. Physical separation of soil organic matter. Agric. Ecosyst. Envir. 34:407-419.

Feller, C., E. Fritsch, R. Poss, and C. Valentin. 1991. Effet de la texture sur le stockage et la dynamique des matières organiques dans quelques sols ferrugineux et ferrallitiques. Cahier Orstom Ser. Pedologie 26:25-36.

Gregorich, E.G., R.P. Voroney, and R.G. Kachanoski. 1991. Turnover of carbon through the microbial biomass in soils with different textures. Soil Biol. Biochem. 23:799-805.

Hamzehi, E., and W. Pflug. 1981. Sorption and binding mechanisms of polysaccharide cleaving soil enzymes by clay minerals. Z. Pflanzenernaehr. Bodenkd. 144:505-513.

Harter, R.D., and G. Stotzky. 1971. Formation of clay-protein complexes. Soil Sci. Soc. Am. Proc. 35:383-388.

Hassink, J. 1994. Effects of soil texture and grassland management on soil organic C and N and rates of C and N mineralization. Soil Biol. Biochem. 26:1221-1231.

Hassink, J. 1995[a]. The capacity of soils to preserve organic C and N by their association with clay and silt particles. Subm. to Soil Sci. Soc. Am. J.

Hassink, J. 1995[b]. The degree of saturation of the protective capacity of a soil controls the preservation of ^{14}C-labelled residues. Subm. to Soil Sci. Soc. Am. J.

Hassink, J., L.A. Bouwman, K.B. Zwart, J. Bloem, and L. Brussaard. 1993. Relationships between soil texture, physical protection of organic matter, soil biota and C and N mineralization in grassland soils. Geoderma 57:105-128.

Hassink, J., F.J. Matus, C. Chenu, and J.W. Dalenberg. 1995. Interactions between soil biota, soil organic matter and soil structure. Accepted by Adv. Agroecology.

Huang, P.M. 1990. Role of soil minerals in transformations of natural organics and xenobiotics in soil. p. 29-115. *In* J.M. Bollag and G. Stotzky (ed.) Soil Biochemistry Vol. 6. Marcel Dekker, Inc., New York and Basel.

Hughes, T.D., and G.H. Simpson. 1978. Arylsulfatase-clay interactions. II. The effect of kaolinite and montmorillonite on arylsulfatase activity. Aust. J. Soil Res. 16:35-40.

Jenkinson, D.S. 1977. Studies on the decomposition of plant material in soil. V. The effects of plant cover and soil type on the loss of carbon from ^{14}C labelled ryegrass decomposing under field conditions. J. Soil Sci. 28:424-434.

Jenkinson, D.S. 1988. Soil organic matter and its dynamics. p. 564-607. *In* A. Wild (ed.) Russel's soil conditions and plant growth, 11th ed. Longman, New York.

Jenkinson, D.S. 1990. The turnover of organic carbon and nitrogen in soil. Phil. Trans. R. Soc. Lond. B 329:361-368.

Kortleven, J. 1963. Quantitative aspects of humus accumulation and decomposition. Versl. Landb. Ond. nr. 69.1 PUDOC, Wageningen (in Dutch).

Ladd, J.N., J.M. Oades, and M. Amato. 1981. Microbial biomass formed from ^{14}C, ^{15}N-labelled plant material decomposing in soils in the field. Soil Biol. Biochem. 13:119-126.

Ladd, J.N., M. Amato., and J.M. Oades. 1985. Decomposition of plant materials in Australian soils III Residual organic and microbial biomass C and N from isotope-labelled plant material and soil organic matter decomposing under field conditions. Austr. J. Soil Res. 23:603-611.

Marshman, N.A., and K.C. Marshall. 1981. Bacterial growth on proteins in the presence of clay minerals. Soil Biol. Biochem. 13:127-134.

Martin, J.P., and K. Haider. 1986. Influence of mineral colloids on turnover rates of soil organic carbon. p. 283-304. *In* P.M. Huang and M. Schnitzer (ed.) Interactions of soil minerals with natural organics and microbes. Soil Science Society of America, Inc. Madison, Wisconsin, USA.

McLaren, A.D., and G.H. Peterson. 1965. Physical chemistry and biological chemistry of clay

mineral-organic nitrogen complexes. p. 259-284. *In* W.V. Bartholomew and F.E. Clark (ed.) Soil nitrogen. American Society of Agronomy, Madison.

Matus, F.J. 1995. The distribution of soil organic matter of various aggregate size classes in arable soils. I. Relationships between clay content of aggregates of a sand and a clay soil and carbon mineralization, nitrogen mineralization and microbial biomass carbon. Subm. to Soil Biol. Biochem.

Mebius, L.J. 1960. A rapid method for the determination of organic carbon in soil. Anal. Chim. Acta 22:120-124.

Parton, W.J., D.S. Schimel, C.V. Cole, and D.S. Ojima. 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. Soil Sci. Soc. Am. J. 51:1173-1179.

Pinck, L.A., R.S. Dyal, and F.E. Allison. 1954. Protein-montmorillonite complexes, their preparation and the effects of soil microorganisms on their decomposition. Soil Sci. 78:109-118.

Press, W.H., B.P. Flannery, S.A. Teukolsky, and W.T. Vetterling. 1986. Numerical recipes, the art of scientific computing. Cambridge University Press, Cambridge.

Schnitzer, M., and S.U. Kahn. 1972. Humic substances in the environment. Marcel Dekker, New York.

Skjemstad, J.O., L.J. Janik, M.J. Head, and S.G. McClure. 1993. High energy ultraviolet photo-oxidation: a novel technique for studying physically protected organic matter in clay- and silt-sized aggregates. J. Soil Sci. 44:485-499.

Spain, A.V. 1990. Influence of environmental conditions and some soil chemical properties on the carbon and nitrogen contents of some tropical Australian rainforest soils. Aust. J. Soil Res. 28:825-839.

Stol, W., D.I. Rouse, D.W.G. van Kralingen, and O. Klepper. 1992. FSEOPT a FORTRAN Program for calibration and uncertainty analysis of simulation models. Simulation report CABO-TT no 24.

Stotzky, G. 1986. Influence of soil mineral colloids on metabolic processes, growth, adhesion, and ecology of microbes and viruses. p. 305-428. *In* P.M. Huang and M. Schnitzer (ed) Interactions of soil minerals with natural organics and microbes. Soil Sci. Soc. Am. Madison, Wisconsin USA.

Theng, B.K.G. 1979. Formation and properties of clay-polymer complexes. Elsevier, Amsterdam.

Theng, B.K.G., G.J. Churchman, and R.H. Newman. 1986. The occurrence of interlayer clay-organic complexes in two New Zealand soils. Soil Sci. 142:262-266.

Tisdall, J.M., and J.M. Oades. 1982. Organic matter and water-stable aggregates in soils. J. Soil Sci. 33:141-163.

Van Veen, J.A., and E.A. Paul. 1981. Organic carbon dynamics in grassland soils: I. Background information and computer simulation. Can. J. Soil Sci. 61:185-201.

Van Veen, J.A., and P.J. Kuikman. 1990. Soil structural aspects of decomposition of organic matter by microorganisms. Biogeochemistry 11:213-233.

Van Veen, J.A., J.N. Ladd, and M. Amato. 1985. Turnover of carbon and nitrogen through

the microbial biomass in a sandy loam and a clay soil incubated with [^{14}C(U)]glucose and [^{15}N]$(NH_4)_2SO_4$ under different moisture regimes. Soil Biol. Biochem. 17:257-274.

Varadachari, C., A.H. Mondal, D.C. Nayak, and K. Ghosh. 1994. Clay-humus complexation: effect of pH and the nature of bonding. Soil Biol Biochem. 26:1145-1149.

Verberne, E.L.J., J. Hassink, P. de Willigen, J.J.R. Groot and J.A. van Veen. 1990. Modelling organic matter dynamics in different soils. Neth. J. Agric. Sci. 38:221-238.

Weber, J.B. 1970. Mechanisms of adsorption of s-triazines by clay colloids and factors affecting plant availability. Residue Rev. 32:93-130.

Whitmore, A.P. 1995. MOTOR: A Modular description of Organic matter TurnOveR in soil. Subm. to Soil Biol. Biochem.

CHAPTER 11

PREDICTION OF THE NON-FERTILIZER N SUPPLY OF GRASSLAND SOILS

By: J. Hassink. Submitted to Plant and Soil

PREDICTION OF THE NON-FERTILIZER N SUPPLY OF GRASSLAND SOILS

ABSTRACT

Different methods to estimate the non-fertilizer N supply (NFNS) of mineral and peaty grassland soils were compared. NFNS was defined as the N uptake on unfertilized plots. For mineral soils the potential mineralization rate (0-12 weeks), macroorganic matter and active microbial biomass (determined by the substrate-induced respiration method; SIR) were correlated positively with NFNS. The difference between the actual soil organic N or microbial N content (determined by the fumigation incubation method) and their contents under equilibrium conditions (Δ org. N and Δ MB-N), however, gave the best estimations of NFNS. For field conditions the best estimation for NFNS was: NFNS (kg N ha^{-1} yr^{-1}) = 132.3 + 42.1 * Δ org. N (g kg^{-1} soil; r = 0.80). This method is based on the observation that under old grassland swards close relationships exist between soil texture and the amounts of soil organic N and microbial N. These relationships are assumed to represent equilibrium conditions as under old swards under constant management, the gain in soil organic N and microbial N equals the losses. Soils under young grassland and recently reclaimed soils contained less soil organic N and microbial N. In such soils the amounts of organic N and microbial N increase with time, which is reflected in a lower NFNS. The annual accumulation of organic and microbial N gradually becomes smaller until organic N, microbial N and NFNS reach equilibrium. The main advantage of the "difference method" in comparison with the other methods is its simplicity and fastness.

For peat soils no relationship was found between soil texture and the amount of soil organic N. The NFNS of peat soils could easily be estimated from the average deepest groundwater table: NFNS (kg N ha^{-1} yr^{-1}) = 188.8 + 3.1 * deepest groundwater table (cm below the surface; r = 0.86).

INTRODUCTION

On unfertilized fields, the non-fertilizer N supply (NFNS) consists of soil organic N that is mineralized during the growing season, mineral N that is present in the soil profile in spring, N in dry and wet deposition, and N fixation by free and symbiotic living microbes in the soil. Soils differ greatly in the amounts of nitrogen made available for uptake by plants during the growing season; the contribution of deposition and N fixation to NFNS, however, is quite constant or small (in the absence of clover), respectively. So, the amount of N taken up from plots receiving no fertilizer N provides a good indication of the NFNS (Warren and Whitehead, 1988). Annual N uptake rates on unfertilized plots can range between 10 and 900 kg N per ha in grassland soils (Brockman, 1969; Richards and Hobson, 1977). A reliable

prediction of NFNS would be very helpful to advise farmers on the optimum fertilizer application rate. Different methods have been proposed to predict NFNS, such as quantification of actual mineralization rates in the field (Raison et al., 1987) and potential mineralization rates in the laboratory (Stanford and Smith, 1972; Nordmeyer and Richter, 1985). The determination of potential mineralization rates in the laboratory has the advantage that soils from different locations can be compared relatively simply under the same conditions. Hassink (1994) incubated field-moist, homogenized samples from different grassland sites and determined the potential mineralization rate as the increase in inorganic N after 2 and 12 weeks incubation. When field-moist samples are incubated, N mineralization rates remain relatively constant during incubation (Addiscott, 1983; Hassink, 1994). So a short incubation period might be sufficient to give a good estimation of NFNS.

The active organic matter fractions (such as macroorganic matter and the microbial biomass) are assumed to play a dominant role in the availability of nutrients (Janzen et al., 1992). Warren and Whitehead (1988) observed that the amount of N in macroorganic matter contributes substantially to available N. It has been found that for soils differing in texture especially the light fraction of macroorganic matter (> 150 μm; Meijboom et al., 1995) and the active microbial biomass (determined by the substrate-induced respiration method; Anderson and Domsch, 1978) correlate very well with their potential mineralization rates (Hassink, 1995). So the amounts of active organic matter and microbial biomass could also give a good estimation of the amount of available soil N. A drawback of the developed methods to estimate NFNS is that they are time-consuming and consequently not very attractive for general application.

A new approach to estimate NFNS is based on the observation that in old grasslands a close relationship exists between the organic N content and the granular fraction < 50 μm (clay and silt fraction; Hassink, 1994). The increase in organic N content with increasing clay and silt content is due to the higher physical protection of organic matter in fine-textured soils than in coarse-textured soils (Tisdall and Oades, 1982; Hassink et al., 1995). In addition to organic N, microbial N generally also correlates positively with the clay and silt content of a soil (Gregorich et al., 1991; Amato and Ladd, 1992; Van Veen et al., 1985; Hassink et al., 1995). It may be assumed that in grassland soils, where the amount and quality of inputs of organic residues to the soil is relatively constant, the amount of both organic N and microbial N reach an equilibrium that is mainly controlled by soil texture (Hassink, 1994). When the organic N content is in equilibrium, the annual amount of N that is incorporated into the organic matter pool balances the amount of N that mineralizes in the same year. Soils that have an organic N content that is below the equilibrium level will mineralize less N than is incorporated into the organic N pool and will consequently increase their organic N content (Ryden, 1984; Hassink et al., 1990; Cuttle and Bourne, 1992). It was found that under young swards and in recently reclaimed polder soils in the Netherlands, the amount of soil organic N in grasslands increased with more than 100 kg N per ha annually (Hassink and Neeteson,1991; Hassink, 1994). During the first years after arable soil is sown to grass, the difference between the actual soil organic N content and the equilibrium value is at its

maximum and the accumulation of organic N is high. Accumulation of organic N is finite, however, and the annual increases become smaller with time, and the organic N content reaches equilibrium asymptotically (Jenkinson, 1988; Ryden, 1984). In line with this, the difference between the actual amount of organic and microbial N, and the amounts under equilibrium conditions might give a good estimation of the NFNS. The great advantage of this new approach to estimate NFNS is its simplicity and fastness.

The objective of this paper is to test which approach gives the best estimation of the non-fertilizer N supply of grassland soils under field conditions. In order to test this, estimates of N availability obtained with the different approaches were correlated with the amounts of N taken up by unfertilized perennial ryegrass growing under uniform environmental conditions in the greenhouse and under field conditions.

MATERIALS AND METHODS

Fields sampled for incubation and determination of potential N mineralization rates and soil organic N

In March 1989 and in March 1991 samples were collected from mineral grassland soils that were located in different areas in the Netherlands. The land was grazed by dairy cattle and received 400-500 kg fertilizer-N per ha per year. Three mixed samples, each consisting of 20 bulked cores, were taken from the 0-10 and 10-25 cm soil layer at each location. Characteristics of the soils are given by Hassink (1994).

Soil samples were sieved through a 0.008-m mesh screen; roots and stubble were removed. N mineralization was determined by measuring the increase in mineral N after 2 and 12 weeks of incubation of soil samples in glass jars at 20 °C. The exact procedure is given by Hassink (1994).

Mineral N was measured colorimetrically after extraction with 1N KCl solution for 1 h using a soil:water ratio of 1:2.5.

Total soil N, including mineral N, was determined according to Deys (1961), after destruction with sulfuric acid and salycylic acid.

Determination of the microbial biomass and the active microbial biomass

The amount of N in the microbial biomass was determined in field-moist samples by the chloroform fumigation-incubation (FI) technique (Jenkinson and Powlson, 1976). A k value of 0.4 was used to calculate the biomass from the flush. The exact procedure has been described by Hassink et al. (1991).

The active microbial biomass was determined by the substrate-induced respiration (SIR) method (Anderson and Domsch, 1978). The exact procedure has was described by Hassink

(1993). The active microbial biomass was only determined in the grassland soils that were sampled in 1991.

Determination of the amount of macroorganic matter and its light fraction

Rewetted soil samples (250 g) were washed on two sieves (top sieve: mesh size 250 μm; bottom sieve: 150 μm) till the washings became clear. By pushing the soil through the top sieve all the macroaggregates (> 250 μm) that can be destroyed by agricultural practices (Tisdall and Oades, 1982) were destroyed. When a sieve with a mesh size < 150 μm was used, clogging occurred which made the washing procedure much more time-consuming. The mineral fraction was discarded by decantation. The organic fraction > 150 μm was called macroorganic matter. After combining the organic fraction from both sieves it was fractionated in silica suspensions with a density of 1.13 g cm^{-3}. The light fraction is the fraction with a density < 1.13 g cm^{-3}. The exact procedure of the density fractionation has been described by Meijboom et al. (1995). Macroorganic matter was only determined in a selection of the soils sampled in 1989 and 1991. The characteristics of these soils are given by Hassink (1995).

Assessment of the non-fertilizer N supply (NFNS) of soils

NFNS is defined as the amount of N taken up by ryegrass from unfertilized plots during a growing season. Information on NFNS was obtained from different sources:

(i) In March 1989 and in March 1991 soil samples were taken for the determination of potential N mineralization. At the same time undisturbed soil columns with a diameter of 20 cm and a height of 30 cm were pushed into those soils. From each location 5 undisturbed columns were taken to determine the N uptake by the herbage. They were installed in a greenhouse with a sliding roof that was closed automatically when it started raining. The temperature in the greenhouse was the same as outside. The columns were placed in distilled water, so that the water table was constant at 30 cm below the soil surface. Every week some distilled water was given on top of the columns to prevent salt accumulation in the top layer of the soil. All columns were fertilized with P and K to prevent these nutrients becoming limiting. The grass was harvested by cutting 2 cm above the soil surface at five successive occasions. As mineral N was determined at the time of sampling, NFNS could be corrected for the initial amount of mineral N.

At some of the sites where columns were taken in 1991, N uptake by the herbage was also determined on unfertilized plots in the field. At the selected sites unfertilized plots were laid out in triplicate, and these were cut at five successive occasions.

(ii) Information on NFNS under field conditions of other mineral grassland soils was obtained from fertilization experiments on mown grassland fields that were performed by the Nutrient Management Institute (NMI, Wageningen) and the Research Station for Cattle, Sheep and Horse Husbandry (PR, Lelystad) at different locations in the Netherlands. At all locations

plots that received no fertilizer were laid out in triplicate. From these locations only the organic N and clay and silt content of the top 20 cm of the soil were determined. When N uptake on unfertilized plots was determined in more than one year, the average value was taken. Some characteristics of these soils are given in Table 1.

NFNS in peat soils was determined at 9 locations (6 in the southwestern part and 3 in the northern part of the Netherlands) as N uptake on unfertilized plots in the field in 1993. Only the organic N and clay content and the water table during the growing season were determined. Some characteristics of these peat soils are given in Table 2. The results obtained from these sites were combined with data of peat soils that were sampled by Schothorst (1977).

Table 1. Some soil characteristics of the top 20 cm of mineral grassland soils where NFNS was determined under field conditions (experiments of NMI and PR)

	Years of experiment	Total N (g kg^{-1})	Particle size distribution, % particles <	
			2 µm	50 µm
Sandy soils				
Gortel	1973-83	1.4	4	12
Finsterwolde	1977-78	1.0	10	23
Hemrik	1978-81	2.1	3	15
Den Ham	1979-83	4.2	8	35
Ruurlo	1980-84	2.0	4	30
Achterberg	1988	1.6	4	9
Peest	1988	2.7	5	17
Cranendonck	1989-92	1.1	3	15
Tynaarlo	1989-93	2.3	3	25
Dalfsen	1991	1.9	2	10
Slootdorp	1992	1.6	5	45
Wageningen	1993	1.0	4	10
Loams and clays				
Finsterwolde	1977-78	1.4	59	85
Ten Boer	1977-78	4.0	40	63
Friens	1981-83	3.8	25	70
Burum	1982-91	4.1	29	72
Aduard	1987-88	3.4	30	70
Marknesse	1988	1.8	23	67
Swifterbant	1988	1.8	21	72
Zaltbommel	1991	4.0	51	91
Lelystad	1991	5.0	22	60
Haskerdijk	1991	4.4	54	89

Table 2. Some soil characteristics of the top 20 cm of the peat soils in the southwestern part and the northern part of the Netherlands where NFNS was determined under field conditions in 1993

	Total N ($g\ kg^{-1}$)	Particle size distribution, % particles <	
		2 μm	16 μm
South West			
Zegveld 1	21.0	28.1	40.3
Zegveld 2	26.9	17.0	28.1
Zegveld 3	13.5	38.7	57.3
Zegveld 4	19.8	31.3	44.2
Zegveld 5	17.5	22.4	38.2
Mijdrecht	16.3	15.3	26.7
North			
Onnen	24.2	16.6	27.4
De Wilgen	12.9	8.5	12.4
Boornzwaag	11.4	35.3	50.0

Statistical analysis

The relationships between NFNS and soil characteristics and between soil texture and soil organic N and N in microbial biomass were analyzed by correlation and stepwise multiple regression techniques (Genstat, 1987). The fraction < 50 μm (clay + silt content) was taken as an index of soil texture (Hassink, 1994).

RESULTS

Relationship between soil texture and soil organic N content and N in microbial biomass

Mineral soils: There were highly significant ($p < 0.05$) positive correlations between (i) the organic N content and (ii) the amount of N in the microbial biomass and the fraction < 50 μm (clay + silt content) for both the top 10 cm and the top 25 cm of old (soils more than 10 years under grass) grassland soils that were sampled in 1989 and 1991 to determine potential N mineralization (Table 3; Figs. 1, 2). Recently reclaimed polder soils and arable soils that had recently been sown to grass had lower organic N contents and less microbial biomass than other grassland soils with the same texture. It is assumed that the relationship between soil texture and organic N and microbial N of the old grassland soils represents equilibrium

conditions. This allows the calculation of the difference between the actual amounts of microbial and organic N and the amounts under equilibrium conditions for each soil.

In a previous paper the relationship between the organic N content in the top 20 cm and the fraction < 50 µm of old grasland soils with a deep groundwater table was given as N organic (%) = 0.147 (0.014) + 0.0026 (0.00039) x % < 50 µm (r^2 = 0.76; Hassink, 1994). This relationship was assumed to represent the equilibrium conditions for the soils that were sampled by NMI and PR (soils of Table 1).

Table 3. Correlations and relationships between soil organic N (g kg^{-1}), microbial biomass-N (g kg^{-1}) and the fraction < 50 µm (clay and silt content) for the top 10 cm and the 0-25 cm layer of old grassland soils sampled in 1989 and 1991 for the determination of potential N mineralization

0-10 cm:
Soil organic N (g kg^{-1}) = 1.4 (0.2) + 0.03564 (0.005) * % < 50 µm; r = 0.87
Microbial biomass-N (g kg^{-1}) = 0.0465 (0.0168) + 0.00285 (0.00034) * % < 50 µm; r = 0.90
0-25 cm:
Soil organic N (g kg^{-1}) = 1.2 (0.2) + 0.02833 (0.0037) * % < 50 µm; r = 0.88
Microbial biomass-N (g kg^{-1}) = 0.0404 (0.0139) + 0.001775 (0.000285) * % < 50 µm; r = 0.83

( ) = standard error of difference; r = coefficient of correlation

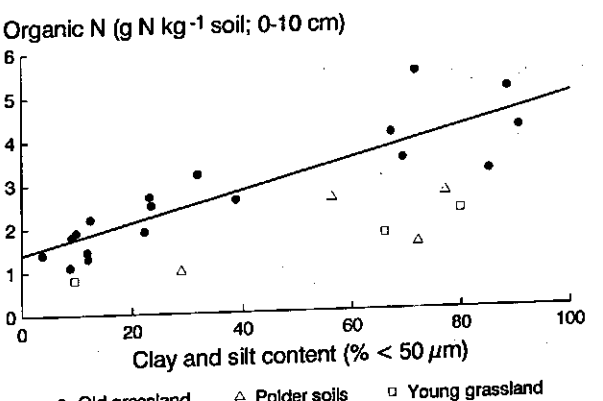


Fig. 1. Relationship between soil texture (% soil particles < 50 µm) and the organic N content (g N kg^{-1}) in the top 10 cm of the grassland soils sampled in 1989 and 1991. Old grassland soils have been under grass for more than 10 years, young grassland soils for less than 10 years. Polder soils were reclaimed from the sea less than 50 years ago. Significant relationship: N = 1.4 (0.2) + 0.03564 (0.005) * % particles < 50 µm.
( ) = standard error of difference.

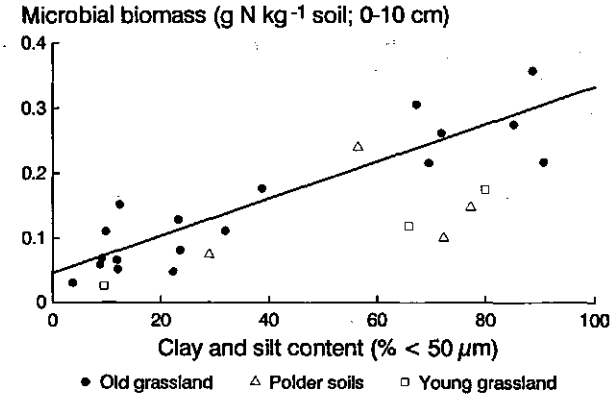


Fig. 2. Relationship between soil texture (% soil particles < 50 µm) and the amount of microbial biomass (g N kg^{-1}) in the top 10 cm of the grassland soils sampled in 1989 and 1991. Significant relationship: microbial biomass N = 0.0465 (0.0168) + 0.00285 (0.00034) * % particles < 50 µm. ( ) = standard error of difference.

Peat soils: Of the peat soils that were sampled in 1993, only clay and organic N contents of the top 20 cm and the groundwater table were determined. Peat soils had higher organic N contents than mineral soils. In contrast with minerals soils, the organic N content of peat soils did not correlate with soil texture. There was neither a significant correlation between the groundwater table and soil organic N.

Non fertilizer N supply (NFNS)

NFNS, defined as the amount of N taken up by unfertilized ryegrass, ranged from 58 to 208 kg N per ha per growing season in the soil columns that were taken from the mineral soils in 1989 and 1991 and which were placed in a greenhouse. For the sandy and loamy soils the field N uptake on unfertilized plots was not significantly different from the amounts taken up in the greenhouse (Table 4). The N uptake on the clay soils, however, was significantly higher (average 30 kg N ha^{-1}) under field conditions than under the non-limiting conditions in the greenhouse (Table 4). It is assumed that the lower N uptake in the greenhouse was due to the wet conditions in the greenhouse experiment which caused denitrification (see discussion). It was, therefore, assumed that for clay soils, NFNS was 30 kg higher than the N uptake by the sward.

For the mineral soils from the field experiments of NMI and PR (soils of Table 1), NFNS ranged from 45 to 233 kg N ha^{-1}. For the peat soils (soils of Table 2) it ranged from 171 to 500 kg N ha^{-1}.

Table 4. Comparison of N uptake by the herbage on unfertilized columns incubated in the greenhouse and on unfertilized plots in the field (kg N ha^{-1} yr^{-1})

	Greenhouse	Field
Sand		
Achterberg	145	108
Crandendonck	127	126
Tynaarlo	134	166
Dalfsen	208	175
Average	154	144
Loam		
Burum	181	181
Swifterbant	134	91
Slootdorp	58	67
Lelystad	180	166
Average	138	126
Clay		
Zaltbommel	134	151
Haskerdijk	163	208
Average	149	180

Correlation between herbage N uptake in soil columns incubated in the greenhouse under non-limiting water conditions and estimates of NFNS by different approaches

When all soils were pooled, N mineralized during an incubation period of 12 weeks correlated best with N uptake by the herbage (NFNS), but the coefficient of correlation was still only 0.60 for the top 10 cm and 0.63 for the top 25 cm (Table 5). As NFNS is affected by the amount of mineral N in the soil at the time of sampling, NFNS was also expressed as total N uptake minus mineral N in the top 30 cm. However, when NFNS was defined in such a way, correlation coefficients between NFNS and soil characteristics actually decreased (Table 5). Higher correlation coefficients were obtained when NFNS was defined as total N uptake minus N uptake in the first cut (Table 5). The highest correlation coefficients with NFNS were obtained with the difference between the amount of N in the microbial biomass and the amount under equilibrium conditions (Δ MB-N; $r = 0.75$), for N mineralized during an incubation period of 12 weeks ($r = 0.74$), for the amount of macroorganic N ($r = 0.69$), for the difference between the amount of organic N and the amount of organic N at equilibrium ($r = 0.65$) and for the amount of active microbial biomass ($r = 0.65$; Table 5). The correlations of NFNS with the light fraction of the macro-organic matter and the amount of

N mineralized during an incubation period of two weeks were considerably lower. Correlations between NFNS and soil characteristics were stronger for the top 10 cm than for the top 25 cm (Table 5).

The correlation with NFNS increased when the sandy soils and loams and clays were analyzed as two separate groups (Table 5). N in the light fraction of macroorganic matter was not analyzed, as the correlation with NFNS was low when all soils were pooled. For the sandy soils, the highest correlation with NFNS was obtained with the difference between the amount of N in the microbial biomass in the top 10 cm of the soil and the amount of N in the microbial biomass under equilibrium conditions (Δ MB-N). The correlation was higher again when NFNS was expressed as total uptake minus N in the first cut (r = 0.90) than when expressed as total N uptake (r = 0.71). The relationship giving the best estimation of NFNS (N uptake minus N in first cut) was: NFNS (kg N ha^{-1}) = 115 (4) + 740 (100) * Δ MB-N (g N kg^{-1} soil; Table 5).

For the loams and clays, correlations of soil characteristics with NFNS were higher again when NFNS was defined as N uptake minus N in the first cut, than as total N uptake or total N uptake minus the initial amount of mineral N (Table 5). The difference between the soil organic N content of the top 10 cm and its organic N content under equilibrium conditions (Δ org. N) showed a better correlation (r = 0.89) with NFNS (N uptake minus N in the first cut) than any other soil characteristic. The best estimation of NFNS was: NFNS (kg N ha^{-1}) = 114 (3) + 12.0 (3.1) * Δ org. N (g N kg^{-1} soil) + 130 (50) * Δ MB-N (g N kg^{-1} soil; Table 5).

Correlation between NFNS and Δ org. N under field conditions for the soils of Table 1

As we had only data on the organic N content of the top 20 cm of the soils and total N uptake on unfertilized plots, we correlated the N uptake on unfertilized plots (including the first cut; NFNS) only with the Δ org. N values of the soils. When all soils were pooled, the correlation between NFNS and Δ organic N was high (r = 0.80). Correlation coefficients were higher for the loams and clays (0.84) than for the sandy soils (0.72). The relationship giving the best estimation of NFNS was: NFNS (kg N ha^{-1} yr^{-1}) = 132.3 (7.3) + 42.1 (7.1) * Δ org. N (g N kg^{-1} soil; Fig. 3).

Correlation between NFNS and soil characteristics of peat soils

Since no correlation was found between soil texture and organic N content in the peat soils, the equilibrium concept could not be used. It appeared that the groundwater table correlated negatively with NFNS (total uptake of N on unfertilized plots). When the results of the soils of Table 2 (sampled in 1993) and the results of the soils sampled by Schothorst (1977) were analyzed together, the best correlation with NFNS was found for the average deepest groundwater table (r = 0.86; Fig. 4). The best estimate of NFNS was: NFNS (kg N ha^{-1} yr^{-1}) = 188.8 (27.7) + 3.1 (0.44)* deepest groundwater table (in cm below the surface).

Table 5. Correlations between soil characteristics and NFNS calculated as the amount of N harvested in herbage (total amount or corrected) in unfertilized columns incubated in the greenhouse. The highest correlation coefficients are underlined

Soil characteristic and soil depth	NFNS calculated according to: All soils			Sandy soils			Loams and Clays		
	a	b	c	a	b	c	a	b	c
A. Potential mineralization									
0-2w 0-10 cm	0.18	0.48	0.22	0.28	0.51	0.28	0.27	0.61	0.11
0-2w 0-25 cm	0.30	0.51	0.43	0.52	0.60	0.55	0.31	0.55	0.34
0-12w 0-10 cm	0.60	<u>0.74</u>	0.49	0.62	0.71	0.51	0.58	0.78	0.48
0-12w 0-25 cm	0.63	0.62	0.61	0.65	0.67	0.56	0.70	0.58	0.68
B. Active microbial biomass (SIR)									
0-10 cm	0.34	<u>0.65</u>	0	0.55	0.65	0.39	0.13	0.67	-0.36
0-25 cm	0.26	0.60	0	0.46	0.65	0.29	0.19	0.68	-0.29
C. N in L fraction of macroorganic matter									
0-10 cm	0.24	0.43	0.25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0-25 cm	0.19	0.35	0.21	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D. N in total macroorganic matter									
0-10 cm	0.49	<u>0.69</u>	0.17	0.54	0.79	0.51	0.60	0.79	0.22
0-25 cm	0.44	<u>0.66</u>	0.17	0.51	0.74	0.50	0.58	0.75	0.22
E. Δ soil organic N									
0-10 cm	0.40	<u>0.65</u>	0.34	0.50	0.56	0.41	0.48	<u>0.89</u>	0.30
0-25 cm	0.27	0.56	0.24	0.51	0.54	0.44	0.21	0.70	0.06
F. Δ MB-N									
0-10 cm	0.57	<u>0.75</u>	0.51	0.71	<u>0.90</u>	0.67	0.63	0.83	0.51
0-25 cm	0.39	<u>0.68</u>	0.39	0.53	0.77	0.53	0.35	0.65	0.26

Sandy soils:
Best relationship: N uptake (minus cut 1; kg N ha^{-1}) = 114.6 (3.9) + 740 (100) * Δ MB-N (g N kg^{-1})

Loams and Clays:
Best relationship N uptake (minus cut 1; kg N ha^{-1}) = 113.9 (3.1) + 12.0 (3.1) * Δ org. N (g N kg^{-1})+ 130 (50) * Δ MB-N (g N kg^{-1})

a = total N harvested
b = total N harvested minus N harvested in the first cut
c = total N harvested min the initial amount of inorganic N present
n.d. = not determined.
( ) = standard error of difference

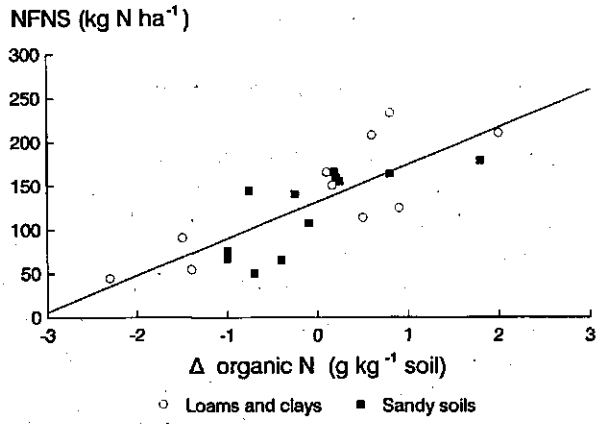


Fig. 3. Relationship between the N uptake by the herbage (NFNS in kg N ha^{-1} yr^{-1}) on unfertilized field plots located on different mineral soils (soils of Table 1) and the difference between the actual organic N content in the top 20 cm and the organic N content at equilibrium (g N kg^{-1} soil). Significant relationship: NFNS = 132.3 (7.3) + 42.1 (7.1) * Δ org. N
( ) = standard error of difference.

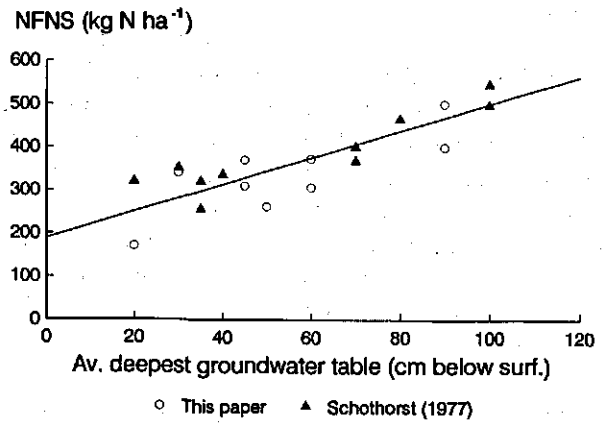


Fig. 4. Relationship between the N uptake by the herbage (NFNS in kg N ha^{-1} yr^{-1}) on unfertilized field plots located on different peat soils (soils of Table 2 and soils sampled by Schothorst, 1977) and the average deepest groundwater table (cm below surface). Significant relationship: NFNS = 188.8 (27.7) + 3.1 (0.44) * deepest groundwater table (cm below the surface).
( ) = standard error of difference.

DISCUSSION

Estimate of the non-fertilizer N supply (NFNS) in mineral soils

The objective of this study was to test which approach gives the best estimate of the non-fertilizer N supply of grassland soils under field conditions. Although the amount of N mineralized in 7- to 14-d incubations is generally considered to be the most accurate method currently available for assessing the N availability of soils (Keeney, 1982), the potential N mineralization rate determined by incubating samples for 14 days did not give a good estimate of NFNS: apparently the time of incubation was too short. The correlation with NFNS was better when an incubation period of 12 weeks was used. In previous experiments both close (Warren and Whitehead, 1988) and very poor (Fox and Piekielek, 1984) correlations were observed between N mineralized during a short incubation and NFNS. The goodness of the correlations found is expected to depend on the range of soils that are used and the variation in mineral N in spring (Machet, 1991). Although it has been found that the light fraction of macroorganic matter correlates better with the potential mineralization rate (12 weeks incubation) than the total amount of macroorganic matter (Hassink, 1995) the opposite was found for the correlation with NFNS. This might be due to the fact that the light fraction can change considerably during the growing season (Table 6), whereas the total amount of macroorganic matter changes less rapidly.

The difference between the actual amount of soil organic N and microbial biomass and their amounts under equilibrium conditions correlated better with NFNS than the commonly determined potential mineralization rate and the amounts of macroorganic matter and active microbial biomass. For all mineral soils, the difference between the actual soil organic N content and the content under equilibrium conditions correlated well with NFNS. The results suggest that for sandy soils the difference between the actual amount of microbial N and microbial N under equilibrium conditions might even improve the correlation with NFNS. The advantage of this new method is that the difference between the actual organic N and microbial N contents and their equilibrium values is easy to determine and that it is less time-consuming than determination of the potential N mineralization rate or the amount of macroorganic N.

The mineral soils that were sampled all had a deep groundwater table (average highest water table > 40 cm below the surface). Soils with a higher water table have a higher organic N content under equilibrium conditions (Hassink, 1994). This means that for soils with a different water table level another equation for equilibrium contents should be used (Hassink, 1994).

Calculation of NFNS in mineral soils under equilibrium conditions

It is accepted that the accumulation of organic N is controlled by the input of organics and is independent of the input of mineral N (Clement and Williams, 1967; Hassink and Neeteson, 1991; Ryden, 1984). On intensively managed grasslands, the input of organic C and N consists of dying roots, decaying leaves, stubble, and manure. According to Hansson and Andren (1986) the production of grassroot material is 4600 kg dry matter per year. Assuming that the biomass of roots remains constant, the same amount will decompose in the soil. Deinum (1985) came up with the same estimate. Assuming that roots have an N content of 15 g kg^{-1} (Whitehead, 1986), this corresponds with about 80 kg N ha^{-1} yr^{-1}. It is assumed that decaying leaves and stubble add considerable amounts of organic matter to the soil. According to Aarts et al. (1988) the annual input of aboveground plant residues amounts to 165 kg organic N ha^{-1}. The average amount of organic N returned to the soil in dung during grazing, and by application of manure was estimated to be 70 and 120 kg N ha^{-1} yr^{-1}, respectively (Deenen and Lantinga, 1993; Aarts et al., 1992). Assuming that 50% of the N in the applied manure is present in the organic form (Beauchamp and Paul, 1989), the annual organic N input into the soil as manure is approximately 130 kg N ha^{-1}. Most of the organic N input into the soil will be mineralized during the same growing season and will not contribute to the increase of the soil organic N pool. According to Jenkinson (1977) approximately 70% of ryegrass roots and tops decompose within 0.5 to 1 year; approximately 20% of the organic N in manure will mineralize during the year of application (Beauchamp and Paul, 1989). This means that about 375 kg organic N will be incorporated into the soil per ha every year, but only 178 kg of this input will contribute to the soil organic N pool. The rest (approximately 200 kg N ha^{-1}) will mineralize in the year of application (Table 6). So, when we assume that the amount of soil organic N does not change, annual net mineralization from the soil organic N pool should amount to approximately 178 kg N per ha. The N input by atmospheric deposition (approx. 40 kg N ha^{-1} annually, Aarts et al., 1992) should be added to this amount. Under field conditions, the annual N uptake on unfertilized plots that are in equilibrium was found to be 138 kg N ha^{-1} on loams and clays, and 126 kg N ha^{-1} on sandy soils. This amount is lower than the sum of the estimated net mineralization (178 kg N ha^{-1} yr^{-1}) and deposition. This might be due to the fact that the efficiency of uptake of available N is generally less than 100% and that mineralization rates are often depressed by moisture limitations during dry periods in summer.

Variation in NFNS in individual grassland soils

To exclude differences in soil temperature and water status between sites, soil columns were incubated under uniform environmental conditions in the greenhouse. At the beginning of the incubation, the amount of mineral N differed considerably between columns of different sites. Mineral N concentrations were highest in the clays. For the clays, N uptake in the first cut was considerably lower than the initial amount of mineral N in the soil. The water table was

Table 6. Estimate of the organic N input into the soil (kg ha^{-1} yr^{-1}) on intensive managed grasslands, the amount mineralized during the first year and the amount contributing to the soil organic matter pool

Source	Organic N input	Mineralization organic N	
		during first yr	left after 1 yr
Roots[1]	80	56[5]	24
Grass residues[2]	165	116[5]	50
Dung patches[3]	70	14[6]	56
Manure[4]	60	12[6]	48
Total	375	198	178

[1] Hansson and Andren, 1986; Deinum, 1985; Whitehead, 1986
[2] Aarts et al., 1988
[3] Deenen and Lantinga, 1993
[4] Aarts et al., 1992
[5] Jenkinson, 1977
[6] Beauchamp and Paul, 1989

kept at only 30 cm below the surface, leading to partially anaerobic conditions in the clay soils. This suggests that at least a part of the mineral N initially present was denitrified in the fine-textured soils. This is in line with the observation that for clays, N uptake under field conditions was higher than N uptake in the greenhouse, while this was not found for loams and sandy soils. Partial denitrification of mineral N in the fine-textured soils at the beginning of the incubation might also explain why the correlation of soil parameters with NFNS increased especially for the fine-textured soils when the first cut was not taken into account.

It was expected that the NFNS in the field would vary between years as temperature and moisture conditions are different every year. At four locations NFNS was determined during four or five years. In these soils NFNS could differ 40% from the average value in individual years. The variation was higher in the soils with a low moisture supplying capacity (Den Ham and Tynaarlo) than in soils with a high moisture supplying capacity (Hemrik and Ruurlo). Variations in nitrogen supply between years were caused by differences in available N in spring and differences in N uptake during the summer period (June-September). As an example the cumulative NFNS in five successive years in Den Ham is presented in Figure 5). Variations in NFNS during the summer period were related to the amount of rainfall between June and September; 1982 and 1983 were dry years. Variations in NFNS in spring could not be explained from differences in temperature or moisture conditions.

Another aspect to take into account is the fact that mineralized N not only accumulates in aboveground plant parts that are harvested, but also in stubble, roots and plant residues (light

fraction of the macroorganic matter pool; Meijboom et al., 1995). When the sum of the amount of N in roots, stubble and plant residues at the end of the growing season differs from the amount at the beginning, NFNS is under- or overestimated. For a sandy soil and a loam, the amounts of N in roots, stubble and light fraction were determined, and they were not significantly different between spring and autumn (Table 7).

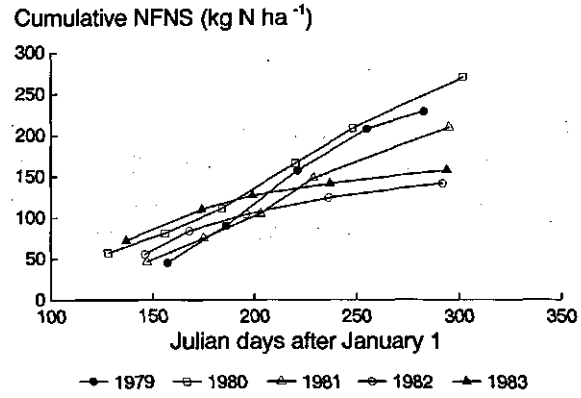


Fig. 5. Cumulative N uptake by the herbage on unfertilized plots in Den Ham (kg N ha^{-1}) in 1979, 1980, 1981, 1982 and 1983.

Table 7. Amount of N (kg ha^{-1}) in stubble, roots and the light fraction of macroorganic matter (light M.O.M.) in the top 25 cm of a sandy (Tynaarlo) and loamy (Lelystad) grassland soil in spring and autumn of 1992

	Tynaarlo		Lelystad	
	spring	autumn	spring	autumn
Stubble	17	19	15	20
Roots	121	55	112	41
Light M.O.M.	49	117	50	83
Total	187	191	177	144

Estimation of NFNS in peat soils

In peat soils no relation was found between soil texture and organic N content. Peat soils have been formed under (partially) anaerobic conditions. Decomposition of organic matter is less complete and slower under anaerobic conditions than under aerobic conditions (Jenkinson, 1988) and due to the wet conditions organic N contents are higher in peat soils than in mineral soils. The organic N contents reflect the drainage conditions of the past. The amounts of organic N in peat soils are higher than the equilibrium level, as NFNS was generally higher

than 200 kg N ha^{-1} yr^{-1}, thus leading to a decrease in the amount of soil organic N.

The observation that NFNS correlated with the average deepest water table is in line with the conclusions of Schothorst (1977) and Moore and Dalva (1993) that the water table and the drainage conditions affect the decomposition rate of peat.

CONCLUSION

It may be concluded that the "difference method" gives a good estimate of NFNS (r = 0.80) on mineral grassland soils. The method has advantages over other methods because it is less time-consuming and simpler. NFNS on peat soils can not be estimated with the "difference method", but here NFNS can easily be estimated from the average deepest groundwater table (r = 0.86).

ACKNOWLEDGEMENTS

B. Wouters, T. Vellinga, T. Kraak, J. Everts and G. Remmelink of the Research Station for Cattle, Sheep and Horse Husbandry and O. Oenema of the Nutrient Management Institute (NMI) are gratefully acknowledged for supplying data of the field experiments. L. Brussaard, J.J. Neeteson, A.P.M. Whitmore and K.B. Zwart are gratefully acknowledged for critically reading the manuscript.

REFERENCES

Aarts H F M, Biewinga E E, Bruin G, Edel B and Korevaar H 1988 Dairy farming and environment. Rapport nr. 111, Proefstation voor de Rundveehouderij, Schapenhouderij en Paardenhouderij (PR), Lelystad, 136 p. (In Dutch.)

Aarts H F M, Biewinga E E and Van Keulen H 1992 Dairy farming systems based on efficient nutrient management. Neth. J. Agric. Sci. 40, 285-299.

Addiscott T M 1983 Kinetics and temperature relationships of mineralization and nitrification in Rothamsted soils with differing histories. J. Soil Sci. 34, 343-353.

Amato M and Ladd J N 1992 Decomposition of ^{14}C-labelled glucose and legume material in soil: properties influencing the accumulations of organic residue C and microbial biomass C. Soil Biol. Biochem. 24, 455-464.

Anderson J P E and Domsch K H 1978 A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol. Biochem. 10, 215-221.

Beauchamp E G and Paul J W 1989 A simple model to predict manure N availability to crops in the field. *In* Nitrogen in Organic Wastes Applied to Soils. Eds. J A Hansen and K

Henriksen. pp 140-149. Academic Press, London, New York.
Brockman J S 1969 The relationships between total N input and the yield of cut grass. J. British Grassland Soc. 24, 89-96.
Clement C R and Williams T E 1967 Leys and soil organic matter. II. The accumulation of nitrogen in soils under different leys. J. Agric. Sci. (Camb.) 69, 133-138.
Cuttle S P and Bourne P C 1992 Nitrogen Immobilisation and Leaching in Pasture Soils. Proc. No. 325. The Fertilizer Society, London, 30 p.
Deenen P J A G and Lantinga E A 1993 Herbage and animal production responses to fertilizer nitrogen in perennial ryegrass swards. I. Continuous grazing and cutting. Neth. J. Agric. Sci. 41, 179-203.
Deinum B 1985 Root mass of grass swards in different grazing systems. Neth. J. Agric. Sci. 33, 377-384.
Deys W B 1961 The determination of total nitrogen in herbage samples. IBS Jaarboek Med. 89, 158. (In Dutch.)
Fox R H and Piekielek W P 1984 Relationships among anaerobically mineralized nitrogen, chemical indexes, and nitrogen availability to corn. Soil Sci. Soc. Am. J. 48, 1087-1090.
Genstat Manual 1987 Genstat. A General Statistical Program. Clarendon, Oxford.
Gregorich E G, Voroney R P and Kachanoski R G 1991 Turnover of carbon through the microbial biomass in soils with different textures. Soil Biol. Biochem. 23, 799-805.
Hansson A C and Andren O 1986 Below-ground plant production in a perennial grass ley assessed with different methods. J. Appl. Ecol. 23, 657-666.
Hassink J 1993 Relationships between the amount and the activity of the microbial biomass in Dutch grassland soils: comparison of the fumigation-incubation method and the substrate-induced respiration method. Soil Biol. Biochem. 25, 533-538.
Hassink J 1994 Effects of soil texture and grassland management on soil organic C and N and rates of C and N mineralization. Soil Biol. Biochem. 26, 1221-1231.
Hassink J 1995 Density fractions of soil macroorganic matter and microbial biomass as predictors of C and N mineralization. Submitted to Soil Biol. Biochem.
Hassink J and Neeteson J J 1991 Effect of grassland management on the amounts of soil organic N and C. Neth. J. Agric. Sci. 39, 225-236.
Hassink J, Scholefield D and Blantern P 1990 Nitrogen mineralization in grassland soils. *In* Proc 13th General Meeting of the European Grassland Federation, vol II. Eds. N Gaborcik, V Krajcovic and M Zimkova. pp 25-32. Banscka Bystrica, Tsjechoslovakia.
Hassink J, Lebbink G, and Van Veen J A 1991 Microbial biomass and activity of a reclaimed-polder soil under a conventional or a reduced-input farming system. Soil Biol. Biochem. 23, 507-513.
Hassink J, Matus F J, Chenu C and Dalenberg J W 1995 Interactions between soil biota, soil organic matter and soil structure. Submitted to Advances in Agroecology.
Janzen H H, Campbell C A, Brandt S A, Lafond G P and Townley-Smith L 1992 Light-fraction organic matter in soils from long-term crop rotations. Soil Sci. Soc. Am. J. 56, 1799-1806.

Jenkinson D S 1977 Studies on the decomposition of plant material in soil. V. The effects of plant cover and soil type on the loss of carbon from ^{14}C labelled ryegrass decomposing under field conditions. J. Soil Sci. 28, 424-434.

Jenkinson D S 1988 Soil organic matter and its dynamics. *In* Russels's Soil Conditions and Plant Growth. 11th edition, chapter 18. Ed. A. Wild. pp. 564-607. Longman, New York.

Jenkinson D S and Powlson D S 1976 The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. Soil Biol. Biochem. 8, 209-213.

Keeney D R 1982 Nitrogen-availability indexes. *In* Methods of Soil Analysis, part 2. Chemical and Microbiological Properties. Agronomy Monograph No. 9. Ed. A L Page. pp 711-734. American Society of Agronomy, Madison, Wisconsin.

Machet J M 1991 Pourquoi et quand mesurer l'état minéral du soil en azote. *In* L'azote et le Soufre dans le Sol. Ed. Gemas. pp 151-161.

Meijboom F W Hassink J and Van Noordwijk M 1995 Density fractionation of soil macroorganic matter using silica suspensions. Submitted to Soil Biol. Biochem.

Moore T R and Dalva M 1993 The influence of temperature and water table position on carbon dioxide and methane emissions from laboratory columns of peatland soils. J. Soil Sci. 44, 651-664.

Nordmeyer H and Richter J 1985 Incubation experiments on nitrogen mineralization in loess and sandy soils. Plant and Soil 83, 433-445.

Raison R J Connell M H and Khanna P K 1987 Methodology for studying fluxes of soil mineral-N in situ. Soil Biol. Biochem. 19, 521-530.

Richards I R and Hobson R D 1977 The relationships between nitrogen supply and the nitrogen yield of cut grass swards. J. Agric. Sci. Cambridge 88, 289-292.

Ryden J C 1984 The flow of Nitrogen in Grassland. Proc. no 229, The Fertiliser Society, London, 44 p.

Schothorst C J 1977 Subsidence of low moor peat soils in the western Netherlands. Geoderma 17, 265-291.

Stanford G and Smith S J 1972 Nitrogen mineralization potentials of soils. Soil Sci. Soc. Am. Proc. 36, 465-472.

Tisdall J M and Oades J M 1982 Organic matter and water stable aggregates in soils. J. Soil Sci. 33, 141-163.

Van Veen J A, Ladd J N and Amato M 1985 Turnover of carbon and nitrogen through the microbial biomass in a sandy loam and a clay soil incubated with [$^{14}C(U)$]glucose and [^{15}N]$(NH_4)_2SO_4$ under different moisture regimes. Soil Biol. Biochem. 17, 257-274.

Warren G P and Whitehead D C 1988 Available soil nitrogen in relation to fractions of soil nitrogen and other soil properties. Plant and Soil 112, 155-165.

Whitehead D C 1986 Sources and transformations of organic nitrogen in intensively managed grassland soils. *In* Nitrogen Fluxes in Intensive Grassland Systems. Developments in Plant and Soil Sciences, Vol. 23. Eds. H G van der Meer, J C Ryden and G C Ennik. pp. 47-58. Martinus Nijhoff Publishers, Dordrecht.

CHAPTER 12

EFFECT OF THE NON-FERTILIZER N SUPPLY ON THE RESPONSE OF HERBAGE TO N FERTILIZATION UNDER MOWING CONDITIONS

By: J. Hassink. Accepted by Plant and Soil

EFFECT OF THE NON-FERTILIZER N SUPPLY OF GRASSLAND SOILS ON THE RESPONSE OF HERBAGE TO N FERTILIZATION UNDER MOWING CONDITIONS

ABSTRACT

We tested whether the non-fertilizer N supply of grassland soils (NFNS; N uptake on unfertilized plots) affects the relationships between N uptake and dry matter production, N application and N uptake, N application and dry matter production, as well as the optimum fertilizer application rate.

At low N uptake rates the amount of dry matter production per kg of N uptake was negatively correlated with NFNS; at higher N uptake levels the correlation was not significant. The apparent nitrogen recovery of fertilizer N was not correlated with NFNS. The optimum fertilizer application rate was correlated positively with the maximum dry matter production (Max DM) and negatively with NFNS. The relationship optimum fertilizer application = -81 - 0.8 * NFNS + 0.0375 * Max DM accounted for 89% of the variance in optimum fertilizer application rate between soils at a marginal N effect of 7.5 kg dry matter per kg N applied. So an increase in NFNS of 100 kg N resulted in a decrease of the optimum N application rate of 80 kg N.

INTRODUCTION

Until recently, the N fertilizer recommendation for intensively managed mown grasslands on mineral soils was 400 kg N ha^{-1} yr^{-1} in the Netherlands, irrespective of soil characteristics. This recommendation was based on the results of field experiments in the early seventies; the optimum application was defined as the level of N input at which the response falls to less than 7.5 kg dry matter per kg N applied ($N_{7.5}$); in the UK the optimum was defined at (N_{10}) (Prins et al., 1980; Prins, 1983; Morrison et al., 1980). It is now realized that this recommendation was too general and that soil characteristics should be taken into account to improve the recommendation for N fertilizer application (Ruitenberg et al., 1991). The N uptake on unfertilized fields gives a good estimation of the non-fertilizer N supply (NFNS; Warren and Whitehead, 1988). NFNS consists of soil organic N that is mineralized during the growing season, inorganic N present in the soil profile in spring, dry and wet deposition and biological N fixation (Ruitenberg et al., 1991). In the absence of clover, differences in NFNS between locations are mainly due to differences in mineralization of soil organic N. As grass derives its N not only from applied fertilizer, but also from sources that are included in NFNS, the level of NFNS should affect the response of herbage to N fertilization and the optimum N fertilizer application rate. Optimum fertilizer application rates in the Netherlands and in the UK range from 260 to 540 kg N ha^{-1} yr^{-1} for mineral soils (Ruitenberg et al., 1991)

and are affected by NFNS and water supply (Whitehead et al., 1981; Salette, 1988; Ruitenberg et al., 1991).

The relationship between N application rate and dry matter production is the result of NFNS, the relationship between N application and N uptake and the relationship between N uptake and dry matter production (Frankena and De Wit, 1958; Fig. 1). Environmental conditions affect the apparent recovery of N fertilizer (ANR; i.e. N uptake by the fertilized sward minus that by the control expressed as a percentage of the N applied) and the efficiency of the N taken up (NUE; nitrogen use efficiency, i.e. the response in dry matter production per kg of N taken up by the sward); ANR and NUE are higher at optimum conditions for plant growth than at suboptimal growing conditions (Garwood et al., 1980; Ruitenberg et al., 1991). The level of NFNS could also affect the ANR and NUE. Salette (1988) observed that at low N uptake levels, NUE was higher on a soil with a low organic matter content than on a soil with a high organic matter content. When NFNS affects NUE or ANR, an increase in NFNS does not necessarily lead to a corresponding decrease in the optimum fertilizer application rate.

The aim of this paper is to test whether NUE and ANR are affected by NFNS and to what extent differences in NFNS affect the optimum fertilizer N application rate.

MATERIALS AND METHODS

Field experiments

We analyzed N fertilization of mown grasslands in experiments that were performed by the Nutrient Management Institute (NMI-Wageningen) and the Research Station for Cattle, Sheep and Horse Husbandry (PR-Lelystad) at different locations in the Netherlands. Information about the characteristics of these soils is given by Hassink (1995). The rates of fertilizer N that were applied ranged from 0 to 550 kg N per ha per year. In spring, fertilizer N (calcium ammonium nitrate) was applied in all treatments when the accumulated mean daily air temperature above 0 °C since 1 January was approximately 200 (Jagtenberg, 1970). Thereafter, applications were made in diminishing amounts until target rates were reached. Grass was mown when the treatments with the highest N application rate reached a dry matter yield of approximately 2500 kg dry matter per ha. Fertilizer was again applied immediately thereafter.

In March 1991, tensiometers were installed at 20 cm depth at four different sites to determine the water potential three times a week.

Statistical analysis

For each soil and in each year, the relationships between N uptake and dry matter production and N application and dry matter production were fitted with a Mitscherlich curve (Genstat,

1987). From the relationship obtained between N application and dry matter production the maximum dry matter production (Max DM) on a soil was estimated. To test whether NFNS affected the relationship between N application and dry matter production, the N application rate at which the marginal N effect was 15, 10 and 7.5 kg dry matter per kg of N applied was determined.

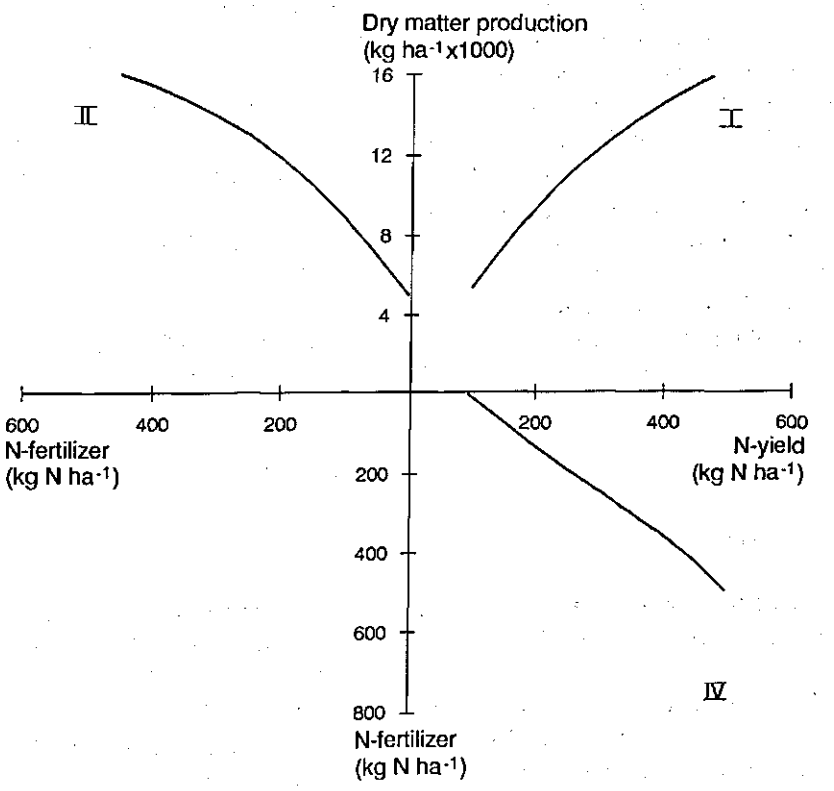


Fig. 1. Relationships between N uptake and dry matter production (quadrant I), N application and N uptake (quadrant IV) and N application and dry matter production (quadrant II).

The following relationships were analyzed with correlation techniques (Genstat, 1987): i) between NFNS and the apparent recovery of fertilizer N, ii) between NFNS and the dry matter production at N fertilizer application levels of 0, 100, 200, 300 and 400 kg N ha^{-1}, iii) between NFNS and the dry matter production of the herbage for the first and each of the following amounts of 100 kg N ha^{-1} taken up by the herbage, and iv) between NFNS and $N_{7.5}$, N_{10} and N_{15}.

Not only NFNS, but also Max DM was correlated with the optimum fertilizer N application rate, as it has been observed that $N_{7.5}$ for sandy grassland soils is related to

maximum dry matter production (Noy, 1989).

The relationships between NFNS, Max DM and optimum N fertilizer application rates set at $N_{7.5}$, N_{10} and N_{15} were also analyzed with multiple regression techniques (Genstat, 1987).

For all soils the analyses mentioned above were performed on the average values for all the experimental years. Results of five experimental years were available for the soils of Den Ham, Ruurlo and Tynaarlo; for these locations the effects of NFNS in different years on the relationship between N uptake and dry matter production by the herbage and on the optimum N application rate were also determined by correlation analysis (Genstat, 1987).

RESULTS

Effect of NFNS on the relationship between N uptake and dry matter production

When the N uptake amounted to 100 kg ha^{-1}, the amount of dry matter produced per kg N uptake (nitrogen use efficiency; NUE) showed a significant ($p < 0.05$) negative correlation with the NFNS of the soil (Fig. 2). The amount of herbage dry matter produced at an uptake of 100 kg N ha^{-1} ranged from 4000 to 6300 kg dry matter ha^{-1} (Fig. 2). The NUE decreased with increasing amounts of N taken up and the relationship between NFNS and NUE disappeared (Fig. 2). Variations in NFNS between years in the soil from Tynaarlo, Den Ham and Ruurlo had no significant effect on the NUE. At low levels of N uptake, NUE was constant between years in Tynaarlo and Den Ham (variation less than 6%); in Ruurlo the variation was larger (14%).

Effect of NFNS on the apparent N recovery (ANR)

The ANR ranged from 64 to 103% among different grassland sites with fertilizer application rates up to 400 kg N ha^{-1}. There was no significant correlation between NFNS and ANR of a particular soil. Variations in ANR between years in Tynaarlo, Den Ham and Ruurlo did not correlate significantly with differences in NFNS.

Effect of NFNS on the relationship between N application and dry matter production

On unfertilized plots, dry matter production increased with increasing NFNS of the soil. The correlation between NFNS and dry matter production decreased with increasing level of N application (Fig. 3).

The optimum fertilizer N application rates (at $N_{7.5}$, N_{10} and N_{15}) showed a significant ($p < 0.05$) negative correlation with the NFNS of a particular soil; the coefficient of correlation decreased with decreasing marginal N effect (Fig. 4).

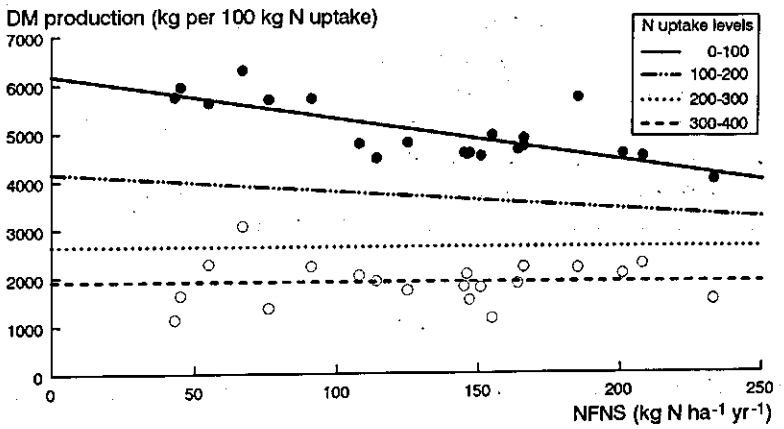


Fig. 2. Relationship between NFNS (kg N ha^{-1} yr^{-1}) and the amount of dry matter production on that soil for each 100 kg N ha^{-1} yr^{-1} that has been taken up by the herbage (kg ha^{-1} yr^{-1}).
0 - 100 : 6166 (253) -8.58 (1.76) * NFNS; r = -0.75
100 - 200 : 4152 (441) -3.56 (1.70) * NFNS; r = -0.49
200 - 300 : 2642; n.s.
300 - 400 : 1910; n.s.
() = standard error of difference; n.s. = non-significant relationship with NFNS.
The results of the individual soils are only given for 0-100 kg N uptake (●) and 300-400 kg N uptake (O).

In contrast with NFNS, the optimum N application rates (at $N_{7.5}$, N_{10} and N_{15}) showed a significant positive correlation ($p < 0.05$) with the maximum dry matter production on each soil (Fig. 5).

For $N_{7.5}$, N_{10} and N_{15} more than 89% of the variation in optimum N application rate was explained by differences in maximum dry matter production and NFNS (Table 1).

Table 1. Equations calculated with multiple regression analysis giving the best predictions of N fertilization (kg N ha^{-1} yr^{-1}) at marginal dry matter productions of 7.5, 10 and 15 kg DM per kg N applied for grassland soils

N application for $N_{7.5}$ = -81.4 (68.8)	-	0.803 (0.199)	*	NFNS +	0.03751 (0.00354)	* Max DM
N application for N_{10} = -61.8 (42.6)	-	0.805 (0.123)	*	NFNS +	0.0318 (0.00219)	* Max DM
N application for N_{15} = -33.2 (25.4)	-	0.813 (0.073)	*	NFNS +	0.0238 (0.0013)	* Max DM

() = Standard error of difference; 89, 94 and 97% of the variation in the optimum fertilizer application rate between sites was explained by the equations at a marginal DM production of 7.5, 10 and 15 kg DM per kg N applied, respectively.
Max DM = Maximum dry matter production in kg ha^{-1}.

The difference between the measured and predicted (according to the equation in Table 1) $N_{7,5}$ (the marginal response commonly used in the Netherlands) was generally less than 30 kg N ha^{-1} (Fig. 6). For this marginal response the optimum fertilizer application rate ranged from 247 to 750 kg N ha^{-1} yr^{-1} (Fig. 6). An increase in NFNS of 100 kg N resulted in a decrease of the optimum N application rate of 80 kg N. Maximum dry matter productions that were reached on the sites in 1991 were related to moisture stress. Table 2 shows the relationship between the maximum dry matter production and the length of the period that the soil water potential was above 50 kPa.

Table 2. Maximum dry matter production in 1991 (Max DM in kg ha^{-1}) and the numbers of days with the water potential at 20 cm depth above 50 kPa

Location	Soil type	Max DM	Number of days with water potential > 50 kPa
Tynaarlo	Sand	14938	27
Burum	Loam	11490	70
Haskerdijk	Clay	19036	0
Zaltbommel	Clay	14087	60

The variation in NFNS between years for the soils of Tynaarlo, Den Ham and Ruurlo did not correlate significantly with $N_{7,5}$, N_{10} and N_{15}. For Tynaarlo the variation in optimum N application rate between years could be explained by differences in the maximum dry matter production and NFNS, but not for Den Ham and Ruurlo. Although the variation in $N_{7,5}$ between years at one location did often not correlate with variations in maximum dry matter production and NFNS, the differences between predicted and measured $N_{7,5}$ in individual years was generally less than 50 kg N ha^{-1}.

DISCUSSION

Effect of NFNS on NUE

One of the aims of this paper was to test whether NFNS affected NUE. We observed that at low levels of N uptake, NUE was negatively correlated with the NFNS of the soil.

It has been shown before that at low N fertilizer levels the variation in dry matter production per kg N taken up by the herbage (NUE) can be considerable between soils, whereas at high N fertilizer levels, the variation is small (Lantinga, 1985; Wieling and De Wit, 1987; Salette, 1988). These studies did not reveal the cause of the variation in NUE and it was concluded that better knowledge of the underlying causes was required (Salette, 1988). The present study showed that the variation in NUE was negatively related with differences in NFNS among soils. The difference in NUE can be due to the fact that in soils with a low

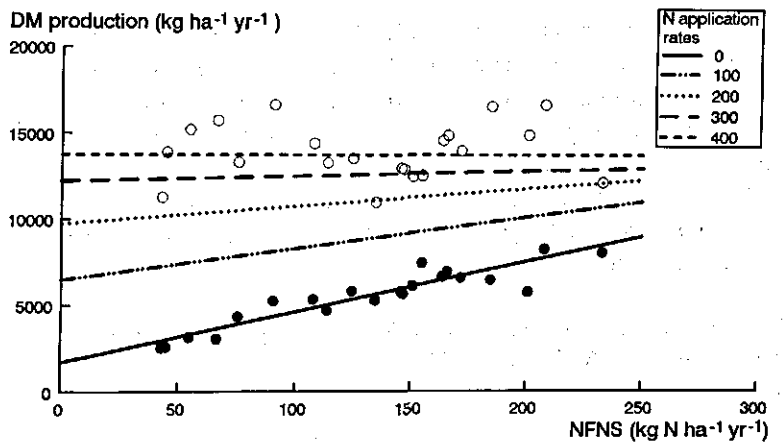


Fig. 3. Relationship between the NFNS of a soil and the dry matter production at annual N application rates of 0, 100, 200, 300 and 400 kg N ha^{-1}.
0 : DM = 1691 (1688) + 29.3 (2.8) * NFNS; r = 0.92
100 : DM = 6482 (1036) + 18.2 (4.2) * NFNS; r = 0.77
200 : DM = 9752 (1381) + 10.2 (5.6) * NFNS; r = 0.51
300 : DM = 12082 (1657); n.s.
400 : DM = 13751 (1555); n.s.
() standard error of difference; n.s.= non-significant relationship with NFNS.
The results of the individual soils are only given for application rates of 0 (●) and 400 kg N (O).

NFNS, herbage derives most of its N from the fertilizer application at the beginning of the growth period after a cut. On soils with a high NFNS, herbage derives relatively more of its N from mineralized organic matter that becomes available during the whole growing period of a cut. This relatively higher availability of N during later periods of a cut in soils with a high NFNS might increase the N content of the herbage. This phenomenon has been observed for lettuce (Bakker et al., 1984). It is also in line with the observation that when herbage is deprived of mineral N during the second part of the growth period of a cut, herbage dry matter production is affected much less than N uptake (Jarvis and Macduff, 1989). At the beginning of a cut the availability of N affects dry matter production to a much greater extent. The photosynthetic activity is low after mowing as the leaf area index is low and not all light can be intercepted. With high N availability at the beginning of the growth period of a cut, the stage of maximum light interception is reached earlier than when N availability is low and more dry matter can be produced during a growing period (Gosse et al., 1986; Gastal and Lemaire, 1988; Rabbinge et al., 1989). The observation that NUE was not affected by the NFNS of a soil at higher levels of N uptake, is in line with this concept. At high N uptake levels, the proportion of N taken up that was derived from fertilizer N must have been high on all soils.

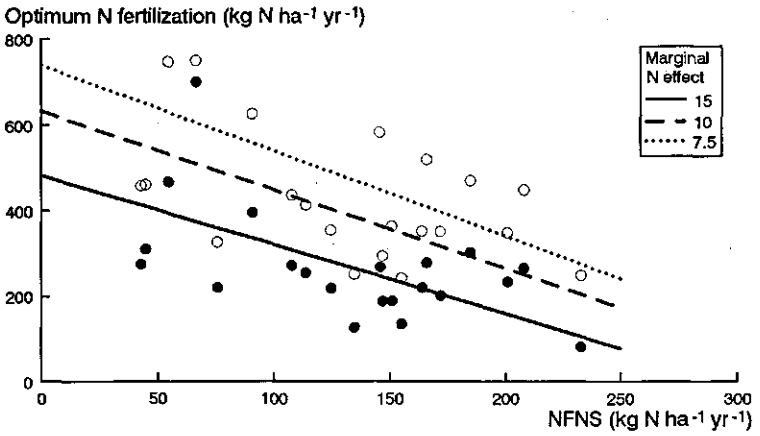


Fig. 4. Relationship between the NFNS (kg N ha^{-1} yr^{-1}) of a soil and the optimum fertilizer application rate at $N_{7.5}$, N_{10} and N_{15} (kg N $ha^{-1}yr^{-1}$).
7.5 : 739 (252) -2.0 (1.0) * NFNS;r=-0.51
10 : 632 (206) -1.84 (0.84) * NFNS;r=-0.57
15 : 482 (144) -1.62 (0.59) * NFNS;r=-0.65
() standard error of difference.
The results of the individual soils are only given for N_{15} (●) and $N_{7.5}$ (O).

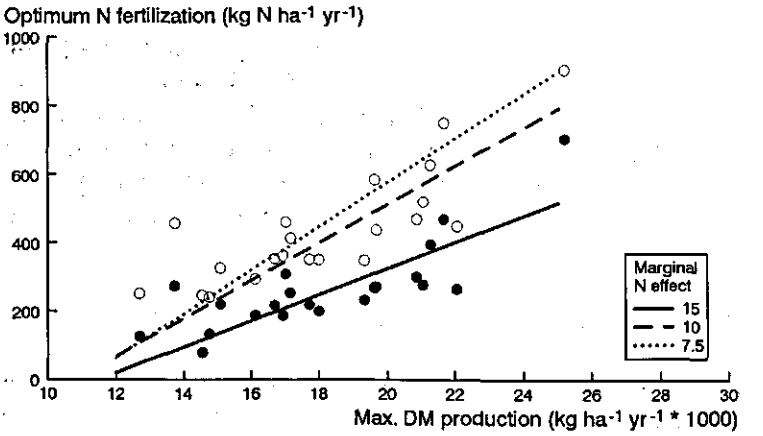


Fig. 5. Relationship between max DM production (kg DM ha^{-1} yr^{-1}) of a soil and the optimum fertilizer application rate at $N_{7.5}$, N_{10} and N_{15} (kg N $ha^{-1}yr^{-1}$).
7.5 : -705 (120) + 0.064 (0.0086) * Max DM; r = 0.91
10 : -597 (116) + 0.055 (0.007) * Max DM; r = 0.90
15 : -436 (93) + 0.038 (0.0058)* Max DM; r = 0.88
() standard error of difference.
The results of the individual soils are only given for N_{15} (●) and $N_{7.5}$ (O).

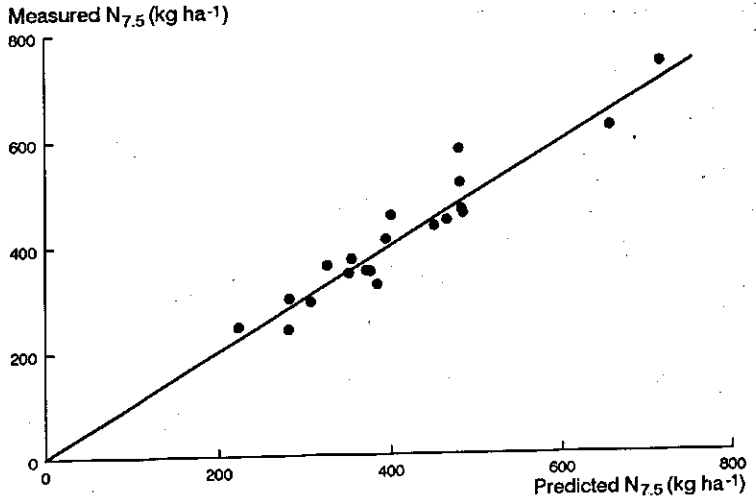


Fig. 6. Relationship between the observed and predicted optimum fertilizer application rate ($N_{7.5}$) for different grassland soils (kg N ha^{-1} yr^{-1}).

Effect of NFNS on the apparent nitrogen recovery (ANR)

We also tested whether NFNS affected the apparent recovery of fertilizer N (ANR) on a soil and found that ANR was not affected by the NFNS of the soil. This is in agreement with the results of other studies (Whitehead, 1984; Herlihy et al., 1978). It confirms the observations that accumulation of N in the soil is essentially independent of the input of mineral N (Clement and Williams, 1967; Hassink and Neeteson, 1991). ANR ranged between 64 and 103%. These are common values in the Netherlands (Van der Meer and Van Uum-van Lohuyzen, 1986).

Effects of NFNS and Max DM on the optimum fertilizer N application rate

Finally, we tested to what extent differences in NFNS and Max DM affected the optimum fertilizer N application rate. Herbage derives its N not only from applied fertilizer, but also from the pools that are defined as NFNS. As at N uptake levels higher than approximately 200 kg ha^{-1}, no effect of NFNS on NUE was found while ANR was not affected by NFNS at all, the level of NFNS of a soil should affect the optimum N fertilizer application rate. We found that the correlation between NFNS and the optimum fertilizer application rate decreased with decreasing marginal N response. At low marginal N response, the variation in the maximum dry matter production affected the optimum N application rate to a greater extent

than NFNS. We obtained some evidence that variations in the maximum dry matter production were related to the availability of moisture. This is in agreement with the results of Morrison (1980) and Noy (1989) who observed that the annual yield was related to the amount of rainfall from April to September plus the water-holding capacity of the soil and Garwood et al. (1977) who found that 67% of the variation in yield on a site could be accounted for by soil moisture conditions.

Multiple regression analysis showed that when the variation in maximum dry matter production was taken into account, a change in NFNS of 100 kg resulted in a change in the optimum fertilizer application rate of 80 kg N, irrespective of the level of marginal N response. More than 89% of the variation in optimum fertilizer application rates between locations was explained by differences in NFNS and Max DM.

Concluding remarks

NFNS can be estimated from the difference between the actual soil organic N content and the organic N content when equilibrium has been reached (Hassink, 1995). In years with favourable moisture conditions Max DM can be obtained from results of previous years and thus the optimum N fertilizer application rate can be calculated very simply for each location using the equation presented in this paper. In years with less favourable moisture conditions it is more difficult to estimate the optimum N fertilizer application rate.

The original N fertilizer recommendation for intensively used mown grasslands on mineral soils was 400 kg N ha^{-1} yr^{-1}. We observed that the optimum N fertilizer application for the soils in this study ranged from 247 to 750 kg N ha^{-1} yr^{-1}. When the maximum application level is set at 400 kg N ha^{-1} yr^{-1}, the average reduction in N fertilizer application on these soils was 42 kg N ha^{-1} yr^{-1} when the calculated optimum amount of fertilizer N was applied instead of the original recommendation of 400 kg. In the Netherlands approximately 1.3 million hectares of mineral soils are under grassland (Ruitenberg et al., 1991). If the soils under study are representative for the mineral grassland soils in the Netherlands, the annual reduction in N fertilizer application is approximately 54.6 million kg N in the Netherlands when the optimum N fertilizer application is given instead of the general 400 kg N ha^{-1} yr^{-1}.

ACKNOWLEDGEMENTS

B. Wouters, Th.V. Vellinga, T. Kraak, H. Everts and G. Remmelink of the Research Station for Cattle, Sheep and Horse Husbandry (PR-Lelystad) and O. Oenema of the Nutrient Management Institute (NMI-Wagenngen) are gratefully acknowledged for supplying the data of the field experiments.

I thank L. Brussaard, J. J. Neeteson, A.P.M. Whitmore of AB-DLO and Th.V. Vellinga of PR-Lelystad for critically reading and improving the manuscript.

REFERENCES

Bakker M J, Slangen J H G and Glas W 1984 Comparative investigation into the effect of fertigation and of broadcast fertilization on the yield and nitrate content of lettuce (*Lactuca sativa* L.). Neth. J. Agric. Sci. 32, 330-333.

Clement C R and Williams T E 1967 Leys and soil organic matter. II. The accumulation of nitrogen in soils under different leys. J. Agric. Sci. (Camb.) 69, 133-138.

Frankena H J and De Wit C T 1958 Nitrogen fertilization, uptake of nitrogen and growth of grass in spring (in Dutch). Landbouwkd. Tijdschr. 11, 465-471.

Garwood E A, Tyson K C and Clements C R 1977 A comparison of yield and soil conditions during 20 years of grazed grass and arable cropping. Grassl. Res. Inst. (Hurley), Tech. Rep. No 2, 89 pp.

Garwood E A, Salette J and Lemaire G 1980 The influence of water supply to grass on the response to fertilizer nitrogen and nitrogen recovery. *In* Proceedings International Symposium European Grassland Federation on The Role of Nitrogen in Intensive Grassland Production. Eds. W H Prins and G H Arnold. pp 59-65. PUDOC, Wageningen, the Netherlands.

Gastal F and Lemaire G 1988 Study of a tall fescue sward grown under nitrogen deficiency conditions. Proc. 12th meeting Europ. Grassl. Fed., Dublin, pp 323-327.

Genstat Manual 1987 Genstat. A General Statistical Program. Clarendon, Oxford, 163 p.

Gosse G, Varlet-Grancher C, Bonhamme R, Chartier M, Allirand J and Lemaire G 1986 Production maximale de matière sèche et rayonnement solaire intercepté par un couvert végétal. Agronomie 6, 47-56.

Hassink J 1995 Prediction of the Non Fertilizer N supply of grassland soils. Subm. to Plant and Soil.

Hassink J and Neeteson J J 1991 Effect of grassland management on the amounts of soil organic N and C. Neth. J. Agric. Sci. 39, 225-236.

Herlihy M, Sheehan P and McAleese D M 1978 Nitrogen uptake efficiency by ryegrass in soils of a textural sequence. Ir. J. Agric. Res. 17, 71-77.

Jagtenberg W D 1970 Predicting the best time to apply nitrogen to grassland in spring. J. Br. Grassl. Soc. 25, 266-271.

Jarvis S C and Macduff J H 1989 Nitrate nutrition of grasses from steady-state supplies in flowing solution culture following nitrate deprivation and/or defoliation. J. Exp. Bot. 40, 965-975.

Lantinga E A 1985 Productivity of grasslands under continous and rotational grazing. Thesis Wageningen Agricultural University, the Netherlands, 111 p.

Morrison J 1980 The influence of climate and soil on the yield of grass and its response to fertilizer nitrogen. *In* Proceedings International Symposium European Grassland Federation on The Role of Nitrogen in Intensive Grassland Production. Eds. W H Prins and G H Arnold. pp 51-57. PUDOC, Wageningen, the Netherlands.

Morrison J, Jackson M V and Sparrow P E 1980 The response of perennial ryegrass to

fertiliser nitrogen in relation to climate and soil. Grassland Res. Inst. (Hurley), Techn. Rep. No. 27, 71 p.

Noy I G A M 1989 Refinement of N recommendations for grassland according to soil type and soil water table (in Dutch). Internal Nota Consulentschap in Algemene Dienst voor Bodem-, Water- en Bemestingszaken in de Veehouderij.

Prins W H 1983 Limits to nitrogen fertilizer on grassland. Thesis Wageningen Agricultural University, the Netherlands, 132 p.

Prins W H, Van Burg P F J and Wieling H 1980 The seasonal response of grassland to nitrogen at different intensities of nitrogen fertilization, with special reference to methods of response measurement. *In* Proceedings International Symposium European Grassland Federation on The Role of Nitrogen in Intensive Grassland Production. Eds. W H Prins and G H Arnold. pp 33-49.

Rabbinge R, Ward S A, Van Laar H H 1989 Simulation and systems management in crop protection. Simulation Monograph 32, PUDOC, Wageningen, the Netherlands, 420 p.

Ruitenberg G H, Wopereis F A and Oenema O 1991 Calculated optimum N fertilizer application on grassland as function of soil type (in Dutch). Rapport 173, Staringcentrum, Wageningen, 62 p.

Salette J 1988 Nitrogen research: results and questions. In Nitrogen and Water Use by Grassland. Proc. Coll. AFRC Inst. for Grassl. and Animal Prod. Ed. R J Wilkins. pp 43-57. Hurley, UK.

Van der Meer H G and Van Uum-van Lohuyzen M G 1986 The relationship between inputs and outputs of nitrogen in intensive grassland systems. In Nitrogen Fluxes in Intensive Grassland Systems. Developments in Plant and Soil Sciences, Vol. 23. Eds. H G Van der Meer, J C Ryden and G C Ennik. pp 1-18. Martinus Nijhoff Publishers, Dordrecht, the Netherlands.

Warren G P and Whitehead D C 1988 Available soil nitrogen in relation to fractions of soil nitrogen and other soil properties. Plant and Soil 112, 155-165.

Whitehead D C 1984 Interactions between soil and fertilizer in the supply of nitrogen to ryegrass grown on 21 soils. J. Sci. Food Agric. 35, 1067-1075.

Whitehead D C, Barnes R J and Morrison J 1981 An investigation of analytical procedures for predicting soil nitrogen supply to grass in the field. J. Sci. Food Agric. 32, 211-218.

Wieling H and De Wit M A E 1987 Course of grass growth during the season (in Dutch). Rapport 105, Research Station for Cattle, Sheep and Horse Husbandry, Lelystad, the Netherlands, 105 p.

CHAPTER 13

GENERAL DISCUSSION AND SUMMARY

GENERAL DISCUSSION AND SUMMARY

GENERAL

The N demand of grass is mainly satisfied by the uptake of fertilizer N and inorganic N produced by mineralization when organic matter is decomposed in the soil. Annual N mineralization rates may vary dramatically, and values between 10 and 900 kg N ha^{-1} have been reported (Brockman, 1969; Richards and Hobson, 1977). When differences in N mineralization would be taken into account, a better match could be reached between fertilizer supply and grass demand, and consequently the losses to the environment would be reduced. A major problem, however, is the prediction of N mineralization in grassland soils.

N mineralization is driven by the decomposition of organic matter (C) by soil organisms. This means that the prediction of N mineralization requires understanding of the process of organic matter decomposition. Several models have been constructed to predict organic matter dynamics and N mineralization. There are, however, several problems associated with modeling soil organic matter dynamics and N mineralization:

i) it is widely recognized that soil texture and soil structure have a predominant effect on the activity of soil biota, organic matter decomposition and N mineralization, but the extent of the interactions between soil texture, soil structure, soil organic matter, soil biota and mineralization are insufficiently known;

ii) the contribution of the soil microfauna to organic matter dynamics and N mineralization in different soil types is not clear, and

iii) models cannot be verified properly because the soil organic matter pools that are distinguished in soil organic matter models can not be determined directly (Chapter 1; Buurman, 1994).

The aims of this thesis are i) to improve our understanding of the interactions between soil texture, soil structure, organic matter, soil biota and mineralization, ii) to develop a procedure that yields soil organic matter fractions that can be determined directly and that can be used in soil organic matter models, iii) to develop a model that predicts the long-term dynamics in soil organic matter, iv) to develop a simple model that can be used by farmers and advisers to predict the non-fertilizer N supply of grassland soils, and v) to quantify the effect of the non-fertilizer N supply of grassland soils on the optimum fertilizer application rate. The results obtained were presented and discussed in the preceding chapters. In this chapter we summarize and discuss the main findings and give some suggestions for further research.

INTERACTIONS BETWEEN SOIL TEXTURE, SOIL STRUCTURE, SOIL ORGANIC MATTER, SOIL BIOTA AND MINERALIZATION

During the last decade it became generally accepted that soil structure exerts dominant control over microbially mediated decomposition processes in terrestrial ecosystems (Van Veen and Kuikman, 1990; Ladd et al., 1993). In order to understand some of the interrelationships between soil structure/texture and microbial/microfaunal activity, it is necessary to quantify pore and aggregate size distributions, microbial and microfaunal populations, and C and N dynamics; it is also necessary to integrate the different concepts of physical protection (Ladd et al., 1993; Juma, 1993; Hassink et al., 1995). We studied soil structure, soil biology and organic matter dynamics in fine- and coarse-textured soils.

Mineralization

In mineral soils we found a positive relationship between the amount of organic N in the soil and the clay + silt content, and a negative relationship between the percentage of soil N mineralizing during incubation and the clay + silt content of the soil (Chapter 2). This indicates that there is more protection of organic matter in fine-textured soils than in coarse-textured soils. For C the relationships with soil texture were less clear as a result of differences in the C:N ratio of the organic matter in sandy soils. The relationships between soil texture and organic C and N were affected by the soil moisture regime. The effects of the rate of N fertilization and grassland management on the amounts of soil organic C and N, and the rates of C and N mineralization were small and non-consistent.

Soil structure

Pore and aggregate size distributions are very different between fine- and coarse-texture soils (Chapter 3). In loamy and clay soils, pores with neck size diameters < 0.2 μm were dominant. Also, pores with neck size diameters between 0.2 and 1.2 μm and between 1.2 and 6 μm were more abundant in these soils than in sandy soils. In sandy soils pores with neck size diameters between 6 and 30 μm were most abundant; the pore size class between 30 and 90 μm also made up a larger part of soil volume in the sandy soils than in the loamy and clay soils.

In the sandy soils microaggregates with diameters between 50 and 250 μm were dominant, while in the loamy and clay soils macroaggregates (diameters > 250 μm) made up approximately 50% of total soil weight (Chapter 3).

Physical protection

Several mechanisms have been suggested to explain the physical protection of soil organic matter against decomposition: adsorption to or coating by clay particles (Waksman, 1936;

Tisdall and Oades, 1982), entrapment in small pores or incorporation into soil aggregates (Tisdall and Oades, 1982; Elliott and Coleman, 1988; Golchin et al., 1994). From these studies it is not clear, however, to which extent the different mechanisms apply to different soils. In sandy soils, the C and N contents of the clay-sized particles were much higher than the C and N contents of the total soil; in the loamy soils the differences were less pronounced, while in the clay soils the C and N contents of the clay fraction did not differ from the C and N contents of the total soil (Chapter 3). In sandy soils, the C contents of the aggregates were closely correlated with their clay content and the decomposition rate of aggregate-C was negatively correlated with the clay content of the aggregate. In loamy and clay soils C contents and decomposition rates of aggregate-C were not correlated with the clay content of the aggregates (Chapter 3). Fine-sieving increased mineralization in loams and clays (the increase being highest in clays), while the mineralization rate in sandy soils was not affected (Chapter 3). We assume that disruption of the soil structure by fine sieving would release some of the organic material that was physically protected by its location in small pores or in aggregates. The results of the particle and aggregate size analysis and the disruption experiment (Chapter 3) suggest that in sandy soils organic matter is physically protected by its association with clay particles, while in fine-textured soils organic matter is also protected by its location in small pores and in aggregates.

Estimation of the protective capacity of soils

Although it has been recognized that there is more physical protection of organic matter in fine-textured soils than in coarse-textured soils (Jenkinson, 1988; Van Veen and Kuikman, 1990), it has not been studied whether the capacity of soils to physically protect organic matter is limited and whether this capacity can be quantified. Soil organic matter can be physically protected by incorporation into microaggregates and adsorption on or coating by clay and silt particles (Tisdall and Oades, 1982; Golchin et al., 1994). As microaggregates consist mainly of clay and silt particles (Skjemstad et al., 1993), the amounts of C and N associated with clay and silt particles should correspond with the amounts of C and N protected in microaggregates. Laboratory studies showed that the amount of organics that can be bound to dispersed clay particles is limited (Pinck et al., 1954; Harter and Stotzky, 1971). We determined the amounts of C and N that are preserved by association with clay and silt particles in old grassland soils, and compared those with published results of corresponding measurements in uncultivated soils in temperate and tropical regions (Chapter 4). We assumed that the amounts of clay- and silt-associated C and N are at equilibrium and at a maximum in these soils. We observed close relationships between the proportion of clay and silt particles in a soil and the amounts of C and N that are associated with these particles (r^2 = 0.81). The capacities of soils to preserve C and N by association with clay and silt particles were estimated from the observed relationships. The relationships were valid for soils from both temperate and tropical regions, showing that equilibrium levels of organic matter are not

necessarily lower in tropical soils than in soils of temperate regions (Greenland et al., 1992).

Organic C and N in the size fraction > 20 μm was not correlated with soil texture. The results confirmed the hypothesis that the amounts of C and N in coarser size fractions depend primarily on the amount of residues incorporated into the soil and are not affected by soil texture (Garwood et al., 1972). Converting grassland and uncultivated soils to cultivated arable land decreased C and N in the particle size fraction > 20 μm to a larger extent than C and N associated with clay and silt particles (< 20 μm). This confirmed that particles in the clay and silt size fraction are better protected against microbial degradation than C and N in larger size fractions.

Preservation of residue C

We hypothesized that the degree of saturation of the protective capacity of a soil affects the decomposition rate and stabilization of freshly applied residues: less of the applied C is thought to be preserved in the soil when more of the protective sites are occupied. This is in conflict with the general assumption that the preservation of organic residues in soil is directly positively correlated with its clay content (Ladd et al., 1981, 1985; Merckx et al., 1985; Van Veen et al., 1985; Jenkinson et al., 1987; Verberne et al., 1990; Jocteur Monrozier et al., 1991; Amato and Ladd, 1992). To test the hypothesis we mixed ^{14}C-labeled ryegrass through soil samples that differed in soil texture and in saturation deficit (difference between the maximum and actual amount of clay and silt associated C; Chapter 5). Our hypothesis was generally confirmed: the saturation deficit of the soil was much better correlated with $^{14}CO_2$ production than soil texture *per se*.

The observation that the saturation deficit affects the preservation of residue C in soil can explain the results of experiments where no effect of soil texture on the preservation of residue C in soil was observed (Gregorich et al., 1991; Bremer and Kuikman, 1994). Most studies in which the preservation of residue C in soil was positively correlated with the clay content of the soil were carried out with Australian soils, and it seems that in these soils the protective capacities of fine-textured soils were saturated to a lesser extent than those of the coarse-textured soils (Ladd et al., 1981, 1985; Merckx et al., 1985; Van Veen et al., 1985; Jocteur Monrozier et al., 1991; Amato and Ladd, 1992). In the study of Gregorich et al. (1991) the protective capacity of coarse- and fine-textured soils seems to be saturated to the same extent (Hassink et al., 1995). The results of our study are in agreement with laboratory studies with pure clays where it was observed that at low protein:clay ratios added protein was protected, whereas at high protein:clay ratios the clays had little effect on biodegradation of added protein (Pinck et al., 1954; Marshman and Marshall, 1981).

Soil organisms

The soil microfauna may stimulate microbial growth rates through grazing (Clarholm, 1985; Coleman et al., 1978), and predation of microbes by protozoa and nematodes can increase C

and N mineralization (Coleman et al., 1978; Clarholm, 1985; Brussaard et al., 1991; Rutherford and Juma, 1992; Bouwman and Zwart, 1994; Brussaard et al., 1995). We tried to determine whether soil structure affects the biomass of soil organisms and the interactions between microbes and soil microfauna, and whether differerences in C and N mineralization rates between soils are caused by differences in the grazing activity of the soil microfauna. We found that the biomass of bacteria was closely correlated ($r^2 = 0.91$) with the soil volume made up by pores with diameters between 0.2 and 1.2 μm and the biomass of nematodes with the soil volume made up by pores with diameters between 30-90 μm ($r^2 = 0.84$; Chapter 3). The biomass of fungi and protozoa showed a poor correlation with any of the pore size classes. The results suggest that bacteria and nematodes are spatially separated and that most bacteria are physically protected against grazing by nematodes. In agreement with this we found no correlation between the grazing pressure of the nematodes and the activity of the bacteria (expressed as the amount of CO_2 produced per unit of bacterial biomass; Chapter 3).

Analysis with a soil food web model indicated that in intensively managed grasslands, soil microfauna did not contribute significantly to C and N mineralization and that differences in microfaunal activity could not explain the differences in mineralization between soils. According to the calculations with the food web, bacterial feeding activity is high in grassland soils, reducing the relative importance of other groups (Chapter 6). These results are in contrast with most studies of natural and agro-ecosystems, where the contribution of the fauna to the transfer of nitrogen in the soil approximates 30% (Verhoef and Brussaard, 1990). This discrepancy might be related to the continuous high input of easily decomposable root and leaf material in grassland soils, whereas in arable soils the input of organic residues is much lower and is concentrated at harvest time. This high input does not seem to lead to a high biomass of bacteria but to a high activity.

In contrast with our results, it is generally found in microcosm studies that microfaunal grazing increases the activity of the microbes. In all microcosm studies bacteria and microfauna have been applied to sterilized soil. These conditions are very different from the natural situation in our studies. The indigenous bacterial population will mainly be present in protective niches, whereas inoculated bacteria are located in more open areas (Postma, 1989). The inoculated bacteria can be grazed upon to a much higher extent than the indigenous bacterial population. The inoculated bacteria can find more protective niches in fine-textured soils than in coarse-textured soils, leading to a higher grazing pressure in the coarse-textured soils (Postma, 1989) and a stronger increase in C mineralization (due to faunal activity) in coarse-textured soils than in fine-textured soils (Rutherford and Juma, 1992). It may be expected, however, that under natural conditions the differences in grazing pressure are much smaller as most bacteria in both fine- and coarse-textured soils are located in protected sites. It should therefore be realized that results of microcosm studies cannot be easily translated to field situations.

Analysis with a food web model showed that the C:N ratio of the microorganisms is a parameter that strongly affects N mineralization (De Ruiter et al., 1993). We observed that

the C:N ratio of the microbial biomass was higher in sandy soils than in loams and clays, and that there was a positive correlation between N mineralization per unit of microbial biomass and the C:N ratio of the microbial biomass. Food web calculations indicated that observed C and N mineralization rates could not be explained by differences in microfaunal activity, but could satisfactorily be calculated using different bacterial C:N ratios as determined for the soil types (Chapter 6).

Suggestions for further research

Soil structure is usually related to soil texture. To gain more insight into the relationships between soil structure, soil organic matter dynamics, soil organisms, and mineralization, measurements should be done in soils with similar textures but with different pore and aggregate size distributions. To obtain more direct proof of the exact location of soil organic matter and soil organisms ultrastructural studies should be performed.

In most physical fractionation studies, clay- and silt-associated C and N, rather than C and N incorporated into aggregates have been determined. Future research might indicate whether it is better to relate the protective capacity of a soil to the content of microaggregates instead of the amount of clay and silt particles. Future research, including microscopic observations and determination of the specific surface area (Burford et al., 1964), might show whether the capacity of soils to preserve organic C and N can be related to specific characteristics of clay and silt particles and microaggregates. Further research might also indicate whether factors such as drainage, pH and climatic conditions affect the capacity of a soil to preserve organic C and N by their association with clay and silt particles or by incorporation into aggregates.

We observed that the biomasses of bacteria, fungi, and soil microfauna in the grassland soils were in the same range as found in a Dutch arable soil (De Ruiter et al., 1993), while the activity of the bacteria and rates of N mineralization were considerably higher. We suggest to determine the biomasses of bacteria, fungi and soil microfauna and rates of C and N mineralization in a range of ecosystems, in which the quantity and quality of inputs of organic residues are very different, to test to what extent the activity and biomass of groups of soil organisms are affected by the input of organic residues.

The cause of the higher C:N ratio of the microbial biomass in sandy soils is not yet clear. Fungi generally have a higher C:N ratio than bacteria, but their contribution to the total microbial biomass was low in all soils studied. As all soils were intensively managed grasslands, the quality of the residues entering the soil was neither expected to be different. The differences in C:N ratio of the microbes between coarse- and fine-textured soils might, however, be related to differences in the composition of soil organic matter, as fine-textured soils generally contain more stabilized clay- and silt-associated organic matter (with a low C:N ratio) than coarse-textured soils, which contain relatively more labile organic matter fractions (with a higher C:N ratio; Chapters 4 and 7). To estimate the dynamics of N mineralization it might be necessary to study the relationships between soil texture and the C:N ratio of the microbial biomass in more detail. By separating bacteria from the soil

(Bakken, 1985) we might be able to determine the C and N contents of bacteria feeding on different soil organic matter pools.

FRACTIONATION OF SOIL ORGANIC MATTER

One of the main drawbacks of models describing soil organic matter turnover is that most pools are hypothetical and cannot be determined directly (Paustian et al., 1992). Successful development of techniques for direct measurement of pool sizes would be a major step towards appropriate verification of models and the revision of inherent concepts (Bonde et al., 1992). Although it is accepted that physical fractionation yields functional soil organic matter fractions, models containing pools that are based on physical fractionation have not been developed so far. This might be due to the lack of data estimating decomposition rates of the individual fractions and the uncertainty of how widely the proposed techniques can be applied (Christensen, 1992). We described a new and relatively simple procedure that separates soil organic matter into size and density fractions using silica suspensions as heavy liquids (Meijboom et al., 1995). The fractionation is based on the phenomenon that plant residues that enter the soil have a low density of approximately 1 g cm^{-3}; residue material remaining in the soil is gradually adsorbed by mineral particles, leading to an increase in density, and gradually reduced in particle size. Two size fractions and three density fractions were distinguished: C in microaggregates with diameters < 20 μm and 20-150 μm and C in light (density < 1.13 g cm^{-3}), intermediate (density 1.13-1.37 g cm^{-3}) and heavy (density > 1.37 g cm^{-3}) fractions of the macroorganic matter pool (> 150 μm). The decomposition rates of the fractions were measured in a sandy and a clay soil that were kept bare for 15 years (Chapter 8). The decomposition rates of the fractions decreased in the order light, intermediate and heavy macroorganic matter (> 150 μm; k = 23.9, 9.8 and 3.8 x 10^{-4} day^{-1}, respectively) and were lowest for C incorporated in microaggregates (< 150 μm; k = 0.5-0.7 x 10^{-4} day^{-1}; Chapter 8). As the rate constants of the fractions were found to be independent of soil texture, the fractions seem to be widely applicable. We therefore propose to use these units in organic matter dynamics models.

C and N mineralization of total soil were positively correlated with the amount of C and N in the light macroorganic matter fraction. The correlation with mineralization decreased with increasing stability of the organic matter fractions (Chapter 7). In agreement with previous results (Dalal and Mayer, 1987; Janzen et al., 1992) we found that macroorganic matter (> 150 μm) was much more affected by residue input than organic matter incorporated in microaggregates (< 150 μm; Chapter 7).

The transformations of plant residues in the soil have received little attention so far. We tested whether applied crop residues (^{14}C-labeled grass) would be transferred from light to intermediate and heavy macroorganic matter fractions and finally become stabilized in microaggregates (Chapter 9). Immediately after application most of the label appeared to be

present in the soluble and light macroorganic matter fractions. Newly synthesized microorganisms fed on the labeled components of these fractions. The amounts of ^{14}C in these fractions decreased fast, while the amounts of ^{14}C in the other fractions remained more stable as a result of the transfer of ^{14}C from the soluble or light macroorganic matter fractions to the other fractions. Six months after the application of ^{14}C-labeled ryegrass, most of the residual ^{14}C was found in the microaggregates. In agreement with Matus (1995) we observed that 180 days after the application of labeled grass, grass-derived C in a fraction was protected to a lesser extent than soil-derived C. The decomposition rate of ^{14}C associated with microaggregates in a soil was negatively correlated with the saturation deficit of the clay and silt fraction of the soil.

Suggestions for further research

Future research should be directed to the comparison of different methods to obtain size and density fractions and to the analysis of decay constants of size and density fractions in a wider range of ecosystems.

MODELING SOIL ORGANIC MATTER DYNAMICS AND N MINERALIZATION

In Chapter 1 we identified the following problems that are associated with modeling soil organic matter dynamics: i) the limited knowledge of the interactions between soil texture/soil structure/soil organic matter/soil biota/mineralization; the mechanisms of physical protection of soil organic matter in different soils are not clear; it is not clear whether the capacity of soils to preserve organic C and N is limited and whether it can be quantified, ii) the limited knowledge about the contribution of the soil microfauna to C and N mineralization, and iii) the fact that organic matter models contain pools that can not be determined directly.

These aspects were studied in this thesis and resulted in the following conclusions:

- The main mechanism of physical protection is partly different between fine- and coarse-textured soils; in coarse-textured soils soil organic matter is protected by association with clay and silt particles and in fine-textured soils also by incorporation in small pores and aggregates.
- Soils have a limited capacity to preserve organic C and N, which is positively correlated with the clay and silt content of the soil.
- The soil microfauna does not contribute significantly to C and N mineralization in intensively managed grassland soils.
- The C:N ratio of the microbial biomass differs between fine- and coarse-textured soils, which explains differences in N mineralization between soils.
- Size and density fractions differ in decomposition rate; such fractions can replace the currently used fictitious fractions in organic matter models.

The prediction of organic matter dynamics and N mineralization can be improved when these findings are included in organic matter models. It should be realized that the aim of a model determines its level of resolution. To predict the long-term dynamics of soil organic matter it might be sufficient to only take the capacity of soils to preserve soil organic matter into consideration. To predict the short-term dynamics of soil organic matter it might be necessary to distinguish different active organic matter pools, and for the prediction of the short-term dynamics in N mineralization it might be necessary to determine the C:N ratio of the microbial biomass feeding on different organic matter pools.

We developed two simple models. The first model predicts the long-term changes of soil organic matter. It includes the assumption that each soil has a maximum capacity to protect soil organic C and that the degree of saturation of the protective capacity determines the degree of physical protection of residue C (Chapter 10). The mechanism of protection of organic matter is adsorption to clay and silt particles or incorporation in microaggregates. The rate at which organic matter becomes protected depends on the amount of free organic matter and the degree of saturation of the microaggregates with organic matter. This new model allows a better prediction of the build-up and decline of soil organic matter in soils of different textures and initial organic matter contents than with the conventional implicit descriptions of protection (Van Veen et al., 1985; Jenkinson et al., 1987; Verberne et al., 1990). Our model makes a distinction between physically protected microaggregate C and non-protected microaggregate C in accordance with the observation that the preservation of ^{14}C in microaggregates is negatively correlated with the degree of saturation of these aggregates with soil organic matter (Chapter 9) and with the findings that microaggregates and clay and silt particles contain external layers of organic matter coatings that are mineralized much faster than organic matter physically protected in internal layers (Skjemstad et al., 1993; Buyanovsky et al., 1994).

The second model is an empirical relationship that can be used by farmers and advisers to estimate the Non Fertilizer Nitrogen Supply (NFNS) of grassland soils under field conditions in order to be able to optimize the fertilizer application rate on grasslands. We defined NFNS as the N uptake on unfertilized plots. The amount of N mineralized during incubations is generally considered to be an accurate method for assessing the N availability of soils (Keeney, 1982). We observed that an incubation period of 2 weeks was too short to give a good estimation of NFNS. Correlations with NFNS were better when an incubation period of 12 weeks was used. An incubation of 12 weeks makes the procedure too time-consuming to be widely applicable, however (Chapter 11). We found that under old grassland swards on mineral soils, close relationships exist between soil texture and the amount of soil organic N and microbial N. We assumed that under old grassland swards the amounts of soil organic N and microbial N were in equilibrium. The difference between the actual soil organic N content and the content under equilibrium conditions (Δ organic N) appeared to give a good estimation of the non-fertilizer N supply of grassland soils ($r^2 = 0.64$; Chapter 11). The advantage of this difference method in comparision with other methods is its

simplicity and fastness. This approach did not work for peat soils. For those soils NFNS could easily be estimated from the average deepest groundwater table.

Suggestions for further research

In the first model a distinction is made between physically protected and non-protected microaggregate C. We propose to test and develop methods, such as the high energy ultraviolet photo-oxidation method (Skjemstad et al., 1993), that can be used to separate protected and non-protected aggregate C and N. We propose that the difference method in the second model be tested on a wider range of soils and that it be tested to what extent the reliability of the difference method is affected by factors such as soil pH and groundwater table. The results suggest that for sandy soils the difference between the actual microbial N content and the content under equilibrium conditions would even give a better estimation of the non-fertilizer N supply of grassland soils than Δ organic N. We propose that this will also be tested. Additional research might make clear whether the difference approach can also be used to estimate the non-fertilizer N supply of other soils than grassland soils.

We propose to also develop models with a higher level of resolution that can predict the short-term dynamics of C and N mineralization. Such models should include the light, intermediate and heavy macroorganic matter fractions and the relationships between soil texture and the C:N ratio of the microbial biomass and organic matter pools.

EFFECTS OF NON-FERTILIZER N SUPPLY ON THE OPTIMUM N FERTILIZER APPLICATION RATE

The aim of the research presented in the last chapter was to determine the effect of differences in NFNS on the optimum fertilizer application rate (Chapter 12). The relationship between N application rate and dry matter production is the result of NFNS, the relationship between N application and N uptake and the relation between N uptake and dry matter production (nitrogen use efficiency; Frankena and De Wit, 1958). We analyzed N fertilization of mown grasslands in experiments that were performed at different locations in the Netherlands. At higher N uptake rates the apparent recovery of N fertilizer and the NUE were not affected by NFNS. The optimum N application rate was not only affected by NFNS, but also by maximum dry matter production. In agreement with Morrison (1980) and Garwood et al. (1977) we observed that the maximum dry matter production was related with the availability of moisture. Multiple regression analysis showed that an annual increase in NFNS of 100 kg per ha resulted in an annual decrease of the optimum N application rate of 80 kg N per ha on mown grassland. When instead of the original N fertilizer recommendation for intensively used mown grasslands, the optimum N fertilizer application is given by taking differences in NFNS into account, the annual reduction in N fertilizer application on mineral grassland soils is more than 50 million kg N in the Netherlands.

MAIN FINDINGS PRESENTED IN THIS THESIS

The main findings of the experiments described in this thesis are listed below:

* Quantitative data on the relationships between soil texture, soil structure, soil organic matter, soil organisms and N mineralization.
* Estimation of the capacities of soils to preserve organic matter.
* Recognition that not soil texture *per se*, but the degree of saturation of the protective capacity of a soil affects the preservation of plant residues in soil.
* Recognition that the soil microfauna does not contribute significantly to C and N mineralization in intensively managed grassland soils.
* Recognition of differences between coarse- and fine-textured soils in the C:N ratio of the microbial biomass and its importance for predicting N mineralization.
* Method to fractionate soil organic matter in biologically meaningful pools that differ in decomposition rate.
* Model that predicts the long-term dynamics of soil organic matter by simple adsorption-desorption kinetics.
* Simple and fast method to estimate the non-fertilizer N supply of mineral and peaty grassland soils.
* Quantification of the effect of differences in the non-fertilizer N supply on the optimum N fertilizer application rate.

REFERENCES

Amato M. and Ladd J. N. (1992) Decomposition of ^{14}C-labelled glucose and legume materials in soil: properties influencing the accumulation of organic residue C and microbial biomass C. Soil Biology & Biochemistry 24, 455-464.

Bakken L. R. (1985) Separation and purification of bacteria from soil. Applied and Environmental Microbiology 49, 1482-1487.

Bonde T. A., Christensen B. T. and Cerri C. C. (1992) Dynamics of soil organic matter as reflected by natural ^{13}C abundance in particle size fractions of forested and cultivated oxisols. Soil Biology & Biochemistry 24, 275-277.

Bouwman L. A. and Zwart K. B. (1994) The ecology of bacterivorous protozoans and nematodes in arable soil. Agriculture Ecosystems and Environment 51, 145-160.

Bremer E. and Kuikman P. (1994) Microbial utilization of ^{14}C[U]glucose in soil is affected by the amount and timing of glucose additions. Soil Biology & Biochemistry 26, 511-517.

Brockman J. S. (1969) The relationships between total N input and the yield of cut grass. Journal of the British Grassland Society 24, 89-96.

Brussaard L., Kools J. P., Bouwman L. A. and De Ruiter P. C. (1991) Population dynamics

and nitrogen mineralization rates in soil as influenced by bacterial grazing nematodes and mites. In: G.K. Veeresh, D. Rajagopal and C.A. Viraktamath (eds), Advances in management and conservation of soil fauna. Oxford & IBH Publ. Co., PVT Ltd, New Delhi, pp. 517-523.

Brussaard L., Noordhuis R., Geurs M. and Bouwman L. A. (1995) Nitrogen mineralization in soil in microcosms with or without bacterivorous nematodes and mites. Accepted by Acta Zoologica Fennica.

Buyanovsky G. A., Aslam M. and Wagner G. H. (1994) Carbon turnover in soil physical fractions. Soil Science Society of America Journal 58, 1167-1173.

Burford J. R., Deshpande T. L., Greenland D. J. and Quirk J. P. (1964) Influence of organic materials on the determination of the specific surface areas of soils. Journal of Soil Science 15, 192-201.

Buurman P. (Ed) (1994) Decomposition and accumulation of organic matter in terrestrial ecosystems. Ecosystem research report no 12. Final report of a concerted activity. Commission of the European Communities.

Clarholm M. (1985) Possible role for roots, bacteria, protozoa and fungi in supplying nitrogen to plants. In: A.H. Fitter, D. Atkinson, D.J. Read, and M.B. Usher (eds), Ecological interactions in soil:plants, microbes and animals. British Ecological Society Special Publications, Oxford, pp. 355-365.

Christensen B. T. (1992) Physical fractionation of soil and organic matter in primary particle size and density separates. Advances in Soil Science 20, 1-89.

Coleman D. C., Cole C. V., Hunt H. W. and Klein D. A. (1978) Trophic interactions in soil as they affect energy and nutrient dynamics. I. Introduction. Microbial Ecology 4, 345-349.

De Ruiter P. C., Moore J. C., Zwart K. B., Bouwman L. A., Hassink J., Bloem J., De Vos J. A., Marinissen J. C. Y., Didden W. A. M., Lebbink G. and Brussaard L. (1993) Simulation of nitrogen mineralization in belowground food webs of two winter wheat fields. Journal of Applied Ecology 30, 95-106.

Elliot E. T. and Coleman D. C. (1988) Let the soil work for us. In: H. Eijsackers and A. Quispel (eds), Ecological implications of contemporary agriculture. Proceedings 4th European Ecology Symposium, Wageningen. Ecological Bulletins, pp. 23-32.

Dalal R. C. and Mayer R. J. (1987) Long-term trends in fertility of soils under continuous cultivation and cereal cropping in Southern Queensland. VI. Loss of total nitrogen from differnet particle-size and density fractions. Australian Journal of Soil Research 25, 83-93.

Frankena H. J. and de Wit C. T. (1958) Nitrogen fertilization, uptake of nitrogen and growth of grass in spring (in Dutch). Landbouwkundig Tijdschrift 11, 465-471.

Garwood E. A., Clement C. R. and Williams T. E. (1972) Leys and soil organic matter, III. The accumulation of macro-organic matter in the soil under different swards. Journal of Agricultural Science Cambridge 78, 333-341.

Garwood E. A., Tyson K. C. and Clements C. R. (1977) A comparison of yield and soil conditions during 20 years of grazed grass and arable cropping. Techn. Rep. no. 2, Grassl. Res. Inst.

Golchin A., Oades J. M., Skjemstad J. O. and Clarke P. (1994) Study of free and occluded particulate organic matter in soils by solid state 13C CP/MAS NMR Spectroscopy and scanning electron microscopy. Australian Journal of Soil Research 32, 285-309.

Greenland D. J, Wild A. and Adams D. (1992) Organic matter dynamics in soils of the tropics-from myth to complex reality. In: R. Lal and P.A. Sanchez (eds), Myths and science of soils of the tropics. Proceedings of an international symposium. American Soc. of Agronomy and Soil Sci. Soc. of America, pp. 17-33.

Gregorich E. G., Voroney R. P. and Kachanoski R. G. (1991) Turnover of carbon through the microbial biomass in soils with different textures. Soil Biology & Biochemistry 23, 799-805.

Harter R. D. and Stotzky G. (1971) Formation of clay-protein complexes. Soil Science Society of America Journal 35, 383-389.

Hassink J., Matus F. J., Chenu C. and Dalenberg J. W. (1995) Interactions between soil biota, soil organic matter and soil structure. Accepted by Advances in Agroecology.

Janzen H. H., Campbell C. A., Brandt S. A., Lafond G. P. and Townley-Smith L. (1992) Light-fraction organic matter in soils from long-term crop rotations. Soil Science Society of America Journal 56, 1799-1806.

Jenkinson D. S. (1988) Soil organic matter and its dynamics. In: A. Wild (ed), Russel's soil conditions and plant growth. 11th ed., Ch. 18. Longman, New York, pp. 564-607.

Jenkinson D. S., Hart P. B. S., Rayner J. H. and Parry L. C. (1987) Modelling the turnover of organic matter in long-term experiments at Rothamsted. Intecol Bulletin 15, 1-8.

Jocteur Monrozier L., Ladd J. N., Fitzpatrich R. W., Foster R. C. and Raupach M. (1991) Components and microbial biomass content of size fractions in soils of contrasting aggregation. Geoderma 49, 37-62.

Juma N. G. (1993) Interrelationships between soil structure/texture, soil biota/soil organic matter and crop production. Geoderma 57, 3-30.

Keeney D. R. (1982) Nitrogen-availability indexes. In: A.L. Page (ed), Methods of soil analysis, part 2. Chemical and microbiological properties. Agronomy Monograph No. 9. American Society of Agronomy, Madison, Wisconsin, pp. 711-734.

Ladd J. N., Oades J. M. and Amato M. (1981) Microbial biomass formed from ^{14}C, ^{15}N-labelled plant material decomposing in soils in the field. Soil Biology & Biochemistry 13, 119-126.

Ladd J. N., Amato M. and Oades J. M. (1985) Decomposition of plant material in Australian soils. III. Residual organic and microbial biomass C and N from isotope-labelled legume material and soil organic matter, decomposing under field conditions. Australian Journal of Soil Research 23, 603-611.

Ladd J. N., Foster R. C. and Skjemstad J. O. (1993) Soil structure: carbon and nitrogen metabolism. Geoderma 56, 401-434.

Marshman N. A. and Marshall K. C. (1981) Bacterial growth on proteins in the presence of clay minerals. Soil Biology & Biochemistry 13, 127-134.

Matus F. J. (1995) The distribution of soil organic matter of various aggregate size classes in arable soils. II. Residual organic ^{14}C, ^{15}N, microbial biomass ^{14}C and ^{14}C and ^{15}N mineralization rates in a sand and a clay soil. Submitted to Soil Biology & Biochemstry.

Merckx R., Den Hartog A. and Van Veen J. A. (1985) Turnover of root-derived material and related microbial biomass formation in soils of different texture. Soil Biology & Biochemistry 17, 565-569.

Meijboom F. W., Hassink J. and Van Noordwijk M. (1995) Density fractionation of soil macroorganic matter using silica suspensions. Accepted by Soil Biology & Biochemistry.

Morrison J. (1980) The influence of climate and soil on the yield of grass and its response to fertilizer nitrogen. In: W.H. Prins and G.H. Arnold (eds), Proc. Int. Syp. European Grassland Federation on the role of nitrogen in intensive grassland production. PUDOC, the Netherlands, pp. 51-57.

Paustian K., Parton W. J. and Persson J. (1992) Modelling soil organic matter in organic-amended and nitrogen fertilized long-term plots. Soil Science Society of America Journal 56, 476-488.

Pinck L. A., Dyal R. S. and Allison F. E. (1954) Protein-montmorillonite complexed, their preparation and the effect of soil microorganisms on their decomposition. Soil Science 78, 109-118.

Postma J. (1989) Distribution and population dynamics of *Rhizobium sp.* introduced into soil. Thesis Agricultural University Wageningen.

Richards I. R. and Hobson R. D. (1977) The relationships between nitrogen supply and the nitrogen yield of cut grass swards. Journal of Agricultural Science, Cambridge 88, 289-292.

Rutherford P. M. and Juma N. G. (1992) Influence of texture on habitable pore space and bacterial-protozoan populations in soil. Biology and Fertility of Soils 12, 221-227.

Skjemstad J. O., Janik L. J., Head M. J. and McClure S. G. (1993) High energy ultraviolet photo-oxidation: a novel technique for studying physically protected organic matter in clay- and silt-sized aggregates. Journal of Soil Science 44, 485-499.

Tisdall J. M. and J. M. Oades. 1982. Organic matter and water-stable aggregates in soils. Journal of Soil Science 33, 141-163.

Van Veen J. A. and Kuikman P. J. (1990) Soil structural aspects of decomposition of organic matter. Biogeochemistry 11, 213-233.

Van Veen J. A., Ladd J. H. and Frissel M. J. (1985) Turnover of carbon and nitrogen through the microbial biomass in a sandy loam and a clay soil incubated with [$^{14}C(U)$] glucose and [^{15}N]$(NH_4)_2SO_4$ under different moisture regimes. Soil Biology & Biochemistry 17, 747-756.

Verberne E. L. J., Hassink J., Willigen P. de, Groot J. J. R. and Van Veen J. A. (1990) Modelling organic matter dynamics in different soils. Netherlands Journal of Agricultural Science 38, 221-238.

Verhoef H. A. and Brussaard L. (1990) Decomposition in natural and agro-ecosystems: the contribution of soil animals. Biogeochemistry 11, 175-211.

Waksman S. A. (1936) Humus: origin, chemical composition, and importance in nature. Williams and Wilkins Company, Baltimore.

SAMENVATTING

Het grootste deel van de stikstof (N) die door gras wordt opgenomen is toegediend als kunstmeststikstof of is beschikbaar gekomen bij de afbraak van organische stof. Graslandbodems bevatten gewoonlijk 5.000-15.000 kg organische N per ha in de bewortelbare zone. De hoeveelheid N die jaarlijks bij de afbraak van organische stof (mineralisatie) beschikbaar komt varieert van 10 tot 900 kg per ha. Als rekening wordt gehouden met de verschillen in N-mineralisatie, kan de kunstmestgift beter op de behoefte van het gras worden afgestemd en zal minder N verloren gaan. Het is echter nog niet goed mogelijk te voorspellen hoeveel N er via mineralisatie beschikbaar komt in verschillende gronden. N-mineralisatie wordt gestuurd door de biologische afbraak van organische stof. Voordat we in staat zullen zijn te voorspellen hoeveel N er jaarlijks zal mineraliseren, is het noodzakelijk het proces van organische-stofafbraak goed te begrijpen. Er zijn verschillende modellen ontwikkeld die de dynamiek van organische stof en N-mineralisatie voorspellen. Bij de modellering zijn er echter verschillende problemen:

i) er wordt algemeen aangenomen dat de bodemtextuur en -structuur grote invloed hebben op de activiteit van bodemorganismen, de afbraak van organische stof en de N-mineralisatie. Het is echter onvoldoende bekend op welke manier textuur en structuur deze invloed uitoefenen en hoe groot die invloed in verschillende gronden is,

ii) het is onduidelijk hoe groot de bijdrage van de bodemmicrofauna aan de organische-stofafbraak en N-mineralisatie is bij verschillende grondsoorten, en

iii) modellen kunnen niet goed getoetst worden omdat de organische-stoffracties die in modellen onderscheiden worden niet direct gemeten kunnen worden.

De doelen van deze studie zijn: i) het inzicht in de interacties tussen bodemtextuur, bodemstructuur, organische stof, bodemorganismen en N-mineralisatie te vergroten, ii) een methode te ontwikkelen waarmee organische stof gescheiden kan worden in fracties met verschillende afbraaksnelheid, iii) een model te ontwikkelen dat de dynamiek van organische stof voorspelt, iv) een eenvoudig model te ontwikkelen dat door boeren en voorlichters gebruikt kan worden om het N-leverend vermogen van graslandgronden te schatten, en v) het effect van verschillen in het N-leverend vermogen van graslandgronden op de optimale kunstmest-N-gift te kwantificeren.

INTERACTIES TUSSEN BODEMTEXTUUR/STRUCTUUR, ORGANISCHE STOF, BODEMORGANISMEN EN N-MINERALISATIE

Om het inzicht in de interacties tussen bodemstructuur/textuur, activiteit van micro-organismen en microfauna en N-mineralisatie te vergroten, bestudeerden we bodemstructuur, bodemorganismen en organische stof afbraak in zand-, zavel- en kleigronden.

Mineralisatie

We vonden een positieve relatie tussen het organische-N-gehalte en de klei- en silt-gehalten van graslandgronden, en een negatieve relatie tussen het percentage van de voorraad aan organische N die mineraliseert en het klei- en silt-gehalte van graslandgronden (Hoofdstuk 2). Er wordt verondersteld dat deze verschillen veroorzaakt worden door de grotere mate van fysieke bescherming van organische stof in kleigronden dan in zandgronden. Voor koolstof (C) waren de relaties met bodemtextuur (klei- en silt-gehalte) minder duidelijk, omdat zandgronden inert C in de vorm van houtskool bevatten. De relaties tussen bodemtextuur en de hoeveelheid bodem-organische C en N werden beïnvloed door de grondwaterstand. De effecten van kunstmeststikstof en graslandbeheer (maaien of beweiden) op de hoeveelheid bodem-organische C en N, en de C- en N-mineralisatie waren gering en niet consistent (Hoofdstuk 2).

Bodemstructuur

Zandgronden hadden een andere bodemstructuur dan zavel- en kleigronden: in zandgronden kwamen poriën met diameters tussen 6 en 30 µm het meest voor; poriën met diameters tussen 30-90 µm waren in zandgronden ook talrijker dan in zavel- en kleigronden. In zavel- en kleigronden kwamen poriën met diameters < 0.2 µm het meest voor, terwijl poriën met diameters van 0.2-1.2 en 1.2-6 µm in deze gronden ook talrijker waren dan in zandgronden. In zandgronden kwamen micro-aggregaten met diameters van 50-250 µm het meest voor, terwijl in zavel- en kleigronden macro-aggregaten (diameters > 250 µm) het meest voorkwamen (Hoofdstuk 3).

Fysieke bescherming

Er wordt aangenomen dat organische stof fysiek beschermd kan worden in de grond door de binding aan kleideeltjes, of door de aanwezigheid in kleine poriën, voornamelijk in aggregaten, waardoor de micro-organismen de organische stof niet kunnen bereiken. We probeerden vast te stellen welk mechanisme in zand,- zavel- en kleigronden verantwoordelijk is voor de fysieke bescherming van de organische stof. De kleideeltjes in zandgronden hadden een veel hoger C- en N-gehalte dan de totale grond; in zavelgronden waren de verschillen minder duidelijk, terwijl de C- en N-gehalten van kleideeltjes in kleigronden gelijk waren aan de C- en N-gehalten van de totale grond (Hoofdstuk 3). In zandgronden was het C-gehalte van bodemaggregaten positief gecorreleerd met het kleigehalte; de afbraaksnelheid van aggregaat-C was negatief gecorreleerd met het kleigehalte van het aggregaat. In zavel- en kleigronden waren C-gehalten van aggregaten en de afbraaksnelheden van aggregaat-C niet gecorreleerd met de kleigehalten van de aggregaten. In kleigronden werd de mineralisatie sterk verhoogd nadat de structuur kapot was gemaakt door de grond door een zeef van 1 mm te wrijven; bij zavelgronden was de toename in mineralisatie geringer, terwijl bij zandgronden het kapotmaken van de structuur de mineralisatie niet beïnvloedde (Hoofdstuk 3). Er wordt aangenomen dat door het kapot maken van de structuur, een deel van de organische stof die

fysiek beschermd was tegen afbraak door bodemorganismen door de lokalisatie in kleine poriën en aggregaten de bescherming zou verliezen en afgebroken zou worden door bodemorganismen. Deze resultaten duiden erop dat in zandgronden organische stof alleen beschermd kan worden door de binding aan kleideeltjes, terwijl in zavel- en kleigronden organische stof ook beschermd wordt in aggregaten en kleine poriën (Hoofdstuk 3).

Beschermingscapaciteit van de grond

Er wordt algemeen aangenomen dat er meer fysieke bescherming van organische stof optreedt in kleigronden dan in zandgronden. Er is echter nooit vastgesteld hoeveel C en N de grond maximaal fysiek kan beschermen tegen afbraak.

We namen aan dat de meeste organische stof die fysiek beschermd is in de grond geassocieerd is met klei- en siltdeeltjes. We bepaalden de hoeveelheid C en N die geassocieerd is met klei- en siltdeeltjes in oude graslandgronden en in gronden onder een natuurlijke vegetatie in gematigde en tropische gebieden. We gingen ervan uit dat in deze gronden de hoeveelheid C en N die gebonden kan worden door klei- en siltdeeltjes het maximum bereikt heeft. We vonden een positieve relatie tussen het klei- en siltgehalte van deze gronden en de hoeveelheid C en N die geassocieerd was met deze fractie (r^2 = 0.81; Hoofdstuk 4). Uit deze relatie tussen de hoeveelheden C en N die geassocieerd was met de klei- en siltfractie in oude graslandgronden en gronden onder een natuurlijke vegetatie, en de hoeveelheid klei- en siltdeeltjes werd geschat hoeveel C en N er in een grond beschermd kan worden ("beschermingscapaciteit").

De hoeveelheden C en N in de fractie > 20 µm waren niet gecorreleerd met de bodemtextuur. De hoeveelheden C en N in de fractie > 20 µm werden voornamelijk beïnvloed door de hoeveelheid gewasresten die aan de grond werd toegevoegd; de hoeveelheden C en N in de fractie < 20 µm werden voornamelijk beïnvloed door de textuur van de grond.

Bescherming van met ^{14}C gelabelde gewasresten

Over het algemeen wordt aangenomen dat het kleigehalte van een grond de afbraaksnelheid van gewasresten beïnvloedt. We testten de hypothese dat niet de textuur als zodanig, maar de mate van verzadiging van de klei- en siltdeeltjes met organische stof de afbraaksnelheid van aan de grond toegevoegd gewasmateriaal bepaalt. We mengden met ^{14}C gelabeld gras door zand- en kleigronden die verschilden in de mate waarin de klei- en siltdeeltjes verzadigd waren met organische stof (Hoofdstuk 5). De hypothese werd bevestigd: de hoeveelheid ^{14}C die vrijkwam als $^{14}CO_2$ was positief gecorreleerd met de mate van verzadiging van de klei- en siltdeeltjes. De correlatie tussen $^{14}CO_2$ en bodemtextuur was minder goed. De waarneming dat de mate van verzadiging van de klei- en siltdeeltjes invloed heeft op de afbraak van gewasresten en niet bodemtextuur als zodanig, verklaart waarom in sommige experimenten geen effect van de textuur op de afbraak van gewasresten werd gevonden, terwijl dat in andere gevallen wel zo was.

Bodemorganismen

We probeerden vast te stellen of er verschillen bestaan in de begrazingsdruk van de bodemmicrofauna op de micro-organismen in zand,- zavel- en kleigronden en of de gemeten verschillen in C- en N-mineralisatie tussen verschillende gronden veroorzaakt kunnen zijn door verschillen in de begrazingsdruk van de bodemmicrofauna. Er was een hoge correlatie tussen de biomassa aan bacteriën en het bodemvolume dat werd ingenomen door de poriën met diameters van 0.2-1.2 μm (r^2 = 0.91); de biomassa aan nematoden vertoonde een hoge correlatie met het bodemvolume dat werd ingenomen door de poriën met diameters van 30-90 μm (r^2 = 0.84; Hoofdstuk 3). De biomassa aan schimmels en protozoën was niet gecorreleerd met een poriëngrootte-klasse. De resultaten suggereren dat de meeste bacteriën en nematoden fysiek gescheiden voorkomen in de grond en dat de meeste bacteriën beschermd zijn tegen begrazing door de nematoden. Berekeningen met een voedselwebmodel toonden aan dat de gemeten verschillen in C- en N-mineralisatie tussen gronden niet verklaard kunnen worden uit verschillen in begrazingsdruk door de bodemmicrofauna (Hoofdstuk 6). De verschillen konden wel verklaard worden door rekening te houden met de gemeten verschillen in de C:N-verhouding van bacteriën tussen zand,- zavel- en kleigronden (Hoofdstuk 6).

FRACTIONERING VAN DE BODEM-ORGANISCHE STOF

Modellen die de opbouw en afbraak van bodem-organische stof beschrijven, bevatten organische-stoffracties die verschillen in afbreekbaarheid. De meeste fracties kunnen echter niet direct gemeten worden. De ontwikkeling van methoden om de theoretische fracties die in de modellen onderscheiden worden te kwantificeren zou een grote stap zijn in het valideren van de concepten in huidige modellen.

We gebruikten een nieuwe methode waarmee de bodem-organische stof wordt gescheiden in fracties op basis van verschillen in deeltjesgrootte en -dichtheid. De fractionering is gebaseerd op het fenomeen dat organisch materiaal dat aan de grond wordt toegevoegd steeds verder wordt verkleind door bodemorganismen en dat de organische stof die overblijft in de grond in dichtheid toeneemt doordat het in steeds grotere mate gebonden wordt aan minerale delen (vnl. klei- en siltdeeltjes). We onderscheidden twee grootte-fracties: C in micro-aggregaten met diameters kleiner dan 20 μm en 20-150 μm, en drie dichtheidsfracties: C in lichte (dichtheid < 1.13 g per cm^3), midden (dichtheid 1.13-1.37 g per cm^3) en zware (dichtheid > 1.37 g per cm^3) fracties van de macro-organische stof (> 150 μm; Hoofdstuk 8). De afbraaksnelheid van de fracties nam af in de volgorde lichte, midden en zware macro-organische stof (k = 23.9, 9.8 en 3.8 x 10^{-4} per dag) en was het laagst voor C in micro-aggregaten (< 150 μm; k = 0.5-0.7 x 10^{-4} per dag). De lichte fractie bestaat uit gedeeltelijk afgebroken gewasresten met een hoge C:N-verhouding; in de midden en zware fractie wordt de structuur van het gewasmateriaal steeds minder duidelijk en is de C:N-verhouding wat lager, terwijl de organische stof in de micro-aggregatenfractie bestaat uit amorf materiaal met

een lage C:N-verhouding. De afbraakconstanten werden niet beïnvloed door de bodemtextuur. We stellen voor om deze fracties te gebruiken in modellen die de organische-stofdynamiek beschrijven (Hoofdstuk 8).

De mineralisatie van C en N in grond was positief gecorreleerd met de hoeveelheid C en N in de lichte fractie. De correlatie met mineralisatie werd minder met toenemende stabiliteit van de organische-stoffracties (Hoofdstuk 7). De fractionering werd ook gebruikt om de omzettingen van met ^{14}C gelabeld gras in de grond te volgen (Hoofdstuk 9). Onmiddellijk na toediening van het gras kwam de meeste ^{14}C voor in de oplosbare en de lichte macro-organische-stoffractie. ^{14}C werd omgezet van deze meest actieve fracties naar de midden en de zware macro-organische-stoffractie. Twee en zes maanden na toediening van het gras werd de meeste residuele ^{14}C aangetroffen in micro-aggregaten. In alle fracties werd residueel ^{14}C veel sneller afgebroken dan C. De afbraaksnelheid van ^{14}C in micro-aggregaten was positief gecorreleerd met de mate van verzadiging van de micro-aggregaten met organische stof (Hoofdstuk 9).

MODELLEN DIE DE DYNAMIEK VAN ORGANISCHE STOF EN HET NIVEAU VAN N-MINERALISATIE VOORSPELLEN

We ontwikkelden twee modellen. Het eerste model voorspelt de dynamiek van organische stof op de lange termijn. In dit model wordt aangenomen dat elke grond een maximum capaciteit heeft om organische stof te beschermen en dat de mate van verzadiging van de beschermende capaciteit bepaalt in welke mate gewasresten fysiek worden beschermd tegen afbraak (Hoofdstuk 10). Met dit model waren we beter in staat de opbouw en afname van organische C in gronden met verschillende textuur en organische-stofgehalten te voorspellen dan conventionele modellen waarin de bescherming impliciet wordt beschreven en gerelateerd is aan het kleigehalte.

Het tweede model is een empirische relatie die door boeren en voorlichters gebruikt kan worden om het N-leverend vermogen van graslandgronden te schatten. We definieerden het N-leverend vermogen als de hoeveelheid N die geoogst wordt op een niet met N bemest object. We vonden dat het organische-N-gehalte van oude minerale graslandgronden positief en goed gecorreleerd was met de bodemtextuur (fractie < 50 µm). We veronderstelden dat onder oude graslanden de hoeveelheid bodemorganische-N in evenwicht is. Het verschil tussen het actuele organische-N-gehalte van de grond en het bodem-organische-N-gehalte onder evenwichtsomstandigheden (oud grasland) bleek goed te correleren met het N-leverend vermogen van minerale graslandgronden (r^2 = 0.64; Hoofdstuk 11). Het voordeel van deze benadering is dat het N-leverend vermogen van gronden snel en eenvoudig te schatten is. Bij veengronden bestond er geen relatie tussen bodemtextuur en organische-N-gehalte; bij veengronden bestond er een goede correlatie tussen de gemiddelde laagste grondwaterstand in de zomer en het N-leverend vermogen van de grond (r^2 = 0.74; Hoofdstuk 11).

EFFECTEN VAN VERSCHILLEN IN HET N-LEVEREND VERMOGEN VAN MINERALE GRASLANDGRONDEN OP DE OPTIMALE N-GIFT

Om het effect van verschillen in N-leverend vermogen op de optimale N-gift vast te stellen hebben we N-bemestingsproeven geanalyseerd die de laatste jaren op gemaaid grasland zijn uitgevoerd in Nederland (Hoofdstuk 12). De optimale N-gift werd beïnvloed door het N-leverend vermogen van de grond en de maximale droge-stof-produktie op een perceel. De maximale droge-stof-produktie bleek sterk beïnvloed te worden door de beschikbaarheid van vocht. Als rekening werd gehouden met verschillen in de maximale produktie, bleek dat op gemaaid grasland een toename in het N-leverend vermogen van de grond met 100 kg per ha per jaar leidde tot een verlaging van de optimale N-gift van 80 kg N per ha per jaar. Door rekening te houden met verschillen in het N-leverend vermogen van gronden zou er jaarlijks in Nederland meer dan 50 miljoen kg kunstmest-N bespaard kunnen worden op minerale graslandgronden, hetgeen tot gevolg kan hebben dat de verliezen naar het milieu verminderen.

BELANGRIJKSTE RESULTATEN DIE HET ONDERZOEK HEEFT OPGELEVERD

* Kwantitatieve gegevens over de relaties tussen bodemtextuur, bodemstructuur, organische stof, bodemorganismen en N-mineralisatie
* Schatting van de capaciteit van gronden om organische stof te beschermen
* Onderkenning dat niet bodemtextuur als zodanig, maar de mate van verzadiging van de beschermingscapaciteit van een grond de afbraak van gewasresten beïnvloedt
* Onderkenning dat de bodemmicrofauna niet significant bijdraagt aan de C- en N-mineralisatie in intensief gebruikte graslandgronden
* Onderkenning van het optreden van verschillen in de C:N-verhouding van micro-organismen tussen zand-, zavel- en kleigronden, en het belang daarvan voor het voorspellen van N-mineralisatie
* Methode om de organische stof te scheiden in biologisch relevante fracties die verschillen in afbreekbaarheid
* Model dat de dynamiek van bodem-organische stof voorspelt
* Eenvoudige en snelle methode om het N-leverend vermogen van minerale en veengraslandgronden te schatten
* Kwantificering van het effect van verschillen in het N-leverend vermogen van minerale graslandgronden op de optimale kunstmest-N-gift

CURRICULUM VITAE

Jan Hassink werd 26 juni 1960 op de ouderlijke boerderij in het Gelderse Wilp geboren. Na het behalen van het VWO-diploma aan de Alexander Hegius Scholengemeenschap te Deventer begon hij in 1978 met de studie Bodemkunde aan de Landbouw Universiteit te Wageningen. Tijdens zijn stage aan de University of Guelph (Canada) en zijn doctoraalfase ontdekte hij de boeiende wereld van de bodembiologie. Nadat hij in 1986 de doctoraalsbul gehaald had, trad hij, als erkend gewetensbezwaarde, in dienst bij het toenmalige ITAL. Hij werkte in de Bodembiologie-groep onder leiding van Hans van Veen mee aan het Lovinkhoeve-project, dat gericht was op de vergelijking tussen twee landbouwsystemen: een 'conventioneel' en een 'geïntegreerd' systeem. Binnen dit project hield hij zich gedurende twee jaar bezig met de biomassa en diversiteit van bacteriën, actinomyceten en schimmels. Daarna verhuisde hij naar het 'hoge Noorden', waar hij in dienst trad bij het toenmalige IB-DLO te Haren om te gaan werken aan het kwantificeren van de N-mineralisatie in graslandgronden. Vanaf september 1987 woont hij met veel plezier in de stad Groningen. Zijn wetenschappelijke interesse gaat uit naar het integreren van het N-mineralisatie-onderzoek met onderzoek naar de afbraak van organische stof, de biomassa van micro-organismen en microfauna en bodemstructuur.

Overige publikaties (chronologische volgorde)

Coleman D. C., Brussaard L., Beare M. H., Hendrix P. F., Hassink J., Heijnen C. E. and Marinissen J. C. Y. 1989. Microbial-faunal interactions as they influence soil organic matter dynamics. *In*: T. Hattori, Y. Ishida, Y Maruyama, R. Y. Morita and A. Uchida (eds), Recent advances in Microbial Ecology. Proc. Vth Int. Symp. Microbial Ecol. Kyoto, pp. 175-179.

Hassink J., Scholefield D. and Blantern P. 1990. Nitrogen mineralization in grassland soils. *In*: N. Gaborcik, V. Krajcovic and M. Zimkova (eds), Proc 13th General Meeting of the European Grassland Federation, vol II. Banscka Bystrica, Tsjechoslovakia, pp. 25-32.

Verberne E. L. J., Hassink J., de Willigen P., Groot J. J. R. and van Veen J. A. 1990. Modelling organic matter dynamics in different soils. Netherlands Journal of Agricultural Science 38, 221-238.

Brussaard L., Bouwman L. A., Geurs M., Hassink J. and Zwart K. B. 1990. Biomass, composition and temporal dynamics of soil organisms of a silt loam soil under conventional and integrated management. Netherlands Journal of Agricultural Science 38, 283-302.

Hassink J. 1991. Effect of grassland management on N mineralization potential, microbial biomass and N yield in the following year. Netherlands Journal of Agricultural Science 40, 173-185.

Hassink J. and Neeteson J. J. 1991. Effect of grassland management on the amounts of soil organic N and C. Netherlands Journal of Agricultural Science 39, 225-236.

Hassink J., Lebbink G. and van Veen J. A. 1991. Microbial biomass and activity of a reclaimed-polder soil under a conventional or a reduced-input farming system. Soil Biology & Biochemistry 23, 507-513.

Hassink J., Oude Voshaar J. H., Nijhuis E. H. and van Veen J. A. 1991. Dynamics of the microbial populations of a reclaimed-polder soil under a conventional and a reduced-input farming system. Soil Biology & Biochemistry 23, 515-524.

Hassink J. 1992. Effects of soil texture and structure on C and N mineralization in grassland soils. Biology & Fertility of Soils 14, 126-134.

Handayanto E., Nuraini Y., Purnomosidi P., Hanegraaf M., Agterberg G., Hassink J. and van Noordwijk M. 1992. Decomposition rates of legume residues and N-mineralization in an ultisol in Lampung. Agrivita 15, 75-86.

Hassink J. 1993. Relationships between the amount and the activity of the microbial biomass in Dutch grassland soils: comparison of the fumigation-incubation method and the substrate-induced respiration method. Soil Biology & Biochemistry 25, 533-538.

Hassink J., Bouwman L. A., Zwart K. B. and Brussaard L. 1993. Relationships between habitable pore space, biomass and activity of bacteria and fauna and mineralization rates in grassland soils. Soil Biology & Biochemistry 25, 47-55.

De Ruiter P. C., Moore J. C., Zwart K. B., Bouwman L. A., Hassink J., Bloem J., de Vos J. A., Marinissen J. C. Y., Didden W. A. M., Lebbink G. and Brussaard L. 1993. Simulation of nitrogen mineralization in the belowground food webs of two winter wheat fields. Journal of Applied Ecology 30, 95-106.

Hassink J. 1994. Active organic matter fractions and microbial biomass as predictors of N mineralization. European Journal of Agronomy 3, 257-265.

Hassink J. 1994. Effect of soil texture on the size of the microbial biomass and on the amount of C and N mineralized per unit of microbial biomass in Dutch grassland soils. Soil Biology & Biochemistry 26, 1573-1581.

Neeteson J. J. and Hassink J. 1994. Nitrogen mineralization in agricultural soils. Proceedings of a symposium held at the Institute for Soil Fertility Research, Haren, the Netherlands.

Hassink J., Matus F. J., Chenu C. and Dalenberg J. W. 1995. Interactions between soil biota, soil organic matter and soil structure. Accepted by Advances in Agroecology.

Meijboom, F. W., Hassink J. and van Noordwijk M. 1995. Density fractionation of soil macroorganic matter using silica suspensions. Accepted by Soil Biology & Biochemistry.