Transfer dynamics of nitrogen in a leek intercropping system

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Summary

The study was part of a project, which focuses on the optimisation of the leek intercropping system. Weeds and pests control are major problems in the production of leek. Intercropping leek with *Trifolium subterraneum* seems to offer a sustainable alternative for the conventional production methods. In the present study the additional value of nitrogen mineralization from incorporated subterranean clover on the N availability for leek was investigated. The aims of this research were (i) to quantify the relation between temperature and rate of mineralization of incorporated subterranean clover (*Trifolium subterraneum* L.), (ii) to construct a model which correctly predicts the course of net mineralization and fits into the leek intercropping simulation model and (iii) to indicate research priorities for the development of a subroutine linking below and above ground interactions specifically important for the leek intercropping system. An incubation experiment with subterranean clover was conducted for five weeks at five constant temperatures, ranging from 10 till 25°C, to measure net mineralization. The Arrhenius equation was used to define the relation between temperature and rate of mineralization. A simple, descriptive model, hereafter referred to as 'MINMOD', constructed by van Schöll (1995), was selected to simulate the N mineralization from the incorporated clover. The data from the executed experiment were used to validate the model. MINMOD was able to simulate the course of mineralization from incorporated subterranean clover at constant temperatures during five weeks adequately. Largest deviations between simulated and experimental values occurred for the 10°C treatment. These deviations might be due to either the model performance under low temperatures or the suboptimal experimental conditions at the 10°C temperature regime. Model performance was evaluated by plotting the simulated values against the measured values of all temperatures in a 1 to 1 plot. A paired t-test showed that the plotted values did not significantly deviate ($p = 0.0018$) from the line $y = x$. It was found that a standard temperature correction function can be used for temperatures between 10 and 25°C when the relation between temperature and mineralization rate is defined on basis of the Arrhenius equation. A first rough investigation of the relation between nitrogen release from the incorporated clover and the demand of leek in a intercropping system showed that not synchronisation but quantity is the bottleneck. The clover does not supply sufficient nitrogen to satisfy the needs of the leek.

Voorwoord

Het onderzoek dat in dit verslag beschreven staat, is een gezamenlijk produkt van twee studenten. Drie maanden waren wij te gast bij de Forschungs Anstalt Wädenswil in Zwitserland om de mineralisatie snelheid van ondergewerkte ondergrondse klaver te bestuderen met behulp van experiment en een simulatie model. Velen hebben op een of andere manier een bijdrage geleverd aan het onderzoek en het aangename verblijf in Zwitserland. Enkelen willen we hier met name noemen.

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Als afsluiting een raadsel voor de lezer: 1 + 1 =....?

Liesje en Barbara,
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Chapter 1

General introduction

1.1 Problem definition

People are used to a wide range of agricultural products which are relatively cheap and satisfy demanding external quality criteria such as uniform appearance and other cosmetic standards. Increasing awareness of environmental issues slowly adds quality requirements pertaining to the way in which the products are grown (Theunissen, 1997). In this perspective, the term `sustainability` is often used. Sustainability assumes that the production methods used can be applied indefinitely without decreasing the productivity of the soil and without degrading the agro ecosystem while allowing the farmer to earn a decent living (Theunissen, 1997).

The production of leek (*Allium porrum* L.) increased significantly in the last decade in Europe (Bosch, 1993). Production by the countries of the European Union reaches about 7 million tonnes per year (Brewster, 1994). However, in general no cropping system has been developed yet that provides sustainable solutions for the problems raised by leek production. Currently, leek is cropped in a conventional (Bosch, 1993) and organic manner (Bokhorst *et al.*, 1992). The organic system excludes the use of synthetic chemical fertilisers and crop protection chemicals and relies upon biological control measures: mechanical cultivation, crop rotation, crop residues, animal and green manure, and mineral bearing rocks (Nguyen *et al.*, 1995). The latter system encounters some different production difficulties but broadly speaking leek cultivation causes three major problems:

Pests and diseases

Populations of trips (*Thrips tabaci* Lindeman) as well as severe infections of rust (*Puccinia allii* Rudolph) cause considerable damage to leek crops. Due to frequent insecticide spraying populations of trips are increasingly becoming resistant and can no longer be controlled by the insecticides that are available (Theunissen & Schelling, 1996). So there is an urgent need to find alternative management methods. Pests and diseases constitute already the biggest problem in organic production of leek. The organic vegetable grower has few possibilities to control plagues; all attention has to be focused on prevention (Bokhorst *et al.*, 1992).

Weed control

Leek is a crop with a low competitive ability. Therefore weeds are a major problem and the control is an important crop management activity. (Theunissen & Schelling, 1996). Since herbicides are the most common tools to control weeds, (Bosch, 1993) leek as poorly competitive crop, strongly contributes to the high herbicide consumption in vegetable production (Baumann *et al.,* in press). In organic farming labour intensive mechanical weeding is applied (Bokhorst *et al.*, 1992).

Nitrogen use efficiency

One of the goals in modern agriculture is an efficient use of nitrogen to prevent losses to the environment. Bosch (1993) stated that in leek cropping the total advised N application rate is 270 kg N ha^{-1} and that at most 160 kg of this application is utilised by the crop. In general the N recovery of leek is a poor 50% (Smit *et al.*, 1996). This implies that for this crop large amounts of nitrogen can be subject to losses to the environment during or after the growing period (Smit *et al.*, 1996; Bosch, 1993). Because the use of nitrogenous fertilisers is restricted under alternative systems, one of the major problems is to ensure adequate N input to crops (Nguyen *et al.*, 1995; Atkinson *et al.*, 1995). If N-rich plant material is incorporated, substantial leaching losses of nitrate-N can occur after ploughing-in of the manure. This is mainly because the pattern of N mineralization is often poorly synchronised with crop N demand (Nguyen *et al.*, 1995).

1.2 Intercropping

Because the leek cropping area still increases (Bosch, 1993), there is an urgent need to develop sustainable cropping methods that tackle the above mentioned problems. Experiments looking for solutions were executed using an intercropping set up (Müller-Schärer *et al.,* 1992a, b, c; Müller-Schärer & Baumann, 1993a,b; Hovenkamp, 1996; Imhof *et al.*, 1996; Theunissen & Schelling 1996; van de Poll, 1998). Intercropping is space-dependent form of multiple cropping, hence growing two or more crops simultaneously on the same field in such a way that they interact agronomically (Vandermeer, 1989). It is a cropping system that has a long history and is widely used in tropical regions (Barney, 1987). Potential advantages of intercropping are: suppression of weeds through shading by complex canopies, better use of (soil) nutrients, improved productivity per unit of land, insurance against

crop failure and reduction of insect and disease incidence (Altieri, 1994). In hilly areas, additional advantages like protection of the soil from solarization and erosion could prove to be important as well (Theunissen, 1997). Within intercropping a distinction is made between `undersowing` and `mixed cropping`. The term undersowing is used to denote the combination of an economically important main crop with an undersown intercrop basically without economic significance but used to diversify the agro-ecosystem or to influence the main crop (Theunissen, 1997). The term `mixed cropping` is used when all components of the system have market value (Theunissen, 1997). Individual crops of an intercropping system will be referred to as `component crops` in this report. Eventually a distinction is made between the primary (or principal) and secondary crop. In many studies executed in Europe, mostly conducted with a crop of the family *Cruciferae*, it was actually proven that diversity created by intercropping reduced diseases and pest insects in agricultural systems (Hovenkamp, 1996; Theunissen & Schelling, 1996; Imhof *et al.* 1996). So far, intercropping in European intensive agriculture has not been applied mainly because of possible yield losses due to competition between the principal and secondary crop, the availability of cheap pesticides and growers` reluctance to use more complicated cropping systems (Theunissen, 1997). According to Kesper & Imhof (1998) Swiss vegetable growers do not consider the system as economically viable. But now that the application of pesticides becomes more and more restricted interest in alternative cropping systems, like intercropping, might increase. In many crops, yield is measured only in terms of weight. In vegetables, a combination of weight and quality determines the economic result. In leek, benefits from increased quality through intercropping can offset, at least partly, loss of weight produced (Theunissen & Schelling, 1996).

1.2.1 Effects of intercropping on pest and disease control

In sustainable agriculture, diversification of the habitat is used as a method to reduce pests (Theunissen & Schelling, 1996). Polycultures, including mixtures of different crops and mixtures of crops and non-crop vegetation, have been applied in a wide variety of crop types. Even though the mechanisms are not completely understood and the number of known successful applications is limited, results are encouraging enough for research to be continued. Investigation in vegetables has shown that several pests, such as cabbage root fly and trips can be considerably reduced by using clover as a living mulch or undersow. It has been shown that predators such as

rove beetles and ground beetles, can be enhanced by intercropping but it is not easy to quantify their role in pest reduction. In a study on the effect of intercropping cabbage with white (*Trifolium repens* L.) and subterranean clover (*Trifolium subterraneum* L.) on epigeic predator activity-density, it was found that the predator activity-density was significantly higher in the intercrop than in the monocrop in spring and early summer (Booij *et al.*, 1997). Theunissen & Schelling (1996) carried out field experiments during three successive years in which leek was intercropped with subterranean clover. The effects on trips population and infection by leek rust were studied. In the experiment no insecticides or fungicides were applied. Undersowing leek with clover drastically reduced trips infestations, which was reflected in reduced leaf damage. Leek rust incidence was only reduced slightly. It seems that the clover influences the attractivity of leek for trips but prove for this assumption has not been found yet. When expressed in marketable weight, the yields of the intercropped leek in all years were much better than of the monoculture in the absence of pesticides. This means that the intercrop performs better in terms of crop quality than the monocrop. But the quantity of crop produced was reduced considerably as a result of plant competition (Theunissen & Schelling, 1996). Hovenkamp (1996) executed a similar experiment. In accordance with the results of Theunissen & Schelling (1996), Hovenkamp (1996) found a strong decrease in the occurrence of trips and a significant lower yield for intercropped leek. Theunissen & Schelling (1996) concluded that the loss of harvestable weight seems to be the penalty that has to be paid for growing first quality produce without the use of pesticides. Eventually it will be the combination of yield quantity and quality, which determines whether or not intercropping can be developed into a common commercial practice in vegetable growing.

1.2.2 Effects of intercropping on weed control

Little research has been conducted on the effects of multiple cropping systems on weeds, and the results obtained so far are variable (Vandermeer, 1989). There are some data showing that using intercropping systems may provide some weed control advantages over mono cultures (Liebman, 1995). The factors that affect the success of weed control in multiple cropping systems are poorly understood. Moody & Shetty (1981) suggested that increased suppression of weeds in multiple cropping systems as compared with monocultures, was largely the result of greater overall crop density in multi cropping systems. Ilnicki & Enache (1992) studied the effect of *Trifolium*

subterraneum as weed control measure in vegetables. The various studies showed that *T. subterraneum* can be used as a living mulch for weed control in vegetable crops. Furthermore, the study showed that mowing was advisable to suppress the growth of the subclover to reduce early competition. It was necessary to strip-till, chemically kill or suppress vegetative growth of the *T. subterraneum.* Theunissen (1997) found that in general clovers are suitable for weed suppression but that low growing clovers, like most cultivars of *T. subterraneum,* are less capable of suppressing weeds because of their low competitive abilities. This attribute makes them suitable, however, for intercropping with slow growing vegetables to control pests (Theunissen, 1997). He suggests mowing the undersow to prevent tall growing weeds from flowering and setting seed. The contradicting results of the two studies are noticed by Theunissen but not discussed. As the exact circumstances of the trials executed by Theunissen are not described, it is impossible to develop a hypothesis about the reasons for the differing results. Besides the choice for a particular secondary crop, the optimal seeding date for sufficient weed reduction conflicts with the aim to avoid yield losses due to competition by the living mulch. In the experiment of Theunissen & Schelling (1996) clover was broadcasted 5-9 weeks before the leek was planted. Significant yield losses were recorded. In Switzerland undersowing experiments were executed to investigate the effect of sowing *L. perenne* and *Trifolium repens* at different points in time during the growing period of leek as well (Müller-Schärer, 1992 a,b,c; Müller-Schärer & Baumann, 1993 a,b). It was concluded that sowing *L. perenne* and *T. repens* five weeks after the leek was planted did not result in a significant yield reduction. Sowing the undersow crop one or three weeks after the leek planting resulted in losses of harvestable weight of 53 and 37 %, respectively. The experiments in Switzerland (Müller-Schärer, 1992 a,b,c; Müller-Schärer & Baumann, 1993 a,b) showed that even when ryegrass and clover were sown five weeks after planting of leek the germination of weeds could still be suppressed sufficiently. In this last treatment, weeds were controlled mechanically three times before the secondary crop was sown.

1.2.3 Effects of intercropping on nitrogen use efficiency

From the experiments described in paragraphs 1.2.1 and 1.2.2 it can be concluded that several species have potential for pest and/or weed suppression in a leek intercropping system. Additional research focused on reduction of the accompanying competition effect of the secondary crop on leek. Different species and sowing

regimes of the secondary crop have been investigated. On basis of the results several management systems were developed. Further optimisation of the leek intercropping system might be possible in the field of nitrogen availability and nitrogen use efficiency. For example, according to the Handbuch Gemüse – Manuel des légumes (1999) and Binkley &Vitousek (1992) more than 100 kg N ha^{-1} can be bound from the air with the cropping of clover species for one year. Bokhorst *et al.* (1992) and Barney (1987) mention an amount of about 50 kg N ha^{-1}. How much of this nitrogen can be taken up by a subsequent or simultaneously grown crop depends on the N mineralization, especially the distribution in time and space (Smit *et al.*, 1996). Moreover, the influence of the optimisation of nitrogen use efficiency on the pest and/or weed suppressing effect of a secondary crop and the summed effect on yield of leek has to taken into consideration as well, for the design of an optimal functioning leek intercropping system.

1.3 Research questions

Entomologists investigated the supplementary value of a leek intercropping system firstly. A clear pest and yield reducing effect was found for several intercrops. To counteract the undesirable yield reducing effect of the leek intercropping system, crop scientists started experiments. From weed science it was known that for every crop, periods can be defined during which the neglect of weed control will have a yield or quality reducing effect, the so called `critical periods` (Kropff *et al.*, 1993). The 'period thresholds' in integrated weed management systems are used to predict when, rather than if weeds must be controlled.

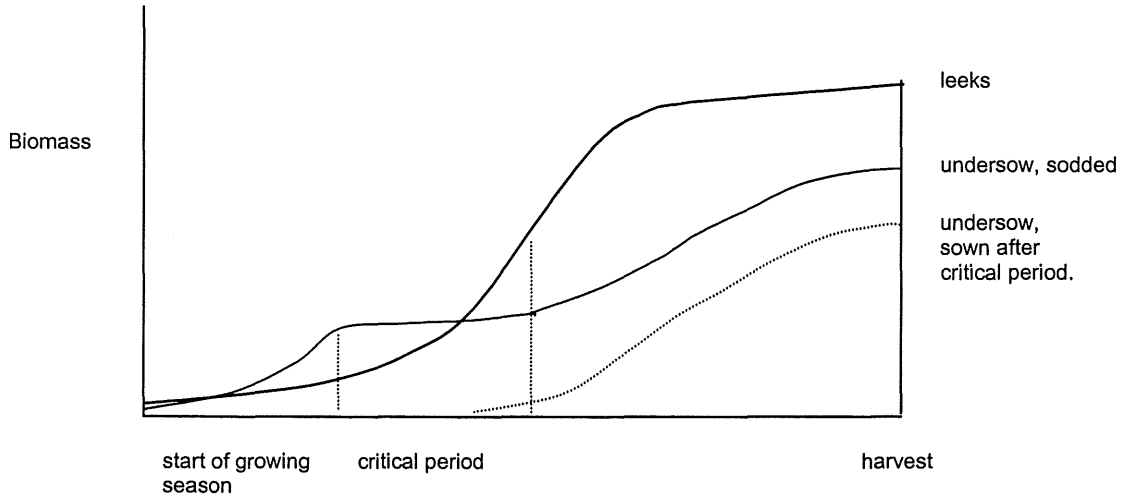


Figure 1.1: Schematic representation of different options for growth of the components in the leek intercropping system as investigated at the FAW.

At the Eidgenössische Forschungsanstalt für Obst-, Wein- und Gartenbau Wädenswil (FAW) the critical periods of several crops, among which leeks, were determined. Based on the results Müller-Schärer & Potter (1991) suggested that cover plants should generally be seeded with a delay, such that they only emerge in the beginning of the second half of the growth period of the main crop (figure 1.1 broken line). Another approach towards reduction of the competition for scarce resources during the critical period is soding the secondary crop. With soding the roots of the crop are cut off at some centimeters depth in the soil. The sods are not removed from the field so that the plants are able to root again. In this way competition is almost completely excluded during the critical period of the crop while the pest reducing effect of intercrop can be used during the complete cultivation period (figure 1.1, continuous line).

Maintenance of soil nutrient status is an important aspect of sustainability (Atkinson *et al.*, 1995). The leek intercropping system as developed at the FAW might be further improved by efficient utilisation of nitrogen fixed in the undersow. As undersow a clover species is the best choice in this case. The incorporation of a legume undersow will add extra nitrogen, bound from the air, to the soil. The clover species *Trifolium subterraneum* L. was selected for this research. An interesting question is whether it is possible to incorporate the undersow in the soil at such a point in time that the leek is capable of efficiently using the released N still in the same cropping period. For that the rate of release of N from an incorporated undersow and uptake of nitrogen by leek have to be quantified.

The understanding of an intercropping system requires a systematic and quantitative study of available experimental data. Straightforward comparison of experimental data is however not simple as many processes and their interactions, influence the results. In such a case, modelling can be a powerful tool. Simulation models can provide understanding of the system in two ways: first, through their construction, where concepts and qualitative (and sometimes conflicting) information must be integrated and questioned, and secondly, through the iterative sequence of hypothesis and experiment resulting in the continuous revision of concepts inclined in the model (Van Veen *et al.*, 1981). As this thesis project is contributing to the leek intercrop research of the FAW, which is meant to gather a better understanding of

the nitrogen dynamics in an intercropping system, simulation of different alternative scenarios is relevant. Once a model has proven to be valid under certain conditions, it can be used to compare various situations which can hardly or cannot be compared by experimental means, to reveal general phenomena of the process under different conditions, and to study functions of single factors (Yang, 1996).

Literature on mineralization and mineralization models was studied. It was decided to work with a subroutine of an existing simulation model on the nitrogen balance in a system of winter wheat and soil, called NWHEAT, to study the fate of nitrogen in soil in this research project (see chapter three). At the FAW a model has been developed to describe the aboveground light competition, caused by differences in light interception and light use efficiency, occurring in an intercropping system. To complete the circle, the step from mineralization of N to uptake by the crop has to be described, linking the modelling of nutrient supply to a simple crop growth model based on light interception and light use efficiency.

As a result of the above described literature review the following questions were defined for this research project:

What is the quantitative relation between temperature and rate of mineralization for incorporated Trifolium subterraneum?
For a prediction of the quantity and rate of N, released from an incorporated green manure, the relation between mineralization rate and temperature is important (Janssen, 1992). Therefore an experiment will be carried out to define the mineralization rate of subterranean clover at five different temperatures relevant for the growing season in Switzerland. From this experiment, the rate constants of mineralization at these specific temperatures will be calculated.

Is it possible to predict the course of nitrogen mineralization from the incorporated crop with the mineralization subroutine in the model NWHEAT as transformed by van Schöll (1995)?
The subroutine used in this thesis, is based on the organic matter dynamics part of NWHEAT, a simulation model for winter wheat growth, crop nitrogen dynamics and soil nitrogen supply by Groot (1987). This subroutine was earlier transformed by van Schöll (1995) to simulate the effect of incorporated winter wheat at low temperatures.

To assess the performance of the subroutine modelling mineralization of the undersow, simulations are made and compared to the experimental data.

Which processes should be taken into account for the development of a link between below- and aboveground interactions specifically important for the studied leek intercropping system?

To link the below- and aboveground interactions in the studied intercropping system, an additional subroutine will have to be developed. The first steps towards such a subroutine are made with an investigation of available literature to define the processes that should be included in a simple submodel, in detail comparable with NWHEAT. Moreover, some schematic calculations are made to check the breakpoints for synchronisation of nitrogen availability and demand of leek.

1.4 Outline of report

Following this introduction, the theoretical backgrounds of mineralization and denitrification will be treated in chapter two. In chapter three an overview of the approaches towards simulation of mineralization of nitrogen and the motivation for the model used in this study will be given. Chapter four deals with the experiment, that was conducted to validate the model. In chapter five, the simulation runs are presented and discussed. In chapter six the step from nitrogen mineralization to plant uptake for the leek intercropping system is shortly discussed and the synchronisation of nitrogen availability and demand of leek is examined.

Chapter 2

Theoretical backgrounds of the nitrogen cycle in the soil

2.1 Introduction

In this chapter the theoretical backgrounds are treated which are necessary to understand the concept and assumptions the used mineralization model is based on. A broad overview of the nitrogen cycle is given, mineralization and denitrification are discussed in detail because these are the processes included in the model.

2.2 Humification of organic matter

The N cycle cannot be studied without having a glance on the organic matter in the soil since the carbon compound of the organic matter is the main energy-carrier in heterotrophic processes (Alexander, 1961; Janssen, 1992; Mary & Recous, 1994; Ruiter & Faassen, 1994; van Schöll, 1995; Yang, 1996). The biological conversion of the organic matter into humus will be referred to as humification. The organic components in the soil can be distinguished into living and dead organic matter (figure 2.1). The living organic matter consists for more than half of roots, for nearly one tenth of soil fauna and for a third part of micro-organisms (Janssen, 1992). The dead organic matter in the literature is often called `soil organic matter` or `humus`. But there are researchers, for whom the concepts "organic matter" and "humus" are not synonymous. For them soil organic matter is all the dead organic material in the soil, while the term `humus` covers the components of soil organic matter which have been converted so far that the plant and animal residues they originate from cannot, visually nor chemically, be recognised anymore (Janssen, 1992). Russel (1973) describes humus as a `state of matter`, with a diffuse boundary. In this report humus is referred to as dead organic matter. Humus may be considered as an intermediate station in the cycles of some elements, especially carbon, oxygen, hydrogen, nitrogen, phosphorus and sulphur. Although Schnitzer (1977) and other research groups all over the world have done much work, the structure of humus molecules is not easy to determine as it changes constantly.

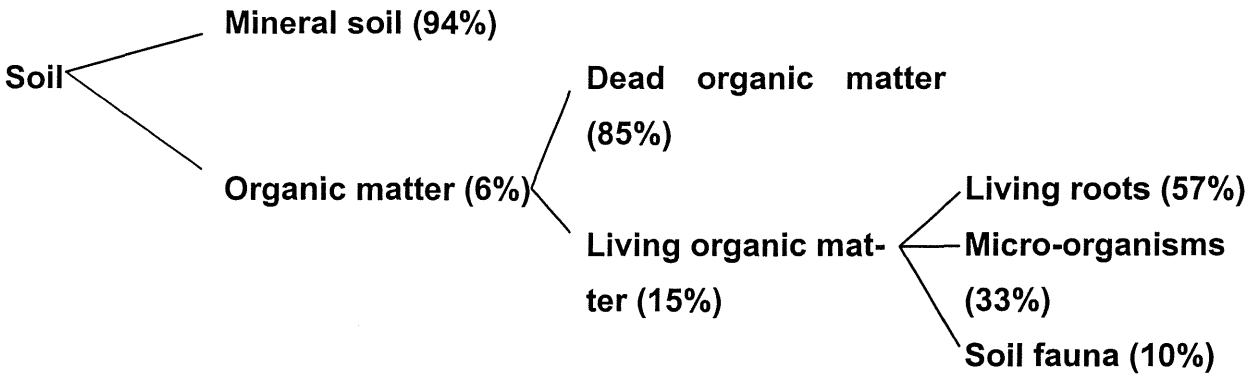


Figure 2.1: Composition of soil organic matter in mass percentages (Revised from Janssen, 1992).

More than 90% of the material that is converted into humus is of vegetable origin (Janssen, 1992; Binkley & Vitousek, 1992). Plant material constitutes a very heterogeneous source of carbonaceous compounds. The chemical composition changes during crop growth. The portion of protein decreases, while the portion of lignin increase with age. The plant material comprises a range of materials with variable degradation rates. The water soluble fraction of plant residues includes sugars, starch, organic acids and proteins. This fraction is readily humificated, followed by fats, waxes, resins and oils. Hemicellulose and cellulose humificate more slowly, followed by lignin and other phenolic compounds (Reber & Schara, 1971; Alexander, 1961. As result of the breakdown of organic material into humus, CO_2 will be released.

Soil fauna and micro flora are highly involved in this successive process, leading to the change of plant and animal materials into soil organic matter. The larger fauna functions at the beginning of the chain. Plant residues are cut into pieces and eaten by animals. Excrements of one group of animals, combined or not with mineral soil parts can become food for a next group, and pass the food channel again and again. The actual decomposition is carried out by the micro flora (Alexander, 1961; Janssen, 1992). The microbes all occupy a small or larger niche in the pathway of degradation.

The active soil flora is made up of three major groups; the bacteria, actinomycetes and fungi. Protozoa are counted as soil fauna. Algae and viruses do not contribute to the mineralization process (Alexander, 1961). The bacteria are numerous but due to their small size they make up less than half of the microbial biomass. The activity of

the bacteria depends on the environment. Actinomycetes are less dependent on environmental factors; they can stand heat and drought. The fungi make up an important part of the biomass due to their size. They are able to decompose material with a high C/N content (Alexander, 1961).

2.3 Mineralization

In this thesis mineralization means that humus is made `mineral`. Mineral is used here as a synonym for inorganic. Decomposition is referred to as the sum of humification and mineralization. Micro-organisms play an essential role in the conversion from organic compounds into inorganic molecules. The humus supplies all nutrients needed by the micro-organisms (table 2.1). The concept of `nutrient` in this context has to be interpreted more widely than `nutritional element`, because it fulfils also other functions such as being electron acceptor.

Table 2.1: Nutrients required by micro-organisms (Alexander, 1961)

Energy source	Organic compounds
	Inorganic compounds
	Sunlight
Electron acceptor	O_2
	Organic compounds
	NO_3^- , NO_2^- N_2O, SO_4^{2-}, CO_2
Carbon source	CO_2, HCO_3^-,
	Organic compounds
Minerals	N, P, K, Mg, S, Fe, Ca, Mn, Zn, Cu, Co, Mo
Growth factors	Amino acids
	Vitamins
	Others

Not all micro-organisms require these `nutrients` to an equal extent. They use what they need and leave the uninteresting parts as waste (Alexander, 1961). The most important function of the organic matter for the micro-organisms is that of provision of carbon and energy source. The micro-organisms can use the organic molecules for these purposes only after uptake into their cells. To that the large humus molecules have to be split in smaller particles such as di- and monomers. Under aerobic conditions the di- and monomers (e.g. glucose, $C_6H_{12}O_6$) will be oxidised to CO_2, H_2O and energy.

2.4 Nitrogen mineralization

N mineralization, the process by which organic N is transformed into the more mobile, inorganic N, may be considered as the driving force behind other soil N transformations (Rees, 1989). Organic N is mainly built into proteins; inorganic N can be found in the soil as ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), nitric oxide (NO), nitrous oxide (N_2O) or molecular nitrogen (N_2). The N transformations occur simultaneously, but individual steps often accomplish opposite goals (Alexander, 1961). The reactions may be viewed in terms of a cycle in which the element is shuttled back and forth at the discretion of the micro flora. Micro-organisms in the soil play a major role in the soil N cycle transforming N compounds into available and unavailable, mobile and immobile, oxidised and reduced and gaseous and solute forms (Bosatta *et al.*, 1980).

As a consequence of N mineralization, ammonium and nitrate accumulate in the soil. In the literature and also in this report the mineralised N, both ammonium and nitrate, are referred to as N_{min}. These products delineate two distinct microbiological processes: ammonification, in which ammonium is formed from organic compounds and nitrification, the oxidation of ammonium into nitrate (Alexander, 1961; Janssen, 1992). The mineralization rate is the velocity at which organic N is converted into ammonium and nitrate.

2.4.1 Ammonification

Ammonium is typically associated with a waste-product overflow in microbial metabolism. The accumulated ammonium represents the quantity of substrate nitrogen in excess of the microbial demand. Almost all bacteria, fungi and actinomycetes take part in the ammonification, but the compounds used and the rate vary with the genus and the species (Alexander, 1961).

2.4.2 Nitrification

Organic nitrogen compounds cannot be directly converted into nitrate, ammonium is the starting point of the nitrification process. Under certain conditions, two separate and distinct steps are distinguishable in nitrification;

Firstly a transformation into nitrite (NO_2^-), secondly in nitrate (NO_3^-).

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + 2\ H^+ + H_2O + 277.2\ kJ$$
$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^- + 75.6\ kJ$$

These reactions yield energy and a source of reducing power for the incorporation of carbon dioxide into the cell. For bacteria. Nitrification is usually associated with the energy-yielding reactions in the metabolism of autotrophic bacteria, although this conversion can also be brought by certain heterotrophics (Alexander, 1961).

2.4.3 Immobilisation

Immobilisation is the conversion of inorganic N into organic N. It results from the microbial assimilation of inorganic nutrients. The process is driven by the fact that micro-organisms cannot multiply unless nitrogen is incorporated into microbial protoplasm in proteins, nucleid acids and other organic complexes (Alexander, 1961). The N uptake by the plant is not considered, only the microbial part (Solomon, 1994). Microbes prefer the uptake of NH_4^+over NO_3^- to incorporate into their cell biomass (Harmsen & Van Schreven, 1955), because ammonium salts are the most readily assimilated nitrogen sources for most micro-organisms (Alexander, 1961).

The three above explained processes occur simultaneously, so that mineralization is the result of the balance between ammonification, nitrification and immobilisation. The total amount of N liberated from organic matter is `gross mineralization`; the quantity remaining after subtraction of immobilisation is referred to as `net mineralization` (Binkley & Vitousek, 1992).

2.5 Factors influencing the nitrogen mineralization

The biochemical heterogeneity of the micro flora bringing about nitrogen mineralization is a critical factor in determining the influence of environmental factors upon the transformation. Because aerobic and anaerobic, acid sensitive and acid-resistant, spore-forming and non-spore-forming micro-organisms function in the degradation pathway, at least some segment of the microbe population is active, regardless of the peculiarities of the habitat (Alexander, 1961). However, the rate is markedly affected by the environment. Soil temperature and moisture are generally assumed as the most important modifiers of the mineralization pattern (Kladivko &

Keeney, 1987; Ellert & Bettany, 1992). In the next section of this chapter the effect of soil temperature and moisture on the mineralization rate are explained as well as the influence of other relevant factors.

2.5.1 The effect of temperature on the relative mineralization rate

A change in temperature will alter the species composition of the active flora and at the same time have a direct influence upon each organism within the population. Each individual microbial species and the bio-chemical capacities of the population as a whole have temperature optima. Because the composition of the flora varies from site to site and is altered at a single site by treatment with different plant residues a single optimum for organic matter breakdown in soil cannot be given (Alexander, 1961). Still, efforts to quantify the relation between temperature and decomposition are undertaken as soil nitrogen mineralization rate is influenced profoundly by temperature within the range normally encountered under field conditions (Stanford *et al.*, 1973).. The Arrhenius function and Q_{10} coefficient are widely applied to describe the temperature dependence of soil biological processes (Ellert & Bettany, 1992). The Q_{10} coefficient is the value *k* (rate constant of mineralization) should be multiplied with, with a rise in temperature of 10°C:

$$k/k_{ref} = Q_{10}^{(IT - Trefl)/10}$$

k = rate constant of mineralization [day-1]
T = temperature [K]

Addiscott (1983) measured mineralization of soil organic nitrogen in laboratory incubation experiments on Rothamsted soils with contrasting histories. The temperature dependence of *k* was best expressed by an Arrhenius-type relationship with absolute temperature (T),

$$k = A*e^{-B/T}$$

$$\text{or, } \ln(k) = \ln A - (B/T)$$

k = rate constant of mineralization [day-1]; A = constant [day-1]; B = E_{act}/R; E_{act} = activation energy or temperature characteristic [J/mol]; R = gas constant [J/mol K]; T = temperature [K].

The Arrhenius A-coefficients are not easy to interpret, the B-coefficients, which measure the temperature sensitivity, are more important (Addiscott, 1983). Stanford *et al.* (1973) measured the mineralization of soil organic nitrogen during several consecutive incubations at 5, 15, 25 and 35°C on 11 soils. Their B-coefficient was an averaged 5294 K^{-1} for all soils as the B did not differ significantly between the soils. The B-coefficient became 6351 K^{-1} when they omitted the 5°C value, which they considered unreliable. Addiscott (1983) found an averaged B value of 6350 K^{-1} for two investigated soils, for another one they found a B-coefficient of 8313 K^{-1}. Van Schöll *et al.* (1997) found the value of 7161K^{-1}.

The results of Stanford *et al.* (1973) indicated that *k* changes approximately twofold for each 10-degree change in temperature (e.g. Q_{10} = 2), independent of soil characteristics. Kladivko & Keeney (1987) and Tyler *et al.* (1959) found confirmation for this value. The Arrhenius function reaches an inflection and approaches a horizontal asymptote, whereas the Q_{10} implies a continuously increase. Under conditions normally encountered in the field, however, the patterns specified by the Arrhenius and Q_{10} are similar, and the inflection in the Arrhenius function usually occurs at temperatures much higher than those tolerated by most biological systems (Ellert & Bettany, 1992). Ellert & Bettany (1992) found most clearly for undisturbed forest soils, but also for disturbed agricultural soils, that both the Q_{10} and E_{act} decreased with increasing temperatures. This indicates that the temperature sensitivity of the mineralization process declines.

Although the Arrhenius function oversimplifies the numerous control mechanisms that interact to determine the temperature responses of complex physiological processes, Ellert & Bettany (1992) concluded that in recently disturbed soils, mineralization conforms to the Arrhenius function because *k* increased exponentially in the range from 5 to 30°C. Van Schöll (1995) analysed seven articles for the relations found for temperature effect. Also this author found that the Arrhenius function is the most appropriate equation to describe the temperature effect on mineralization rate.

2.5.2 The effect of soil moisture and oxygen supply on the relative mineralization rate

The importance of soil water content as a significant factor in the decomposition of organic matter has long been recognised (Alexander, 1961). Actinomycetes and many of the fungi are capable of surviving at very low water potentials (pF 4.6 to 5) but may be metabolically inactive. The activity of bacteria appears to be limited, mainly by the decreasing proportion of water filled pores results in reduced mobility of bacterial cells and limited availability of substrates as caused by reduced diffusion of solutes (Alexander, 1961). It is however difficult to separate the direct effects of the soil water potential from the secondary effects (aeration, diffusion). In this section the soil water tension is converted into pF value [log mbar] for all reviewed experiments. The pF curve describes the relation between soil water content and the logarithm of soil water tension in mbar. A pF value of 2 is assumed to represent field capacity, and at pF 4.2 the permanent wilting point is reached (Wijnja & van Beusichem, 1998).

Stanford & Epstein (1974) analysed nine soils from the United States for relationships between soil N mineralization, soil water content and matrix suction. In the range from optimum soil water content (around pF 2) to pF 4.2 a general expression was derived relating *y*, the nitrogen mineralised, expressed as a proportion of the maximum amount that occurred at optimum moisture conditions, to *x*, the soil water content expressed as a proportion of the optimum soil water content. They obtained the equation: $y = -3.9 + 1.02\ x$, but since the *y* intercept and slope were not significantly different from 0 and 1 respectively, they assumed that $y = x$. Thus only field capacity and nitrogen mineralization rate at field capacity were required to allow calculation of nitrogen mineralised at other soil moisture contents in the range from pF 2 to 4.2. Similar to the findings of Stanford & Epstein (1974), Myers *et al.* (1982) also found that the relationship between nitrogen mineralization and moisture content for various soils was linear over much of the moisture range. But optimum moisture for net nitrogen mineralization corresponded to a pF value of 2 to 2.5. No net nitrogen mineralization occurred close to pF 3.6 (Myers *et al.*, 1982) as microbial actitivity is low due to lack of water (Linn & Doran, 1984). The obtained relationships varied considerably between soils in slope and *y* intercept. Since the

soils used by Stanford & Epstein (1974) were generally light-textured, it was felt that the range of textures investigated by Myers *et al.* (1982) may have contributed to the different results. Myers *et al.* found that the equations in general had negative *y* intercepts. Thus, it appeared that the original negative *y* intercepts obtained by Stanford & Epstein (1974) were real. They concluded that Stanford & Epstein`s (1974) technique for relating net nitrogen mineralization to moisture content is not suitable for generalised use. Myers *et al.* (1982) proposed the polynomial expression $y = bx + (1 - b)x^2$ (where *y* is net nitrogen mineralised expressed as a proportion of the maximum rate; *x* is normalised moisture content) which relates net nitrogen mineralization to available moisture between pF 3.6 and 2.5, which fits many diverse soils. The *b* was added because Myers *et al.* (1982) found a curvilinear relationship for a small group of soils which might have had a high colloid content in common. The variable *b* is a measure of the degree of curvature. In most cases the value of this parameter is one. The authors pointed out that there is an urgent need to define and quantify the soil characteristic(s) that govern the degree of curvature in the proposed equation.

Linn & Doran (1984) found the rate of nitrification to increase linearly until a certain optimum soil moisture content and a decrease thereafter. The optimum soil moisture content these workers defined, cannot be compared with those of others as the percentage of soil pore space filled with water was used as unit. Van Schöll (1995) reviewed seven experiments executed to define the effect of soil moisture content on mineralization. All workers found a linear relation between soil moisture content and mineralization rate for a certain defined pF range. A comparison of the results of the experiments shows that the slope of the line differed due to the fact that the workers did not agree on the soil moisture tension (ranging from pF 2 to 2.7) for optimum mineralization and the point (values ranging from pF 4.2 to 4.9) mineralization ceases.

Kowalenko & Cameron (1976) and Cassman & Munns (1980) found a significant moisture * temperature interaction in laboratory incubation experiments. Also Kladivko & Keeney (1987) studied the hypothesis that water and temperature interact to influence the rate of soil N mineralization. But they criticised the conclusions of former workers because the water-temperature interaction had been described

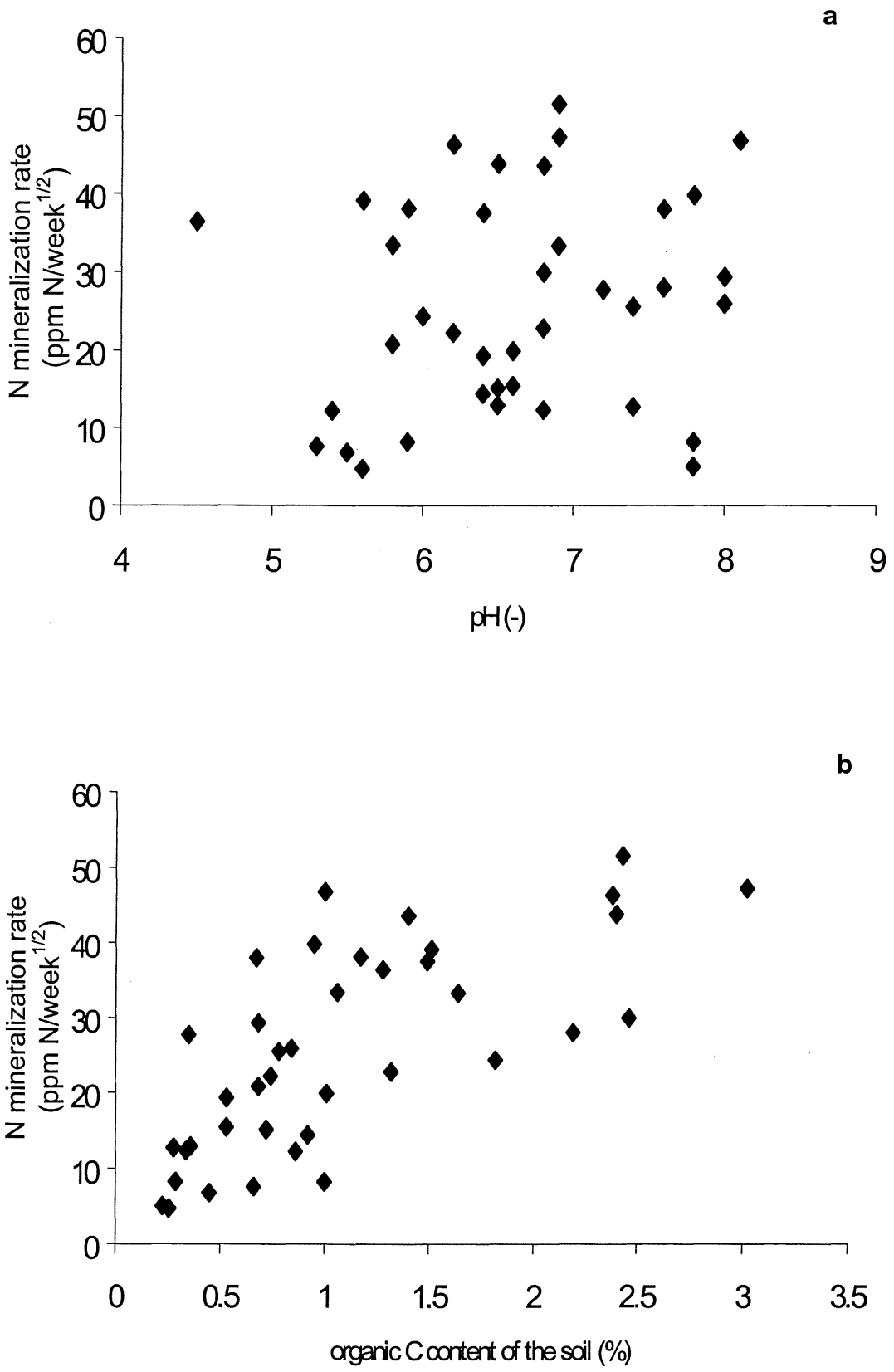


Figure 2.2: Relation between mineralization rate and pH (a) and mineralization rate and organic matter content (b) at week 16 of the incubation experiment executed by Stanford and Smith (1972).

statistically as a linear or quadratic function, while it is known that temperature affects activity exponentially. Kladivko & Keeney (1987) investigated a more mechanistic approach of a possible hydro-thermal factor; if soil water and temperature act independently on microbial activity, the typical temperature relationship of $Q_{10} \approx 2$ should remain constant at all water contents, and the typical linear relationship of microbial activity with water content should be the same at all temperatures. An interaction would be indicated, if the Q_{10} value changed with water content. In the experiments the Q_{10} of N mineralization was approximately 2 for all tested water potentials. No evidence of a hydrothermal factor for N was found below 30°C (Kladivko & Keeney, 1987). Above this temperature the results were not so clear.

2.5.3 The effect of C/N ratio on the relative mineralization rate

The ratio between available C and available N is the main factor determining whether nitrogen mineralization or immobilisation will dominate during decomposition of a substrate (Solomon, 1994; Janssen, 1992; Alexander, 1961; Paul, 1984). The critical level corresponds to a C/N ratio of 20. Wider ratios favour immobilisation; narrower ratios mineralization.

2.5.4 The effect of high pH values on the relative mineralization rate

The soil pH affects the decomposition rate of organic matter (Laura, 1976, Janssen, 1992) because it influences the activity of enzymes, the composition of the micro population and the size and shape of organic particles. The prevailing view is that the organic matter decomposition and N mineralization occur less rapidly in acid soils than in neutral or alkali soils (Alexander, 1961; Laura, 1976; Janssen, 1992). The principal experimental basis for this view is that the amount of mineralized N increases when lime is added to acid soils in short-term experiments (Laura, 1976; pers.com. Braakhekke). From own observations we know that the fallen leaves in woods on lime soils (e.g. Limburg, the Netherlands) decompose much faster than the litter in woods on acid soils (e.g. the Veluwe, the Netherlands). Several investigators (Stanford & Smith, 1972; Laura, 1976; Janssen, 1992; Pathak & Rao, 1998) have studied the effects of alkali soils on the N mineralization. The mechanism of the pH effect has not yet been specified. Even the effects of high pH values in a range from 8 to 10 are still subject of much controversy. Pathak & Rao (1998) reported an increase in NO_3^-, but overall decrease in net N mineralization with increasing pH. Laura (1976) found an increase of net mineralization and only at extreme conditions

(pH > 10.2) an inhibition of the process of nitrification. Stanford & Smith (1972) considered pH to be the most influential factor for N mineralization in their laboratory study for the determination of the potential mineralization of 39 soils. But analysis of their data with linear regression showed (figure 2.2a) that no trend existed between pH and net mineralization ($p = 0.3$). However the organic matter content of the soil seemed to have a relation with net mineralization ($p = 0.0000004$) (figure 2.2b). The figures show the mineralization rates at week 16; for week 4-8-12-30 a similar pattern was found. One of the arguments generally put forward to explain the effect caused by a high pH is that increased alkalinity increases availability of organic matter to microbes and thus accelerates its decomposition (Laura, 1976). The reasons for the higher availability are dissolution, dispersion and chemical hydrolysis of soil organic matter. The enzymes controlling the mineralization process are active within a wide pH range (Janssen, 1992). The optimum for the mineralization process lies at pH 7-8 (Janssen, 1992, Hassink, pers.com).

2.5.5 Other effects

Although all the below mentioned effects were not investigated in this study, they will help the reader to obtain a complete overview of the factors influencing the mineralization process.

Paul (1984) states that a reasonable amount of information is available on the abiotic controls such as above described, but that not enough knowledge is available about soil spatial compartmentalisation to write it off as important factor. The type, content and extent of aggregates of clay continues to be recognised as a major controlling factor affecting soil organic matter dynamics (Tisdall & Oades, 1982). From several experiments it was concluded that bacteria and enzymes can be adsorbed to the clay. It protects them from decomposition, but also limits their activity (Marshman & Marshall, 1981; Janssen, 1992; Breland, 1994b).

Micro-organisms take up the required nutrients for their growth from the soil solution or from the substrate decomposed. The growth of micro-organisms can be slacked by the shortage of the required nutrients (Janssen, 1992). In other words, a shortage of another nutrient than nitrogen might decrease the N mineralization rate.

Studies have shown that soluble C compounds lost from roots can lead to larger and more active microbial populations in the rhizosphere compared with the bulk of the soil. This suggests that carbon lost from a plant root could enhance, via microbial activity, the availability of inorganic N close to the root. After a theoretical analysis Robinson, Griffiths *et al.* (1989) expect any influence of a root on N mineralization to be highly localised and not to extend far into the soil. Griffiths & Robinson (1992) describe two models simulating root-induced nitrogen mineralization. The conclusion is that the majority of N taken up by the plant comes from bulk soil processes and that it is unlikely that altering exudation patterns will influence N supply to the plant to any large extent.

2.5.6 Consequences for research project

Temperature and moisture are two of the most important environmental conditions determining how rapidly organic materials are metabolised. Underlying processes are well described. General consensus was found for the linear relation between soil moisture content and relative mineralization rate. The temperature response of the mineralization rate constant can be adequately described by an Arrhenius equation in the range relevant for this research project. Therefore it seems possible to quantify the relationship between mineralization rate and temperature, one of the questions posed in chapter one. A possible interactive effect of temperature and soil water content was not found for the temperature range normally encountered in the soil. The C/N ratio of *Trifolium subterraneum,* the plant material this thesis focuses on, was found to be approximately 13. From the literature it can be concluded that no immobilisation will occur with the input of organic matter with such a low C/N ratio (Solomon, 1994; Janssen, 1992; Alexander, 1961; Paul, 1984). Apart from the C/N ratio, the chemical composition of the plant material plays a major role in decomposition. As a result, the mineralization rate, which was deduced from the experiment, applies only to the organic substrate examined, *Trifolium subterraneum.* Much controversy exists about the effect of pH on mineralization rate. No clear relation has been defined yet. The pH of the soil used in the experiment had an acidity of 7.8 – 8.0. The variations depend on the time of the year in which the sample is analysed. From the literature could be derived that the mineralization was optimal at this pH, when other conditions, e.g. soil moisture content, were chosen well.

2.6 Denitrification

Denitrification is the anaerobic process in which nitrate is biologically reduced to the gaseous N compounds N_2O and N_2 (Burford & Bremner, 1975; Alexander, 1961). The capacity for denitrification is limited to certain bacteria. Fungi and actinomycetes have not been associated with denitrification (Alexander, 1961). The most abundant denitrifiers are heterotrophs, which require sources of electrons or reducing equivalents contained in soil organic matter, plant residues and root exudates. Soil micro-organisms in general are mostly limited by organic carbon and energy.

2.7 Factors affecting the denitrification process

2.7.1 Moisture content and aeration of the soil

The denitrifying bacteria responsible for reduction of nitrate into gaseous forms of nitrogen are facultative anaerobes that have the ability to use both oxygen and nitrate as electron donors (Burford & Bremner, 1975). Aeration affects the transformation in two apparently contrasting ways. On the one hand denitrification proceeds only when the O_2 supply is insufficient to satisfy the microbial demand (Knowles, 1981); at the same time O_2 is necessary for the formation of nitrite and nitrate, which are essential for denitrification (Velthof, 1997). The existence of anaerobic micro-environments in soil depends greatly on the moisture content of the soil since this is a major factor in limiting the within- and between-aggregate porosity (Knowles, 1981). Aerobic microbial activity increases with soil water content until a point is reached where water displaces air and restricts the diffusion and availability of oxygen (Linn & Doran, 1984). Bremner and Shaw (1958) studied the factors affecting denitrification in soil by determining loss of nitrogen from soil under various conditions by total-N analysis. In agreement with Knowles (1981) and Alexander (1961) they found that the rate of denitrification of nitrate in soil is profoundly affected by the water content of the soil. Even when other conditions (pH, temperature etc.) are very favourable for denitrification, little loss of nitrogen occurs if the moisture content is less than 60% of the water holding capacity of the soil. In addition, Velthof (1997) noticed that denitrification in peat soils water-logged during 3 to 5 months, was hampered as well due to a decreased rate of mineralization of organic carbon and nitrification.

2.7.2 Organic carbon content

Denitrification of nitrate by heterotrophic organisms can not occur unless the substrate contains some organic compound which can support growth of the organisms and act as electron acceptor. Consequently the denitrification is much faster in soils with a high than in a soil with a low organic matter content (Alexander, 1961; Velthof, 1997). Burford & Bremner (1975) investigated the relationship between the denitrification capacities of 17 surface soils and the amounts of total organic carbon, mineralizable carbon, and water-soluble organic carbon in these soils. The results confirmed that denitrification in soils under anaerobic conditions is controlled largely by the supply of readily decomposable organic matter and that analysis of soils for mineralizable carbon or water-soluble organic carbon provides a good index of their capacity for denitrification. The dependence on organic carbon results in higher denitrification potentials found in surface soils (Knowles, 1981; Velthof, 1997). In experiments on grassland executed by Velthof (1997) the relation between organic C mineralization potential and denitrification potential was not the same for all soils, suggesting that other factors than organic carbon influenced denitrification. The researcher hypothesised that differences in the presence and activity of bacterial populations may have contributed to the differences between the soils.

An experiment in which NO_3^- was added to soil samples at various rates and incubated for 24 hours under waterlogged conditions, seemed to show that the nitrate loss rates were dependent on NO_3^- concentrations in case of concentrations below 100 ppm. At higher concentrations the rates appeared to be independent of NO_3^- concentration (Kohl *et al.*, 1976). It became not clear from the literature whether this last result would have been found if more organic carbon would have been added to the soil. In other words, whether the soil was saturated with NO_3^- or that organic carbon was the limiting factor. But in another research mineral N content was found to be a major factor controlling the N_2O emission from grassland besides moisture content. Denitrification is considered to be a major source of N_2O (Velthof, 1997).

The rate of denitrification was also found to vary with the incorporated materials; depending on their resistance to decomposition by soil micro-organisms the rate of

denitrification is highest with cellulose and least rapid with lignin and sawdust, which are highly resistant materials to decomposition (Bremner & Shaw, 1958).

2.7.3 Acidity

Besides moisture and organic matter content of the soil, Bremner & Shaw (1958) investigated two other factors influencing denitrification. It was found that denitrification was considerably slower in a soil of pH 5.8 than in soils with higher pH values, independent of the moisture content. Loss of nitrogen from the soils with pH values higher than 5.8 was very rapid during the first few days of incubation and thereafter very slow. No explanation was given for this observation.

2.7.4 Temperature

Bremner & Shaw (1958) also found that the rate of denitrification increases rapidly with a rise in temperature from 2°C to 25°C. Increase in temperature above 25°C did not have a significant effect. At 70°C denitrification was inhibited.

2.7.5 Soil structure

Denitrification is also favoured by mechanical aggregates greater than 4 mm in diameter, because it is more difficult for O_2 to diffuse through these large particles to the centre where microbial reduction takes place (Alexander, 1961).

2.7.6 Consequences for research project

The literature suggests that high pH, high temperatures and a high mineral N and organic matter content of the soil favour the occurrence of denitrification. For this thesis the high temperature circumstance applied only to the samples incubated at 25°C. All samples have a high pH and a high organic matter content. But without the incidence of anaerobic conditions, no denitrification takes place. Anaerobic conditions are mainly due to a high degree of water saturation (>60%) although mechanical aggregates greater than 4 mm make a contribution as well. To conclude, if anaerobiosis occurs in the samples, it is most likely that denitrification takes place, as all other circumstances in the experiment are favourable for denitrification.

2.8 Estimation of relative mineralization rate

To be able to estimate the relative mineralization rate of *Trifolium subterraneum*, an experiment has to be conducted which allows measurements of net mineralization in

time. Two types of methods are used to assess net mineralization in soils: methods defining `potentially mineralizable N` and methods providing `N availability indexes` (Binkley & Vitousek, 1992).

Potentially mineralizable N

Stanford & Smith (1972) developed an approach for determining potential N availability in soil. These authors used a long-incubation technique at a rather high temperature (35°C), with periodic leaching of the soil to remove N mineralized. The N mineralization kinetics obtained by this technique show that the mineralization rates decrease smoothly and continuously with time. The kinetics fit relatively well with a single exponential model, given by the equation:

$$N = N_0 (1 - e^{-kt})$$

In the formula above N = the amount of mineralized nitrogen, N_0 = the potentially mineralised nitrogen, k = a mineralization constant and t = time. The potential value can be calculated using the formula and the incubation data obtained according to the method of Stanford & Smith (1972). The concept of N_0 has been criticised quite severely. Even if there is a very good fit of the model to the data, the N_0 value cannot be determined accurately because the optimised parameters N_0 and k depend strongly on the incubation time (Mary & Recous, 1994). The leaching test may be useful for estimating the potential mineralization capacity of a soil (Mary & Recous, 1994) but not for estimating the net mineralization. The reason for this is that leaching periodically removes all mineralized N, while the process of net mineralization actually depends on the available N concentration in the soil system (Thorup-Kristensen, 1994). As net mineralization is the object of this research, the leaching method will not be applied.

N availability indexes

The N availability indexes are used as reference values of soil net mineralization capacities. There are two methods of defining these indexes; Incubation experiments followed either by soil analysis to determine soil inorganic nitrogen content or by analysis of the N uptake by test plants (Thorup-Kristensen, 1994). The soil analysis methods vary in the nature of the extractant used (neutral, acidic or alkaline), and the temperature and time of extraction (Mary & Recous, 1994). The biological methods

are incubation tests with plants growing on the incubated soil. Differences between the biological methods consist of aerobic or anaerobic conditions in the soil and with or without leaching (Mary & Recous, 1994).

The major disadvantage of chemical analysis as presented by Thorup-Kristensen (1994) is that it is often questioned whether the recorded NO_3^- and NH_4^+ amounts are really due to the mineralization process. As early as 1955, Harmsen & van Schreven pointed out that the incubation tests with plants do not necessarily reflect the actual N mineralization capacity of the soil neither. The disadvantage put forward by the biological method is that the assumption that the rate of plant uptake equals the rate of N availability is not always valid (Thorup-Kristensen, 1994). For example, at start of the experiment plants can be too small to take up all available N. Precise control of soil temperature and humidity are hard to achieve. Furthermore reliable measurements shortly after incubation are difficult to obtain, because the plant has not had enough time to react on the different N availability's due to the different treatments. Fox & Piekielek (1984) found a very poor correlation between N mineralised in incubation tests analysed with the biological method and field-measured N availability. More generally, the major criticism of most studies on indexes of N availability is that they have not been properly checked (Mary & Recous, 1994). If the index value is a measure of the nitrogen mineralization capacity, it must be correlated with net N mineralization obtained under field conditions. Very few studies have tried to quantify net mineralization *in situ* (Mary & Recous, 1994).

2.8.1 Concluding remarks

After consideration of the above mentioned arguments and practical limitations it was decided that an incubation experiment would serve the aim of the experiment best. For the examination of the soil we chose for chemical analysis. The reason for this was that we countered lack of time to execute a test according to the biological method and that such a test did not prove to yield better results than chemical soil analysis (Mary & Recous, 1994; Binkley & Vitousek, 1992).

Chapter 3

The simulation of mineralization

3.1 The requirements for the mineralization model

The simulation of mineralization is a small component of the model of the overall intercropping system. Final objective of the research on the intercrop model at the FAW is to provide a means for analysis on a higher integration level, namely in the specific management system. Reality is complex and scientific explanation should be based as far as possible on the chemistry and physics of the plant and its environment (van Keulen & Seligman, 1987). Whereas this approach is the essence of process models, it leads to a false realism in whole system crop simulation models because of the inevitably arbitrary mixture of empirical functions and detailed descriptions of selected processes that have been more generally studied (van Keulen & Seligman, 1987). Taking this reasoning into account, for this thesis, the most important outcome of the mineralization model ought to be a good prediction of the rate of mineralization. The output of the subroutine, not the description of the process of mineralization itself, will influence the functioning of the complete intercropping system model.

In the last decade a considerable number of mineralization models has been developed. Therefore we decided to select a model suiting our research best and to evaluate it for the specific circumstances dealt with in this thesis. A number of requirements and practical limitations played a role in the search for the most suitable model; It had to be possible to calculate the release of N from the incorporated clover making use of easy obtainable information. For example, the exact chemical composition of the subterranean clover has not been retrieved, only the C and N content were determined. The influence of texture, temperature and soil moisture content on the decomposition process, as well as denitrification, had to be accounted for in the model, according to conclusions of chapter two.

3.2 Overview of mineralization models

Literature review revealed a large number of diverse mathematical descriptions of subprocesses involved in mineralization. A selected number more recently described models were compared. To obtain an overview of the different approaches and

concepts, it was attempted to classify the models in a useful way for this work. A first, broad distinction was made into two classes; organism-oriented or food web models and process-oriented, also called organic matter models (figure 3.1a&b).

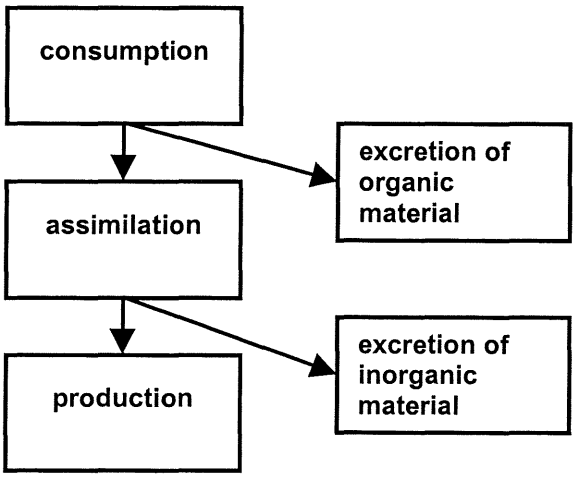


Figure 3.1a: Scheme of a organism-oriented model relating consumption, excretion (of organic material) and mineralization (revised from de Ruiter & van Faassen, 1994).

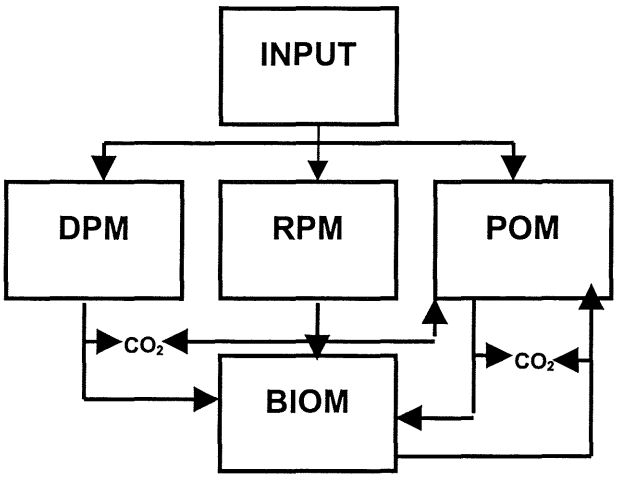


Figure 3.1b:Diagram of a process oriented model (revised from de Ruiter & van Faassen). DPM = decomposable plant material; RPM = resistant, slowly decomposable plant material; POM = physically protected organic material; BIOM =

3.2.1 Organism-oriented models

In this kind of models the energy and matter fluxes are simulated in relation to the abundance and activity of the soil organisms constituting the soil food web (McGill, 1996). A food web can be described in terms of material flows among trophic groups, the influence of a particular group of organisms on the functioning of other groups and that of the system as a whole (de Ruiter *et al.*, 1994). The ecosystems approach is central to the understanding of the energy flow and nutrient cycling in soil, because the dynamics in energy and nutrient flows are direct consequences of the dynamics of organisms, which in turn depend on abiotic factors (de Ruiter *et al.*, 1994). Consumption rates among the groups of organisms are calculated based on biomass and turnover rates. Subsequently, N mineralization rates are derived from consumption rates using information on energy conversion efficiencies and C/N ratios of the organisms (Ruiter & Faassen, 1994).

Key point of the organism-oriented models is the contribution of organisms involved. The species are assembled into functional groups: roughly homogeneous in habitat, food, feeding mode and ecophysiology (de Ruiter *et al.*, 1994). The subdivision is largely determined by taxonomic and ecological knowledge of the micro-organisms,

which in turn is largely determined by the techniques to isolate and quantify organisms from soil (Brussaard, 1991). Mostly these techniques do not result in very detailed information. Therefore the decomposition is often studied in terms of sum parameters such as microbial biomass, soil respiration and enzyme activity (Brussaard, 1991). The drawback of this kind of models is that many data are needed to run the model: e.g. specific death rates, assimilation and production activities, C/N ratio's for the functional groups in the different food webs as well as biomass estimates (Ruiter *et al.*, 1993a). Besides, the food web model, requiring data on the dynamics of the soil biota, is only able to calculate N mineralization rates during the period of observation, and has therefore primarily an explanatory value (Ruiter & Faassen, 1994).

3.2.2 Process-oriented models

The second category models focuses on the processes controlling energy and matter transformations. McGill (1996) proposed a scheme for classifying information on process-oriented models. Defined categories, useful for the selection of the most appropriate model for this study and for the evaluation of the performance of the selected model later on, are discussed.

Regulation by soil properties

Abiotic soil properties regulate biotic behaviour. Inclusion of soil properties might increase the generality of a model by accommodating regional or site-specific soil properties as it implies a more mechanistic approach of the process of mineralization. Two categories were recognised by McGill (1996): none or soil texture (clay or sand content were used as surrogates for texture). Soil texture was incorporated both to protect or slow down decomposition of soil organic components and to regulate the partitioning of C among pools. Soil texture in this context is not related to moisture status of the soil.

Fractionation of organic matter

Most workers distinguish at least a green manure pool and another pool for the microbial biomass. But Johnson *et al.* (1987) argue that the model can be simplified by equating the C/N ratio of the microbial biomass and humus; as a result no separate microbial biomass pool was defined in their model. Reliable methods to partition the fresh organic matter among different pools in a biologically meaningful

way are not available yet (van Schöll *et al.*, 1997). Still the organic matter is divided into different main pools for simulation, each with a specific quality as substrate for the soil biota (Ruiter & Faassen, 1994; Ruiter *et al.*, 1993a). The criteria dividing the green manure in several pools resulted in 4 categories. In the *Kinetic* category components are distinguished just by their turnover rate. For the pools different rate constants are defined on the base of empirical evidence. No additional measurements are done. For example, active organic matter (turnover time 10-50 year) and old organic matter (turnover time, 500-1000 year) are distinguished (Parton *et al.*, 1987). Alternatively the incorporated crop residues are simply separated into decomposable, structural and recalcitrant components. In the *C/N dependent* category pools are made upon the basis of the C/N ratio of the different parts of the plant material. Another category is called *Functional*. Distinction has been made on the base of measurements of not only C/N content, but also lignin and other phenolic compounds. McGill (1996) used the name *Functional* because the biochemical composition is linked with the biological function of the material. To avoid the partitioning of the green matter based just on theoretical grounds, another approach was developed. In this approach the heterogeneity of organic matter is assumed to be a continuously varying quality variable (QV) (Molina *et al.*, 1983; Janssen, 1992). The QV determines the efficiency and rate of substrate utilisation. The QV decreases in time as humification proceeds. The advantage of this approach is that the QV explicitly refers to the continuous spectrum of changing soil organic matter composition rather than to discrete pools, which do not exist in reality. This approach is called *cohort* by McGill (1996).

One other aspect, not evaluated by McGill is important for our choice for a certain kind of model. The process of denitrification plays a relevant role in the nitrogen balance according to chapter two. Therefore an extra column in table 3.1 was added to make note of the existence of a denitrification submodel.

De Willigen and Neeteson (1985) compared the accomplishments of six simulation models on the soil nitrogen cycle, run with the same data set. All six models calculated a correct course of the nitrate content in the upper 60 cm, in the unsaturated zone of the soil profile. The models had in common that transport of water and solutes is essentially in vertical direction.

Table 3.1. Characteristics of 11 mineralization models. For explanation of terms see paragraph 3.2.2.

Model	Regulation of soil processes	Fractionation of organic material	Denitrification
CENTURY (Parton *et al.*, 1987)	Soil texture	Functional	No
DAISY (Hansen *et al.*, 1990)	Soil texture	Functional & Kinetic	No
NCSOIL (Molina, 1996)	None	C/N & Cohort	No
VVV (Verberne *et al.*, 1990)	Soil texture	Functional	No
Veen & Frissel (s.a.)	Soil texture	Functional	Yes
Groot (1987)	None	C/N	No
Van Schöll *et al.* (1997)	None	C/N	Yes
Kersebaum & Richter (1991)	None	Kinetic	No
Johnsson *et al.*(1987)	None	Kinetic	Yes
Van Keulen and Seligman (1987)	None	Functional	No
Habets and Oomen (1994)	Soil texture	Cohort	No

Soil profile is divided into layers, and each layer is assumed to be uniform throughout. Ammonification and nitrification are generally modelled in one step. Denitrification is ignored. Immobilisation (if modelled) is dependent on the C/N ratio of the microbial biomass and the added organic material. The relatively simple models yielded better results than the models requiring many input data; the latter ones calculated a much too high mean content of mineral N. From the field data could be derived that denitrification had taken place during a certain period of time. None of the models was able to calculate this process correctly, as all of them lacked a denitrification component. De Willigen (1991) compared fourteen simulation models. The mineralization part of these models can be labelled as descriptive, as none of them explicitly takes account of the underlying mechanisms of the process involved in the turnover of organic matter, and regarding the fact that they were developed for a practical purpose rather than as research tool. The models were run and compared to a data set. All models were found to give satisfactory results. Total deviation between the simulations and observations were mostly within a range of 20 kg N/ha, which can be considered as the mean confidence level of the nitrogen estimation within the field (Kersebaum & Richter, 1991). Models treating the organic N to consist of three or more pools did not yield a better result than models that treat organic N as a homogeneous mass. Important conclusions from the two comparisons which apply to this research are that a simple model does at least not simulate mineralization worse than a complex model and that the process of denitrification cannot be neglected in the development of a mineralization model.

3.3 Selection of model

The importance of mechanistic models as a very valuable research tool rather than as predicting devices is stressed by their authors several times (Veen *et al.*, 1981, Verberne *et al.*, 1990). As the underlying mechanisms, and not only observations are modelled, the range of conditions that can be examined is wide. But the models require many site-specific input parameters that cannot easily be obtained by simple field measurements (Groot, 1987; Veen *et al.*, 1981). This implies that this type of models will not be very suitable for the goal of this thesis, prediction of the timing and rate of nitrogen mineralization with limited data input. A simple model is preferred in this situation. As shown by de Willigen and Neeteson (1985) simple models give an acceptable prediction of the nitrogen course.

In 1995, Laura van Schöll, at that time a student of the department Theoretical Production Ecology (TPE) studied the mineralization of nitrogen from catch crop material after incorporation in the soil. Her final aim was to predict the amount of nitrogen released to the soil solution in the form of NH_4^+ and NO_3^-. She constructed a descriptive model for the mineralization of incorporated plant material based on the subroutine for organic matter dynamics in the model NWHEAT by Groot (1987). The model performance was evaluated on basis of data from laboratory and field experiments. Mineralization was described reasonable. The subroutine of Laura van Schöll (1995), in the next chapters referred to as MINMOD, suits the requirements as described in the first paragraph of this chapter to a large extent; it is simple, the model just needs a few, simple obtainable inputs, the influence of moisture and temperature on the mineralization rate are taken into account and a denitrification subroutine, although very basic, is included. The influence of texture is neglected and a description of the vertical course of water and solutes as found by de Willigen and Neeteson (1985) in all reviewed models is lacking. These factors might show to be of relevance in the field sitation (see section 2.5.5). Moreover, the latter description could be of use while linking nitrogen mineralization and availability for the crop. Another advantage of MINMOD was that it is written in the simulation language FST, which is available at the department TPE and the experimental station FAW. Therefore, in spite of the negative points, it was felt that MINMOD was the most suitable model to use to simulate the rate of N mineralization of incorporated subterranean clover.

3.4 Concepts of MINMOD

3.4.1 Introduction

The mineralization model is written in the simulation language FST (FST= Fortran Simulation Translator) (Van Kraalingen *et al.*, 1994) The FST model is translated into a structured Fortran subroutine with data files containing timer variables, model parameters and rerun specifications. The MINMOD model consists of an initial and a dynamic part. In the initial part, all variables, parameters and constants are declared and (initial) values are given. In the dynamic part the integration of the state variables over time takes place and two subroutines are called upon. In the two subroutines all rate values are calculated. The first subroutine (MINR) is a modified version of the organic dynamics part of the model NWHEAT by Groot (1987). The second subroutine (DENITR) contains a very simple denitrification description.

3.4.2 Approach of MINMOD

Calculation of the decomposition and mineralization rate

The rate of substrate decomposition (dC/dt) is calculated using first order kinetics; the decomposition rate is proportional to the amount of substrate (C).

$$dC/dt = k_{ref} * f_{T,\theta} * C$$

where k is the rate constant at a reference temperature (d^{-1}) and $f_{T,\theta}$ are the correction functions for temperature and moisture. Note that k as used in the literature (Stanford *et al.*, 1973; Addiscot, 1983; Stanford & Epstein, 1974; Kladivko & Keeney, 1987; Jenkinson, 1990) is built from k_{ref} and the correction functions for temperature and/or moisture.

Nitrogen fluxes are assumed to be proportional to the carbon fluxes. The rate of N release depends on the C/N ratio of the substrate.

A fraction e of the decomposed C is used for biomass growth, the remaining part (1 - e) is used for respiration. There is no flow from the decomposed C to the soil organic matter. The N required for optimal growth of biomass is calculated as: e * [(dC/dt) / (C/N_{biom}). When the C/N ratio is higher than: e * (C/N_{biom}), the decomposition

becomes N-limited and net immobilisation occurs. If the amount of soil N_{min} is not sufficient to cover the N requirement of the biomass, the decomposition rate is reduced.

Temperature and soil moisture correction function

Literature, reviewed in chapter two, revealed conflicting evidence on the existence of an interaction between temperature and moisture content. In the most recent investigation (Kladivko & Keeney, 1987) on this subject a more mechanistic approach was applied to test both own and data of other experiments. No prove of interaction was found. Therefore an interaction term will not be included and separate response functions for soil moisture and temperature will be used.

In most experiments done on the influence of temperature it was found that the relation between the temperature and the rate constant was well described by an Arrhenius equation (Stanford *et al.*, 1973; Addiscott, 1983; Nordmeyer and Richter, 1985; Kladivko and Keeney, 1987; Ellert & Bettany, 1992). So it seems most appropriate to use the Arrhenius equation for the temperature correction factor.

Most investigation on the effect of soil moisture content on the mineralization found mineralization to stop at pF values above 4.2 (wilting point). An optimum value for decomposition is found by all workers at pF 2 (field capacity), while several investigations found this optimum to be a plateau around field capacity (Stanford & Epstein, 1974). Between optimum point or plateau and wilting point the relation between moisture content and decomposition is described as linear. In the correction function used in the model it will be assumed that the mineralization is zero at pF values above 4.2, and proceeds linear with soil moisture content between pF 4.2 and pF 2.4, where the correction factor has the value 1. Between pF 2.4 and pF 2 the correction factor has value 1, while a further increase in soil moisture hampers mineralization, described by a linear decrease of the correction factor that reaches a value of 0.8 at maximum water holding capacity.

Ammonification and nitrification

Both ammonification and nitrification are modelled as separate, one step processes. The intermediate products of ammonification and nitrification are not considered. Modelling the two processes together in one step would indicate that nitrification is

assumed to proceed faster than ammonification, so that all $NH_4^+$is transformed into NO_3^- at the moment it is formed. This is not always true, especially at low temperatures (<7°C as found by experiment) ammonification proceeds faster than nitrification (Tyler *et al.*, 1959; Nemeth *et al.*, 1996), so that the amount of ammonium will build up, to be nitrified with a rise in temperature. Probably the above mentioned situation will not occur in the circumstances this research is dealing with. But as simplifying the description would not change the output of the model, we decided not to adapt it. The same counts for the influence of moisture content of the soil on the two discussed processes. This research focused on the temperature effect. Therefore the validity of the description of the effect of soil moisture content on the rates of ammonification and nitrification was not investigated.

Decomposition of the microbial biomass

The size of the microbial biomass and the microbial activity influenced by the contents of the substrate and environmental factors are not explicitly stated. But the functioning of these variables served as basis for many statements in the model. The N immobilised by the soil micro-organisms is not modelled as a constantly growing pool, as mineralization of this immobilised nitrogen is included in the model. The nitrogen will be "cycled" through the soil system, while carbon is lost as CO_2, so that the C/N ratio of the system decreases.

Denitrification

The model contains a simple denitrification component. Denitrification occurs if soil moisture content is 80% or more of the maximum water holding capacity. It is described as a first order process of nitrate turnover. The relative rate of denitrification is influenced by temperature and soil moisture conditions in the same way as nitrification. Carbon is not a determining factor for denitrification.

3.4.3 Assumptions made in MINMOD (revised from van Schöll, 1995)

The plant material is chemically homogeneous.

Plant material consist of several components, each with its own chemical structure. This assumption is thus a simplification.

The rate constant for decomposition is constant.
The rate constant for decomposition in reality decreases in time, as the remaining components of the organic matter are more resistant to breakdown. This assumption thus follows from the assumption of chemical homogeneous plant material.

The C/N ratio of the plant material and the microbial biomass is constant.
This also follows from the assumption of chemical homogeneous material. In fact, the plant material is built from several components, which have a different C/N ratio, so that the C/N ratio of the plant material will change during the decomposition.

The first three assumptions, mentioned above, could all lead to the same deviation of the simulation from the experimental results. Namely, an underestimation of the rate of mineralization in the beginning and a higher predicted rate in the middle part of the curve. The reasoning behind is that bacteria firstly will eat the easier decomposable parts of the plant, resulting in a fast decomposition rate in the beginning. Later, the more recalcitrant components are broken down, this consumes more time. A lower than simulated mineralization rate is expected in this case. Moreover the decomposition might be found to demand more time than expected. This depends upon the ratio of easy decomposable and recalcitrant components of the organic matter; the more recalcitrant components the more time it will take to break down the complete input.

The plant material is homogeneously distributed within the soil.
For a field situation this will be a simplification, as with rotary cultivating the plant material will be incorporated but not completely mixed with the soil. For simulation of mineralization in experimental conditions, this assumption might be valid as soil and plant material are thoroughly mixed.

The decomposition rate constant is influenced only by soil temperature and soil moisture content.
This means that the model has to be calibrated again when used for situations or soils differing strongly from the one it is designed for (sandy soil in spring in temperate region). The value of k found in the experiment as described in chapter four, takes into account all the effects of the different environmental circumstances, except for temperature and soil moisture content naturally.

The organic C and N are decomposed at parallel rates.
As the organic material is converted, the C and N will be released in the same ratio as they are contained in the organic material. If this is not the case an overestimation can occur of the amount of organic material that is decomposed.

The mineralization rate is only determined by the decomposition rate and the C/N ratio.
Follows from the assumptions of chemically homogeneous material and parallel rates of C and N release from the organic material.

Soil properties and characteristics are constant.
Fluctuations in soil properties and characteristics will be small over the time period that this model is intended to simulate.

The rate of release of nitrogen from soil is not influenced by the incorporation of plant material.
There are indications (see chapter four) that the addition of organic substrate enhances the release of nitrogen from soil. But the contribution of this extra amount of nitrogen is so small, about 1/30 of the total mineralized nitrogen in this research, that serious deviations in the prediction of net mineralization are not expected.

The growth of the microbial biomass is inversely proportional to the available amount of green matter. The activity of the organisms is implicitly described by the relative decomposition rates and the correction factors for temperature and soil moisture.
Because no pool for the microbial biomass and therefore no description of the activity is distinguished an overestimation of the amount of nitrogen mineralised at the start can occur because no lag phase due to adaptation of the organisms to the new situation (incorporation of green manure) is included in the model.

If the nitrogen released from the plant material is not enough to satisfy the demand of the soil micro-organisms that make up the microbial biomass, mineral N from the soil solution is immobilised, and nitrification is halted.
This can give an underestimation of the amount of nitrate, as in reality the nitrification will proceed. As the amounts of ammonium in the soil solution in these circumstances will be low, this will not be a serious deviation.

Nitrate is immobilised only after all ammonium is depleted
Most microbes have a preference for ammonium (Alexander, 1961).

The decomposition serves the single purpose of cell synthesis; the soil micro-organisms do not need the decomposition for maintenance respiration.
This is a simplification. The assumption is based on the idea that C the limiting factor is for growth of the microbial biomass. The energy required for maintenance respiration is expected to be small compared to the energy requirement for cell synthesis.

If the nitrogen demand of the soil micro-organisms cannot be fulfilled, mineralization is reduced.
As nitrogen is merely used for growth, the decomposition capacity decreases with nitrogen supply.

Carbon is not a limiting factor for denitrification.
This is a simplification. As organic matter, which is the input for the system, mainly consists of carbon and the soils in west Europe are relatively C-rich, it is expected the assumption will hold in the situation presented in this thesis.

Partial anaerobiosis is considered to be evoked only by high moisture content of the soil.
Several parameters play a role in anaerobiosis, like water-filled pore space and bulk density of the soil, but as they are not included in the model, it is only possible to describe the process on basis of moisture fluctuations.

As conclusion of this paragraph it should be mentioned that it was not possible to test the bigger part of the assumptions nor the eventual (unexpected) effects on the simulation results. Actually only a smaller part of the subroutine, namely the part describing the rate of decomposition influenced by relatively high temperatures, was validated.

3.5 Description of MINMOD

3.5.1. The initial part

In the initial part, all constants, parameters and initial values of state variables are declared. These are user defined and should be changed when using a different material or another soil (see appendix 1 for explanation of the abbreviations).

```
          INCON IGMLAY=11.1, IMINGM=0.
*         [g d.w./kg soil]
          INCON IIMMOB=0.,IAMMO=0.019, INITR=0.061, INDENI=0.
*         [g N/kg soil]
          PARAMETER RDRGM=0.02, RMRBIO=0.0018, KDENI=0.00017, KNITR=0.2
*         [day-1]
          PARAMETER CCGM=0.415, NCGM=0.0393,
*         [g C/g d.w.] and [g N/g d.w.]
          PARAMETER NCRB=0.1, DISASS=2.
*         [g N/g C], [g C diss/g C ass]
          PARAMETER B=7160.
*         [K-1]
          PARAMETER WCWILT=0.03,WCPF24=0.172,WCACT=0.245,...
                    WCFLDC=0.254, WCMAX=0.428
*         [m3 water/m3 soil]
          PARAMETER T=20., REFT=15., FRZPNT=-2.
*         [deg C]
```

Timer variables are set, and output commands are given. Time is given in Julian day numbers. The time step DELT is taken as 0.4 day, print output PRDELT is given with interval of 1.0 day. The time step is defined on base of the rule of thumb that a first approximation to the time interval is obtained by taking DELT smaller than one tenth of the smallest time coefficient [day] in the model (Leffelaar, 1995). In this case is that the inverse of KNITR. Runs were made to check the influence of the chosen time step on the output of the simulation

```
          TIMER STTIME=40., FINTIM=250., DELT=0.4, PRDEL=1.0.
          PRINT T, ANLAY, AMMO, NITR, IMMOB
```

The integration method 'EUDRIV' is used, meaning that a numerical integration method will be used with time step DELT.

```
          TRANSLATION_GENERAL DRIVER = 'EUDRIV'
```

The daily maximum and minimum temperatures are read from a weather file. When simulating with constant temperatures, this part should be omitted. The temperature should be given as a parameter in that case.

```
          WEATHER WTRDIR='W:\WEATHER\', CNTR='RHIZ', ISTN=1, IYEAR=1995
```

3.5.2. The dynamic part

In the dynamic part of the model all rates, calculated in subroutines, are numerically integrated with time step DELT. The rates are considered to be constant during the time increment of integration. The amount of mineral nitrogen in the soil solution follows from the amounts of ammonium and nitrate.

```
          GMLAY=INTGRL(IGMLAY,DRGM)
*         [g d.w./kg soil]
          MINGM=INTGRL(IMINGM,RMINN)
          IMMOB=INTGRL(IIMMOB,RIMMO)
          AMMO=INTGRL(IAMMO,RAMMO)
          NITR=INTGRL(INITR,RNIT)
          ANLAY=NITR+AMMO
          DENI=INTGRL(INDENI,RDENI)
*         [g N/kg soil]
```

3.5.3. Subroutine MINR; Organic matter humification and mineralization.

The subroutine starts with the calculation of the correction functions for temperature and soil moisture. With these correction factors the potential rates are calculated, which are used as base for the calculation of the actual rates.

Temperature correction function for humification

If temperature is below the actual freezing point of the soil FRZPNT [°C], which is below 0°C because of the osmotic potential due to the salts in solution, humification halts, so temperature correction factor TRFDEC [-] is 0. Between actual soil freezing point and 0°C the correction factor is linear from 0 to the value of the intercept of the Arrhenius equation with Y-axis DECDEF [-]. At temperatures above 0°C the temperature effect is described by a modified Arrhenius function. By normalising the Arrhenius equation to 1 at a reference temperature REFT [°C] , which is the temperature that the rate constant is given for, the correction function becomes:

$TRFDEC = kT/kREFT = A*e^{-BT}/A*e^{-BREFT} = e^{-B*\{1/(T+273)-1/(REFT+273)\}}$

```
IF (T.LE.FRZPNT) THEN
     TRFDEC=0.
ELSEIF (T.LT.0.) THEN
     DECDEF= EXP(-B/273.)/EXP(-B/(REFT+273.))
          TRFDEC=(DECDEF/FRZPNT)*(FRZPNT-T)
ELSE
          TRFDEC=EXP(-B/(T+273.))/EXP(-B/(REFT+273.))
ENDIF
```

Soil moisture correction function for humification and mineralization

At actual soil water content WCACT [cm3/cm3] lower than water content at wilting point, pF 4.2, WCWILT [cm3/cm3], decomposition is halted, so the soil moisture correction factor WRFDEC [-] has the value 0. Between wilting point and the water content at pF 2.4, WCPF24 [cm3/cm3], the correction function proceeds linearly with soil water content, from 0 to 1. The correction factor has the value 1 for soil water contents between pF 2.4 and field capacity at pF 2, WCFLDC [cm3/cm3]. From field capacity to maximum water holding capacity WCMAX [cm3/cm3], the correction function decreases linearly with water content from the value 1 to 0.8. The soil moisture correction factor for nitrification is assumed to be equal to the correction factor for humification. In MINMOD no description of the transport of water in soil is included yet . As the moisture content was kept constant in this research, it was not added either.

```
IF (WCACT.LE.WCWILT) THEN
     WRFDEC=0.
ELSEIF (WCACT.LE.WCPF24) THEN
     WRFDEC=( WCACT/(WCPF24-WCWILT) - WCWILT/(WCPF24-WCWILT))
ELSEIF (WCACT.LE.WCFLDC) THEN
     WRFDEC=1.
ELSE
     WRFDEC=(0.8*WCACT/(WCFLDC-WCMAX) - 0.8*WCMAX/(WCFLDC-WCMAX))
ENDIF
```

Temperature correction function for nitrification

At temperatures above 5°C the nitrification is described by the same correction function as the humification and ammonification. The value of the correction function at temperature 5°C is named NITDEF [-]. At temperatures below 5°C the correction factor for nitrification TRFNIT [-] decreases linearly to reach the value 0 at freezing point [-]. No nitrification is assumed to take place at temperatures below freezing point, so the temperature correction function for nitrification TRFNIT [-] has the value 0.

```
IF (T.LE.FRZPNT) THEN
     TRFNIT= 0.
ELSEIF (T.LE.5.) THEN
     NITDEF=EXP(-B/(273.+5.)/EXP(-B/(REFT+273))
     TRFNIT=(NITDEF/(5.-FRZPNT))*(T-FRZPNT)
ELSE
     TRFNIT=TRFDEC
ENDIF
```

Calculation of the potential rates.

The model calculates potential humification rates PDRGM [g dry weight/kg dry soil day] from the humification rate constant RDRGM [1/day], the temperature correction factor TRFDEC [-], the soil moisture correction factor WRFDEC [-] and the total amount of fresh matter in the soil GMLAY [g dry weight/kg dry soil]. From the potential humification rate and the nitrogen content of the fresh matter NCGM [g N/k dry matter] the potential rate at which nitrogen is released (mineralized) from the fresh matter PMINN [g NH4+-N/kg dry soil day] is calculated. The potential rate at

which nitrogen is required for assimilation in the soil by microbial biomass decomposing the fresh matter, PNDEMB [g N/kg dry soil day] is calculated from the nitrogen to carbon ratio of the microbial biomass NCRB [-], and the amount of carbon that is assimilated in the microbial biomass. This is calculated from the potential humification rate, the carbon content of the fresh matter CCGM [g C/g dry weight], and the dissimilation of the microbial biomass DISASS [-]. The dissimilation of microbial biomass is expressed relative to the assimilation -set to the value 1- so that assim./(dissim.+assim.).

```
            PDRGM=-RDRGM*TRFDEC*WRFDEC*GMLAY
*           potential humification [g d.w./kg soil/day]
            PMINN=-PDRGM*NCGM
*           potential nitrification released as NH4 [g N/kg soil/day]
            PNDEMB=-NCRB*PDRGM*CCGM/(DISASS+1.)
*           potential microbial consumption of N
```

Calculation of actual rates

If the nitrogen is released from the fresh matter at a higher rate than it is assimilated by the biomass, the humiftcation process will take place at potential rate. The actual rate of nitrogen release RMINN [gNH4+ -N/kg dry soil day] minus the rate at which this is assimilated in the microbial biomass NDEMB [g N/kg dry soil day] is the potential rate of net ammonification PRAMMO [g NH4+-N/kg dry soil day]. The ammonium already present AMMO [g NH4+-N/kg dry soil] is subject to nitrification. The rate of nitrification RNITR [g NO3 -N/kg dry soil] is calculated from the rate constant for nitrification KNITR [1/day], the temperature and soil moisture correction factors, TRFNIT [-] and WRFDEC [-] and the amount of ammonium. The loss of mineral N from the soil solution due to assimilation NLOSS [g N/kg dry soil] is 0.

```
          IF (PNDEMB.LE.PMINN) THEN
               DRGM=PDRGM
               RMINN=PMINN
               NDEMB=PNDEMB
               PRAMMO=RMINN-NDEMB
               NLOSS=0.
               RNITR=KNITR*TRFNIT*WRFDEC*AMMO
```

If the potential rate of nitrogen assimilation by the soil microbial biomass is bigger than the rate at which nitrogen is released from the fresh matter, all nitrogen released will be assimilated. As long as the mineral nitrogen in the soil solution ANLAY [mg N mineral/kg dry soil] is sufficient to cover the potential rate of nitrogen requirement of the microbial biomass not covered by the nitrogen that is released from the fresh matter PNDEMS, all processes will proceed at potential rate. If the amount of ammonium in the soil solution is large enough to cover the actual requirement of the microbial biomass of mineral nitrogen NDEMBS [g N/kg dry soil day], ammonium will be used to cover this nitrogen requirement. The nitrification halts. If the amount of ammonium is not sufficient to cover the demand on mineral nitrogen, all ammonium will be assimilated in one time step DELT [day], while the remaining requirement will be covered by the nitrate in the soil solution NITR [g NO_3--N].

```
          ELSE
               PNDEMS=AMAX1(0.,PNDEMB-PMINN)
                    IF (ANLAY.GE.PNDEMS*DELT) THEN
                    DRGM=PDRGM
                    RMINN=PMINN
                    NDEMB=PNDEMB
*                   Total microbial consumption of N
                    NDEMBS=PNDEMS
*                   Microbial consumption of N from the soil solution
                    IF (AMMO.GE.PNDEMS*DELT) THEN
                         PRAMMO=-NDEMBS
                         RNITR=0.
                         NLOSS=NDEMBS
                    ELSE
                         PRAMMO=-AMMO/DELT
                         RNITR=-NDEMBS-PRAMMO
                         NLOSS=NDEMBS
                    ENDIF
```

When the mineral nitrogen in the soil solution is not enough to cover the nitrogen demand of the microbial biomass for assimilation, the rate of decomposition is brought down, so that the resulting nitrogen requirement of the microbial biomass on the mineral nitrogen in the soil solution during one time step can be covered by the amount present. All ammonium and nitrate present in the soil solution will be assimilated.

```
        ELSE
                DRGM=PDRGM*(ANLAY/(PNDEMS*DELT))
                RMINN=-DRGM*NCGM
                NDEMB=-NCRB*DRGM*CCGM/(DISASS+1.)
                NDEMBS=NDEMB-RMINN
                PRAMMO=-AMMO/DELT
                RNITR=-NITR/DELT
                NLOSS=NDEMBS
            ENDIF
        ENDIF
```

Calculation of the mineralization from the microbial biomass

The mineralization rate of the microbial biomass MRBIO [g N/kg dry soil day] is calculated from the rate constant for mineralization of the microbial biomass RMRBIO [1/day], the temperature and soil moisture correction factors and the amount of nitrogen in the microbial biomass IMMO [g N/kg dry soil]. Part of this nitrogen will subsequently be used for the assimilation of new microbial biomass, at a rate NDEMBB [g N/kg dry soil day]. Analogous to the nitrogen demand rising from decomposition of fresh matter, this is calculated from the mineralization rate and the dissimilation to assimilation ratio. The rate at which ammonium is released to the soil solution from the microbial biomass is RAMMOB [g NH4+-N/kg dry soil day].

```
                MRBIO=RMRBIO*TRFDEC*WRFDEC*IMMOB
                NDEMBB=MRBIO/(DISASS+1)
                RAMMOB=MRBIO-NDEMBB
```

Calculation of the actual rates for ammonium and immobilised nitrogen

The actual net rates with which ammonium is added or subtracted from the soil solution RAMMO [g NH4+-N/kg dry soil day] and the rate of nitrogen accumulation in the microbial biomass RIMMO [g N/kg dry soil day] are calculated as additional sources of information.

```
RAMMO=PRAMMO+RAMMOB-RNITR
RIMMO=NDEMB-MRBIO+NDEMBB
RETURN
END
```

3.4.4. Subroutine 2; The denitrification component

The subroutine starts with the calculation of the correction functions for soil moisture content and temperature, after which the denitrification and net nitrification rates are calculated.

Calculation of the correction factors for soil moisture content.

At an actual water content WCACT [cm3/cm3] lower than 80% of the water content at maximum water holding capacity WCMAX [cm3/cm3] denitrification is not assumed to take place so the value of the soil moisture correction factor for denitrification WRFDEN [-] is 0. Between 80% of maximum water holding capacity and maximum water holding capacity the denitrification increases linearly with soil water content.

```
IF (WCACT.GE.(0.8*WCMAX)) THEN
        WRFDEN=(WCACT-0.8*WCMAX)/(0.2*WCMAX)
        RDENI=KDENI*TRFNIT*WRFDEN*NITR
    ELSE
        RDENI =0.
    ENDIF
```

Calculation of the denitrification rate

The denitrification rate RDENI [g N/kg dry soil day] is calculated from the rate constant of denitrification KDENI [1/day], the temperature correction function TRFNIT [-], the soil moisture correction factor for denitrification WRFDEN [-] and the amount of nitrate NITR [g NO3--N/kg dry soil]. The rate of nitrification RNITR [g N/kg dry soil day] is corrected for the rate of denitrification, so that the resulting rate of nitrate accumulation is RNIT [g N/kg dry soil day] .

```
RNIT=RNITR-RDENI
```

3.5 Initialisation of model

To initialise the model, input data were used from the experiment and from the literature. The values of all input parameters are given in table 3.2 and when needed discussed in chapter 5.

Table 3.2: Values of input parameters for MINMOD.

Name	Input value	Dimension	Derived from
IGMLAY	13.48	g dry weight of GM kg^{-1} dry soil	Experiment
IMINGM	0.0	g N kg^{-1} dry soil	Experiment
IIMMOB	0.0	g N kg^{-1} dry soil	Experiment
IAMMO	0.0007	g N kg^{-1} dry soil	Experiment
INITR	0.0715	g N kg^{-1} dry soil	Experiment
NCGM	0.036	g N kg^{-1} fresh matter	Experiment
RDRGM	0.046	d^{-1}	Experiment
RMRBIO	0.0018	d^{-1}	Bradbury (1993)
KNITR	0.2	d^{-1}	Johnsson *et al.* (1987)
CCFM	0.47	g C g^{-1} dry weight of GM	Experiment
DISSASS	2.	-	Janssen (1992)
NCRB	0.1	G N g^{-1} C	Janssen (1992)
B	5321	K	Experiment
REFTT	20	°C	Based on rate constants
FRZPNT	-2	°C	Assessed

Soil data

As the model was run with constant optimal moisture content for this thesis, it was not necessary to define soil characteristics. The actual water content, WCACT [m^3 water/m^3 soil], was defined in such a way that the moisture correction function was a constant 1. As result, moisture content had no influence on the mineralization rate.

Temperature

The temperature was given as a constant. The average values as obtained from the experiment were used.

Chapter 4

Experiment

4.1 Introduction

Aim of the experiment; *To quantify the relative mineralization rate of Trifolium subterraneum L. at 5 constant temperatures for a typical Swiss agricultural soil in order to validate the model for the specific green manure and soil used in this research pro-ject.*

In order to be able to estimate the relative mineralization rate (see section 2.10), an incubation test was executed to determine the net mineralization of *Trifolium subterraneum* L in a typical Swiss soil in time under several constant temperatures regimes. Anaerobic conditions were avoided. The data about mineralization were acquired with chemical soil analysis.

The occurrence of denitrification in the experiment had to be avoided. Nitrogen would disappear from the system and it would become impossible to accurately quantify net mineralization as no instruments available were able to measure gaseous N_2. Anaerobic conditions are the most important prerequisites for the incidence of denitrification (Alexander, 1961; Janssen, 1992). The development of anaerobiosis is influenced mainly by the degree of water saturation of soils. (Bremmer & Shaw, 1958). Therefore the influence of soil moisture content on mineralization rate could not be examined.

While the aim of experiment demanded controlled circumstances, the moisture conditions of the used soil had to be brought to a certain preferred constant level. According to Janssen (1992), Bremner & Shaw (1958), Knowles (1981) and Alexander (1961) the mineralization takes place under the most optimal moisture conditions at about pF 2. The occurrence of anaerobic circumstances is least likely to happen at this specific moisture content. There are several methods to set the moisture conditions of soil. Firstly, there is a method in which the soil is dried at high temperatures and rewetted after until the preferred level is reached (Nordmeyer & Richter, 1985). These measures influence the mineralization considerable. The explanation for this is twofold: (i) high temperatures lead to the decay of large organic

molecules and (ii) the killing of microbes at high temperatures may result in a higher mineralization potential of the soil (Nordmeyer & Richter, 1985). Following a second method the soil has to be air-dried. This changes the proportions of the different micro-organisms developing after incubation compared to incubated samples of fresh soils (de Neve *et al.*, 1996). To avoid the above mentioned uncontrolled processes, the moisture content of the soil for this experiment was determined to see whether there was a need to adapt it.

The soil was sieved with a 4 mm pore sieve. This treatment was applied to remove gravel which might influence the measured values, to loosen the soil and to crumble aggregates in order to avoid the occurrence of partial anaerobe conditions. Fine sieving of the soil causes a temporary increase in the mineralization of N and C. Very small pores contain a fraction of the soil organic matter that cannot be reached by the micro-organisms. Sieving destructs these pores so that this fraction will be attacked by microorganisms, causing an increase in N mineralization (Nordmeyer & Richter, 1985). This, temporary, increase in mineralization rate will also occur in the field situation, because the clover will be incorporated in the top layer of the soil (0-5 cm) with a rotary tiller which crumbles the soil. The effect of the increase caused by the treatment of the soil for the experiment will be measured in both the blank and the treatment samples and will therefore not disturb the comparison of the results.

The treatment of the soil just before the start of the experiment was a point of discussion. In literature almost no information on this subject was available and nowhere the effects of different treatments were discussed. The advantage of preincubation is that the samples become acclimatised and the short, high flush of mineral N, expected at higher temperatures (pers. com. Bodegom), will have taken place before the start of the experiment. This flush is induced when moving soil from a low store temperature to higher temperatures. With preincubation of the soil the micro-organisms have already adapted to the selected temperature at the moment the experiment starts. The disadvantage of this method is that the organic matter content of the soils at different temperatures has shifted and therefore comparison of data acquired at different temperature becomes more complicated. Not preincubating the soil implies that the results of the first week after the start of the experiment will not be reliable because the high flush of mineral N, due to the sudden change in

temperature in a part of the samples, will be incorporated in the measurements. In this experiment we chose to preincubate the soil samples because we wanted to be able to compare the relative mineralization rates at 10°C and 25°C. For the field situation this means that mineralization in April is compared with mineralization in August. As it is inherent to a soil that the organic matter contents change during the growing season, and it is aimed to execute the experiment as close to the field situation as possible, preincubating was preferred to storing the soil at a low temperature till the start of the experiment. By subtracting the mineralised N at different temperatures measured on day 0, the undesirable effects of the preincubation were overcome as well as possible.

Plant material used in incubation experiments is often dried and ground, or cut into small parts. Dried plant parts decompose more slowly than fresh material, whereas grinding will accelerate breakdown due to an increased surface promoting microbial activity (Oglesby & Fownes, 1992; Thorup-Kristensen 1994). Variation in size does not seem to make a difference for the mineralization (Whitmore & Groot, 1994). Breland (1994a) reported that burying the green matter increases the rate of mineralization. This can also be concluded from the data of Maag (1999). The N concentrations found for the blank (without incorporated clover) soil samples at each treatment and each sampling time were subtracted from the N concentrations measured in the soil:clover samples. It is assumed that these corrected N concentrations give the values for N coming from the added material (Breland, 1994a; Marstorp & Kirchman, 1991). In other words, that the addition of green manure to the soil did not significantly influence the rate of mineralization of the soil organic matter (Azam *et al.,* 1993).

The incubation assay method used in this experiment was developed by Heller (1999) (figure 4.1). This method is hereafter referred to as "the tower"-method. Drying and rewetting the soil influences the mineralization (Nordmeyer & Richter, 1985), so loss of water had to be avoided, as well as anaerobe conditions. The "tower-method" of Heller (1999) was developed in such a way that these two processes occur only to a negligible level. Holes in the boxes, together with a high soil area/volume ratio and a sufficient O_2 flux in the climate rooms had to ensure that no anaerobic conditions developed. While only at the bottom of the tower holes were located, the moisture

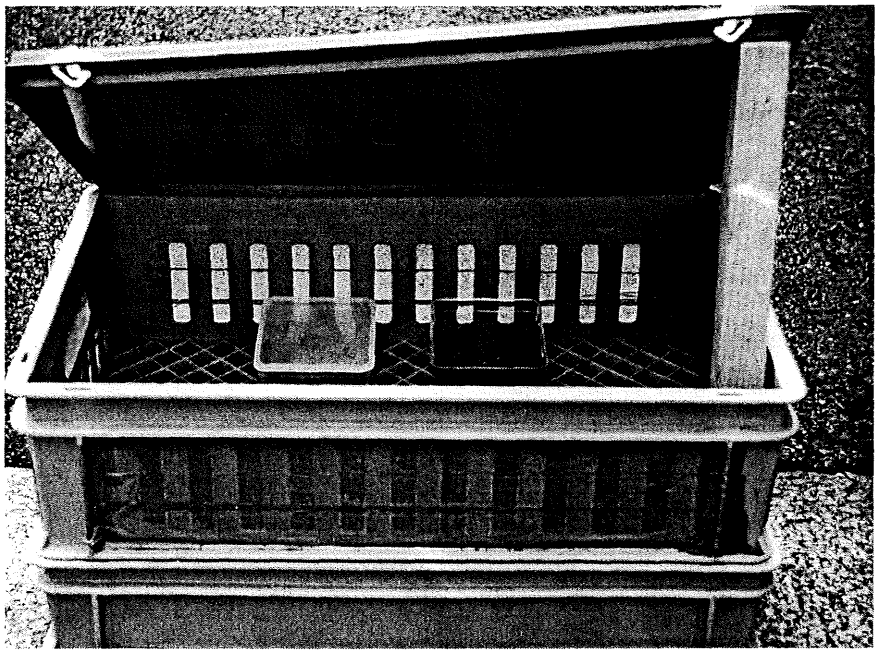


Figure 4.1: Design of incubation assay method, called "the tower" (Heller, 1999). The example on the picture consists of a container with water at the bottom and a crate with a perforated bottom and all round sealed with tape covered by a plastic top. Inside the tower two samples boxes are shown.

content was high within the tower. Heller (pers. com.) experienced that after four weeks only one gram water per box was lost. The crates, each containing 30 samples, were piled. It is probable that the conditions in the respective piled crates differed slightly and a block design is necessary to avoid the influence of differing and uncontrolled circumstances of the experiment on the results (Dourleijn, 1994). The samples were taken at 0-3-7-14-21-28-35 days after incubation. Van Schöll (1997) had a time series up to 10 weeks. After 10 weeks 35% of the total organic N applied had been mineralised. Van Schöll (1997) used root material of winter wheat and incubated the samples at 10°C. Because in this experiment easier decomposable plant material was used and the samples are incubated at higher temperatures, the duration of the experiment was expected to be sufficient. Maag's incubation experiment (1999) lasted only 21 days. The incorporated plant material was also subterraneum clover and the incubation temperature varied between 18 and 20°C. After 21 days the net mineralization curve had flattened. Maag (1999) replicated the treatments four times, van Schöll (1997) and Heller (1999) three times. Thorup-Kristensen (1994) performed incubation experiments in the field with only two repetitions. While no data were available on the expected variation we had to rely on expert judgement. The treatments were replicated four times.

4.2 Materials and methods

4.2.1 Plant material

Subterraneum clover (*Trifolium subterraneum)* or `subclover` is a winter annual forage legume which originates from the Mediterranean region (Hess, 1970). The subterraneum clover used for the experiment is the cultivar Nuba (Otto Hammenstein Seeds) (table 4.1). The clover seeds were sown on 8.9.99 and grown up in a climate

Table 4.1: Fresh and dry weight and C and N content of the subterranean clover portions incorporated for the experiment.

Fresh weight [g]	Dry weight [% fresh weight]	Dry weight [g]	C content [g C/g dry weight]	N content [g N/g dry weight]
16	9.8	1.57	0.47	0.036

chamber with a constant temperature of 23°C, air moisture ca. 60%, and a day/night regime of 14/10 hours. On 4.10.99 plants were harvested. Roots were rinsed to get rid of attached soil, afterwards the complete plants were chopped into pieces of 1-3 cm. At the same date the chopped clover was incorporated in the soil.

4.2.2 Soil

The soil originates from the experimental farm `Sandhof` which belongs to the FAW (table 4.2). The soil was taken from a field where fennel was cropped at the moment the soil for the experiment was removed.

Table 4.2: Characteristics of the soil used for the experiment.

Clay [%][a]	Loam [%][a]	Sand [%][a]	pH[-]	Organic matter [%]
17.0	24.5	54.2	7.8	3.3

[a] Values taken from Weingartner (1996).

4.2.3 Set up of the experiment

Scheme of experiment

- Incubation of soil with incorporated plant material in hard plastic boxes, placed in climate rooms;
- Five constant temperatures: 10-15-17-20-25°C;
- Measurements of mineral N at 7 time intervals: 0-3-7-14-21-28-35 days after incubation;
- Four replicates of every treatment at every time interval.

The soil moisture content had to be set at pF 2. A sample of the same soil that was used in the experiment afterwards was dried at 100°C for 48 hours and the weight was measured before and after drying. When the soil moisture content equals 27% of the dry weight, this soil had a pF of 2 (pers. com. Heller) as a rule of thumb. The moisture content of the soil used for the experiment appeared to be around 27% therefore no further adaptations were needed. The soil was sieved with a 4 mm pore sieve. The soil was preincubated six days before the experiment started. This means that the soil already was put in the boxes and set in the climate rooms at the respective temperatures.

In the executed experiment, the fresh plant material was chopped into pieces of 1-3 cm and immediately incorporated. This is according to the field situation in which the green undersown clover is chopped and incorporated within one action.

For the incubation 150 g of soil was put into hard plastic boxes (Pakoba, 12 x 10 x 5 cm) with lids. Four small holes (diameter 2 mm) were made in both the lids and the bottom of the boxes; this to ensure good aeration within the boxes. The reason for incubating 150 g soil per box was that the method (Heller, 1999) requires so. A larger weight will increase the possibility of an anaerobic situation in the boxes; 150 g soil in the boxes of 12 x 10 x 5 cm gives a proper area/volume ratio. In each box with 150 g soil, the blanks excluded, 16 g fresh, chopped subterranean clover was incorporated. The 16 g incorporated fresh clover corresponded with 0.056 g nitrogen. Heller (pers. com.) experienced that the maximum amount of incorporated mineral N per box should not exceed 3 g. If more mineralizable N is incorporated, organic C will be

limiting, O_2 will be depleted, resulting in anaerobic conditions which might lead to denitrification and ammonia volatilisation. The boxes were stacked up in crates (50 x 40 x 40 cm), in such a manner that a good aeration was provided. The bottoms of the crates were perforated, and the sides sealed with tape. The crates were piled to a tower. In this experiment the tower contained two crates with boxes. The top of the tower was closed. Underneath a container with water was placed. The sides of the container with water were not closed; they contained holes through which gasses could escape. Although the "tower"-method was used several times before we executed our experiment, no checks were made. Therefore some important features for our experiment were measured: the relative humidity content and the temperature at several heights in the tower. Also the weight of the boxes was determined. Samples from the water container underneath were analysed for mineral N to check whether leaching occurred. It was not possible to take gas samples for the detection of eventual concentrations of N_2O and NH_3, while no gas chromatograph was available at FAW.

The set up used was a split-plot experiment in a completely randomised block design with 10 treatments of which each was replicated four times. The treatments consisted of subterraneum clover incorporated in the soil, incubated at five different temperature regimes: 10-15-17-20-25°C. The range is typical for the Swiss situation. These temperatures are encountered in the soil during the growing season from April to August (pers. com. Baumann). The blanks were also incorporated at the five temperature regimes. Of every treatment seven series were made in order to measure the mineralization rate in time. The samples were taken at 0-3-7-14-21-28-35 days after incubation. The treatments were replicated four times. The N concentrations found for the blank (without incorporated clover) soil samples at each treatment and each sampling time were subtracted from the N concentrations measured in the soil:clover samples.

4.2.4 Chemical analyses

Total N content of plant and soil

Samples from the plant material and soil samples taken at the day of the preincubation and at the last sample day, were taken, dried at 70°C, grounded and analysed for N content with the total N method of Kjeldahl (Association of Official

Analytical Chemists, 1975) with a modification. Instead of applying a mercury solution a potassium sulphur-copper sulphur solution was used.

Total C content of plant and soil

Samples of plant and soil were analysed according to the Schweizerische Referenzmethoden der Eidgenössischen Lantwirtschaftlichen Forschungsanstalten (1996b) and measured with an auto analyser (SKALAR 4000, Skalar Analytical, the Netherlands).

Mineral N content of soil

The NO_3^- and NH_4^+ concentrations were measured for the blanks and the clover & soil samples. The N measurements were done with a photometrical auto analyser (SKALAR 4000, Skalar Analytical, the Netherlands) after an 2:1 water volume extraction as described by the Schweizerische Referenzmethoden der Eidgenössischen Lantwirtschaftlichen Forschungsanstalten (1996a). In the scientific world no general agreement exists about the extraction solution used (Houba, 1986). For practical reasons in the Swiss research institutes water is the standard extraction solution.

pH

The soil was analysed for pH with a 2:1 volume extraction with water, according to the Schweizerische Referenzmethoden der Eidgenössischen Lantwirtschaftlichen Forschungsanstalten (1996a).

Leaching test

Concentration of mineral N in the bottom crate with water was determined with the Merck leaching test at the beginning and the end of the incubation period.

4.2.5 Analytical and statistical analysis

Performance of tower

The temperature and relative humidity data, monitored to evaluate the performance of the tower were tested with student-t tests. The data of monitoring the weight were analysed with ANOVA on the regression parameters. The statistical package SPSS (edition 9.0) was used to execute the statistical analysis.

Fitting the rate constant of mineralization (k)

In the literature often it is assumed that the N mineralization follows first-order kinetics (Stanford & Smith, 1972; Smith *et al.*, 1980; Frankenberger & Abdelmagid, 1985; de Neve & Hofman, 1996; van Schöll *et al.*, 1997). Models of mineralization kinetics are attempts to describe soil biological processes, rather than pure mathematical exercises in finding the best fitting curve (Ellert & Bettany, 1988). The simplest first-order mineralization model (Jenny, 1941; as cited in Jenkinson, 1990) postulates that all the organic nitrogen in the soil occupies one single compartment. This approach is followed in this report, in spite of several efforts to develop two-compartment models, multi-compartment models and non-compartment decay models (Jenkinson, 1990 and references therein). The advantage of the simple approach is that even with a limited amount of input parameters and a limited amount of mineralization data the model fits the data set reasonably well (de Neve & Hofman, 1996). The relative mineralization rate (k) in this research was found by fitting the data with the following formula.

$$N(t) = N_{max}(1-e^{-kt})$$

in which N_{max} = the maximum amount of mineralizable N, *k* = the rate constant of mineralization and *t* = the time from the start of the incubation. To obtain the theoretical maximum amount of organic N that can be mineralised (N_{max})(mg N kg^{-1} dry soil) the following equation had to be substituted (van Schöll *et al.*, 1997):

$$N_{max} = G_m * (N_{contGm} - C_{contGm} * ({}^{N}/_{C})_{microo} * ({}^{ass}/_{diss+ass}))$$

G_m = the amount of incorporated green matter (g dry weight kg^{-1} dry soil); N_{contGm} = the N content of the green manure (g N kg^{-1} dry weight); C_{contGm} = the C content of the green manure (g C kg^{-1} dry weight); $({}^{N}/_{C})_{microo}$ the N to C ratio of the microbial biomass, taken as (1:10) (-) (Groot, 1987); $_{diss}$ and $_{ass}$ the dissimilation and assimilation fraction respectively of the microbial biomass, with value of 2 and 1(-) (Groot, 1987). The equation is based on one important assumption; The microbial biomass had a constant size during the incubation period.

Calculating the Arrhenius parameter B

The parameters of the Arrhenius function were found by plotting ln(k) against T [°K].

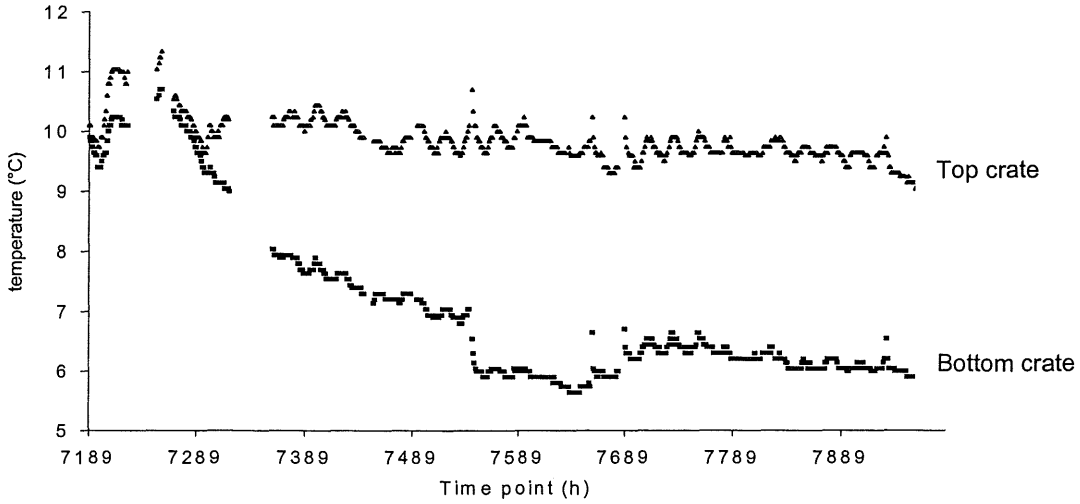


Figure 4.2: Temperature course in time in the two crates of the tower of the 10°C treatment. Time point 7189 is equal to time point 0 in the experiment.

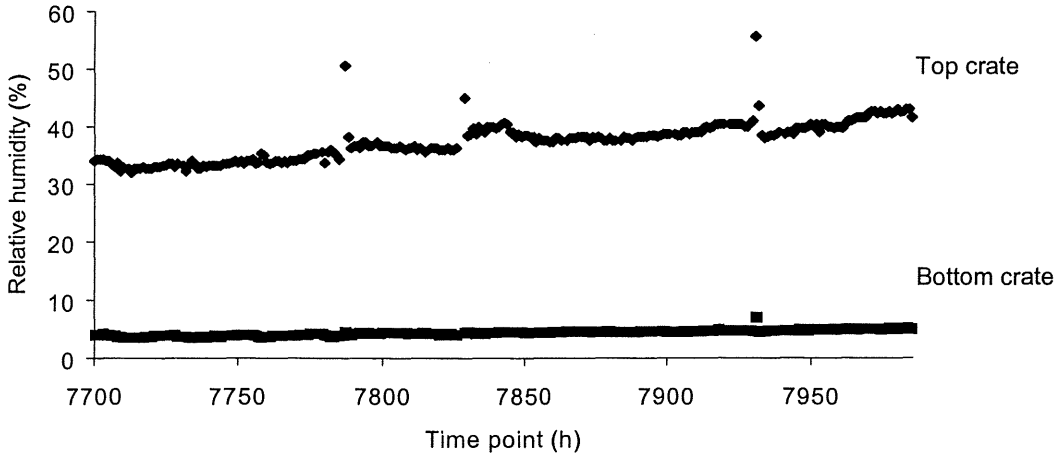


Figure 4.3: Relative humidity course in time in the two crates of the tower of the 10°C treatment. Time point 7700 (in hours) falls within day 21 after incorporation of the clover.

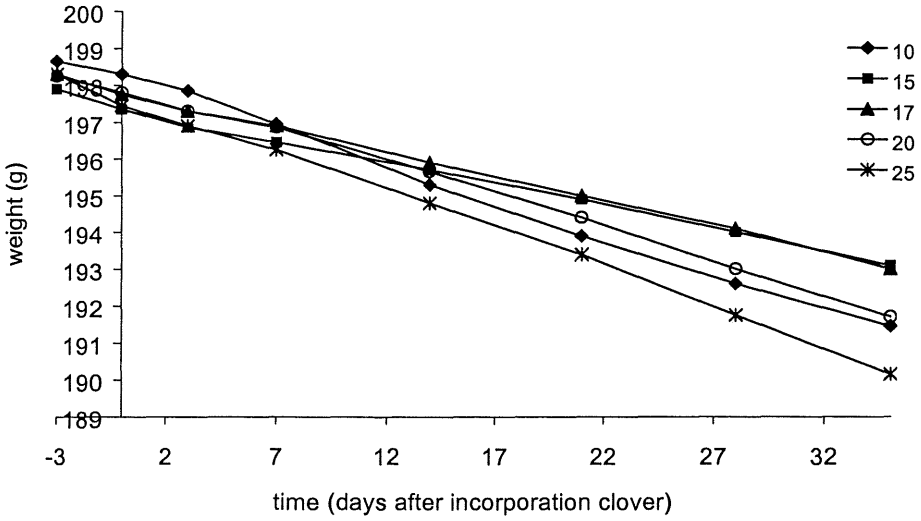


Figure 4.4: Change in weight of monitor boxes placed in climate chambers at five different temperature regimes; 10-15-17-20-25°C.

4.3 Results

4.3.1 Performance of tower

Checking temperature and relative humidity

The "tower-method" of Heller (1999) was developed to provide a system in which anaerobic conditions are not likely to occur and water losses from the samples negligible. The temperature and moisture content in the top and bottom crate of all but the 17°C tower, due to practical limitations, were measured constantly to investigate the tenability of these assumptions. Also the weight of selected boxes was determined regularly, to monitor the change in weight caused by moisture fluctuations of the soil. The measurements were meant to check whether the conditions were homogeneous between the two different crates of a tower.

All measured top and bottom temperature curves differed significantly from each other ($p<0.001$). At 15-20-25 °C the differences between the means were less than 0.1 °C (appendix 4). Therefore the temperature differences between the two crates at these temperatures, although statistically significant, were not assumed to be of demonstrable influence on the results. The significant values of p were found because of the high n values (n = 672) used for the statistical analysis. At 10°C the temperature difference between top and bottom crate is however considerable with means of 9.8 and 6.9 °C respectively (figure 4.2). As consequence of the significant deviating measured temperatures, these ones were used instead of the defined temperatures for the presentation of the results and in the discussion.

The first two weeks of the experiment, the interior of the crates was sprayed with water regularly to increase the relative humidity in the tower. The reason for this intervention was the noticed constant decrease in weight of the monitor boxes. But after dry weight determination of samples, it was decided that the small measured weight decrease due to water evaporation would not influence the process of mineralization as the moisture content of the soil was still in the range for optimal mineralization. The measurements of the relative humidity in the tower are evaluated from time point 7700 on. Day of the experiment corresponds to time point 7190. The selection was made to exclude the large fluctuations in the beginning due to attempts to increase the relative humidity. After time point 7700 the situation was stable. The

relative humidity of the 15-20-25°C towers was maintained at around 70-80 % during the complete experimental period (appendix 4). Although the differences in relative humidity between the crates were significant, again, due to the high number of measurements, no relevant distinction could be made between top and bottom crate

Table 4.3: Weight losses in % of total soil moisture content per box per temperature. In case a block effect was detected with the analysis of the data, top and bottom crate are distinguished.

day	10°C top	10°C bottom	15°C	17°Ctop	17°Cbottom	20°C	25°C
0	1.5	0.6	1.6	1.8	1.5	1.3	2.5
3	2.9	1.8	3.1	2.9	2.9	2.8	4.1
7	5.8	4.1	4.2	4.1	4.1	4.1	6.0
14	11.1	8.5	6.4	7.3	6.7	7.6	10.2
22	15.8	12.0	8.8	9.9	9.4	11.3	14.3
28	20.2	15.2	11.4	12.9	11.7	15.4	19.2
35	24.0	18.1	14.0	16.7	14.3	19.2	23.8

at these temperatures. In the 10°C tower however, much lower values were found for the relative humidity and the difference between top and bottom was considerable; average values of 40 and 6 % respectively were measured (figure 4.3).

Monitoring weight loss from the samples

No significant block effect could be proven. In addition the trendlines for the temperature were analysed (y = ax + b). Slope a differed significantly (p = 0.021) between the temperatures. The value of b stands for the starting weight of the monitor boxes. Figure 4.4 shows that for temperatures 15 and 17°C the moisture losses were smallest. Apparently the laboratory conditions in those cases were best for stabile moisture content of the samples. At temperature 10°C the relative humidity was very low; so this could be the explanation for the higher weight losses. The weight losses at 20 and 25°C might be due to the high evaporation rate from the samples, which is not compensated by the relative high humidity level in these towers. However, the soil moisture content of all treatments was within the optimal range for mineralization during the complete incubation period. Furthermore, although the moisture losses per box were substantial as can be concluded from table 4.3, the losses were neglected at the analysis of the data. During the analyses 200 ml of water was added to soil samples. A loss of 6.4 ml (average value day 35) on 234 ml is not relevant.

4.3.2 Climate chambers

Temperatures of the climate chambers were different from what was supposed. For the analysis of the data the mean values as monitored by the thermometers were used (table 4.4). As no thermometer was installed in the tower placed in the 17°C climate chamber, it was impossible to determine the real temperature in this room.

Table 4.4: Deviation of temperature from the tuned value, measured in the towers.

Supposed temperature (°C)	**Mean actual temperature** (°C)	**Standard error**
10 bottom	6.9	$1.4*10^{-2}$
10 top	9.8	$4.9*10^{-2}$
15	13.8	$1.51*10^{-2}$
20	18.8	$2.71*10^{-2}$
25	24.5	$4.09*10^{-2}$

4.3.3 Net nitrogen mineralization

Nitrogen balance

The definition of the balance is as follows:

$$N_{tot\ soil} + N_{plant} \rightarrow N_{tot\ soil+plant}$$

With the modified Kjeldahl method, as described in section 4.2.4 all nitrogen, organic and mineral is measured. In other words, if the values measured at the beginning and the end of the experiment correspond, no nitrogen has disappeared from the system. In our case, no significant differences in N content were determined. On top of that, no proof of leaching was found with the nitrate-leaching test. Therefore the occurrence of denitrification or volatilisation was assumed not to have taken place in the executed experiment.

Mineralized nitrogen

In all figures based on means, standard errors are included. Only values corrected for the mineralization of the soil are shown unless stated otherwise. A block effect in the data was found for the 10 and 17°C treatments: the curve of net mineralization of the top deviated significantly from the bottom block. Besides the expected concentrations of nitrate and ammonium a certain concentration of nitrite was found in the

samples, mainly in the first 10 days and at the lower temperatures (table 4.5). Figures 4.5a & b show the time course of the components of mineral N (NO_3 + NO_2 + NH_4) at temperatures 10 and 15°C. At the higher temperatures the amount of nitrite was negligible. After 10 days the nitrite had almost completely (99%) disappeared from the samples incubated at the two lowest temperatures as well.

Table 4.5: Mean values and standard errors (se) of accumulation of nitrite in the soil samples at 3-7-14-21-28-35 days after start of incubation. N=4 for treatments 25-20-15°C; n=2 for treatments 15-17°C. Top and bottom refer to the blocks.

Treatment\Days	3	7	14	21	28	35
25	14.90	0.40	0.45	0.12	0.18	0.17
se25	1.64	0.10	0.19	0.00	0.04	0.03
20	20.40	4.04	0.34	0.12	0.12	0.15
se20	2.49	0.35	0.04	0.04	0.03	0.04
17 top	23.55	4.40	0.55	0.25	0.25	0.10
se17 top	0.98	1.51	0.00	0.00	0.00	0.10
17 bottom	24.29	3.94	0.34	0.20	0.10	0.10
se17 bottom	1.20	0.70	0.10	0.10	0.00	0.00
15	15.51	8.38	3.17	0.31	0.20	0.32
se15	1.54	0.90	0.71	0.04	0.02	0.14
10 top	5.78	12.76	14.82	7.29	0.95	0.34
se10 top	1.17	2.43	2.15	1.01	0.15	0.09
10 bottom	6.98	13.75	17.47	17.99	7.56	5.35
se10 bottom	0.22	1.81	0.92	1.08	2.77	0.62

Figure 4.6 shows as example, the difference between concentration mineralized N in the blank samples and the samples with incorporated clover at 25°C. The curves at other temperatures showed the same trend. Based on the assumption that the rate of mineralization of the soil is not influenced by the incorporation of organic matter (section 4.2.3) the amount of mineral N of the blank samples was subtracted from the amount measured in the clover soil samples.

Figure 4.7 shows the increase of the amount of mineral N with temperature and time. The 25°C treatment counts the highest percentage of mineralized clover after 35 days (table 4.6). Determination of the rate constant of mineralization (k) based on temperature

Table 4.6: Percentage nitrogen mineralized of the incorporated amount of nitrogen of the plant material at the different temperatures after five weeks. Amount of incorporated N at start of experiment was 482 mg N/kg dry soil.

Treatment	Mineralised N after 35 days (mg N/kg dry soil)	Percentage mineralised N (%)
10 bottom	138	29
10 top	194	40
15	225	47
17 bottom	231	48
17 top	241	50
20	238	49
25	275	58

Table 4.7 presents the rate constants k and the accompanying R^2 values obtained by fitting the data with a N_{max}, of 0.280 (appendix 3). The *k*`s found for the 17°C are rather strange. They do not fit in the trend of increasing *k* with increasing temperature. The R^2's are in general high, except for the one found for the data of the 25°C treatment. The Q_{10} value which can be calculated from the determined k's (see appendix 3), is 1.89.

Table 4.7: The rate constant of mineralization k, derived from incubation of soil:subterranean clover samples at five different temperatures during five weeks.

Treatment	K (d^{-1})	Asymptotic standard error	R^2
10°C bottom	0.021	0.001	0.95
10°C top	0.032	0.002	0.93
15°C	0.043	0.003	0.90
17°C bottom	0.042	0.003	0.94
17°C top	0.050	0.003	0.93
20°C	0.046	0.002	0.95
25 °C	0.074	0.006	0.82

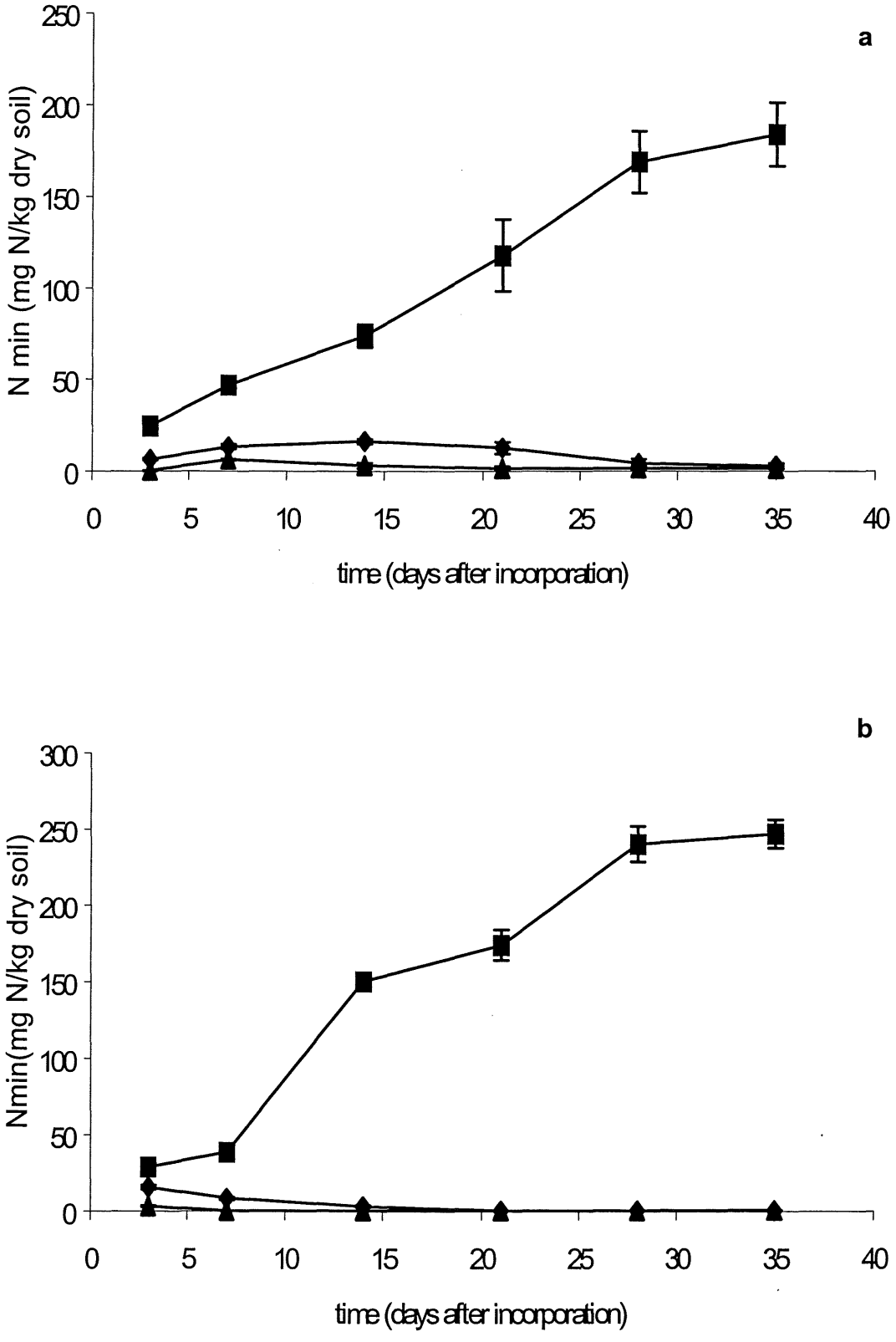


Figure 4.5: NO_3^- (■), NO_2^- (◆)and NH_4^+ (▲) measured in soil:subterranean clover samples of the 10°C (a)and 15°C (b) treatment. Every point is the mean of four replicates.

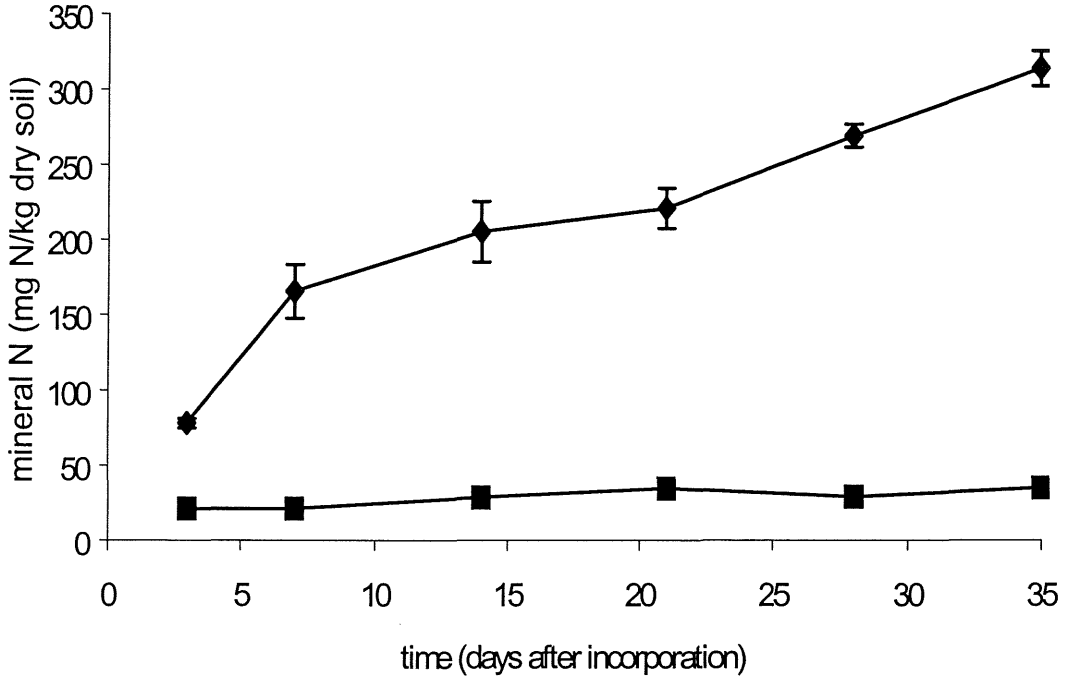


Figure 4.6: Mineral nitrogen measured in the soil:subterranean clover (◆) and blank (■) soil samples, incubated at 24.5°C. Every point is the mean of four replicates.

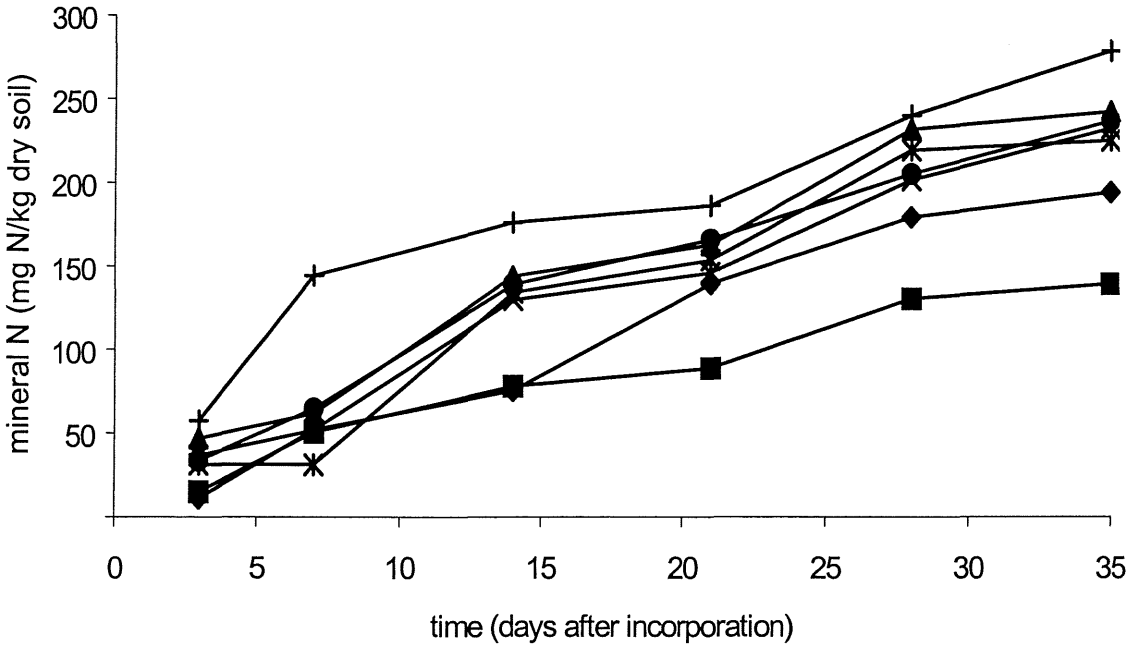


Figure 4.7: Mineral nitrogen released from incorporated clover portions incubated at 6.9 (■) – 9.8 (◆) – 13.8 (✳) – 18.8 (●) – 24.5°C (+). The real temperatures of the 17°C treatment are unknown: top crate = ▲ and bottom crate = x. Every point is the mean of four replicates. Error bars are not reflected.

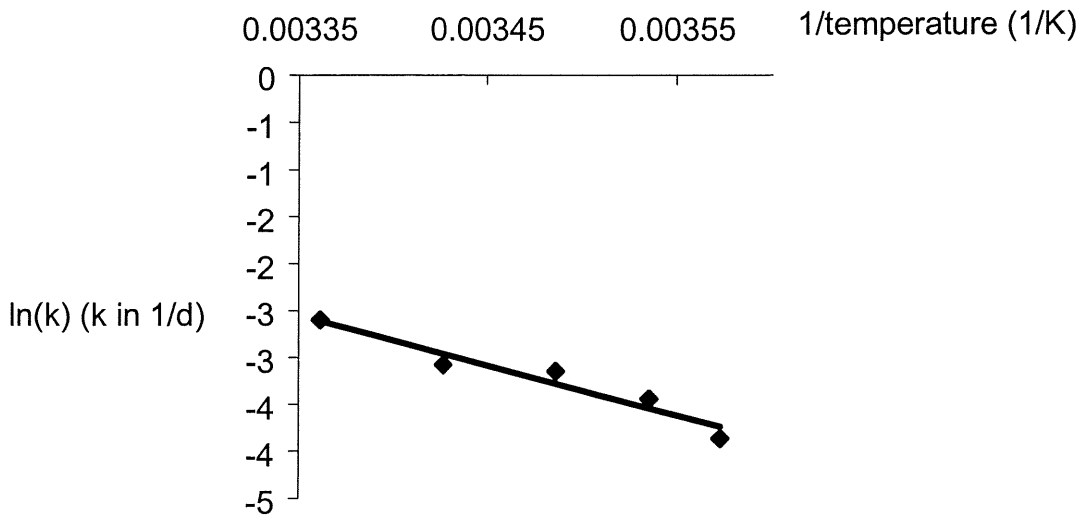


Figure 4.8: Arrhenius plot of ln(k) against T^{-1} with k the rate constant of mineralization, derived from an incubation experiment with clover:soil samples at 6.8-9.8-13.8-18.8-24.5°C during five weeks.

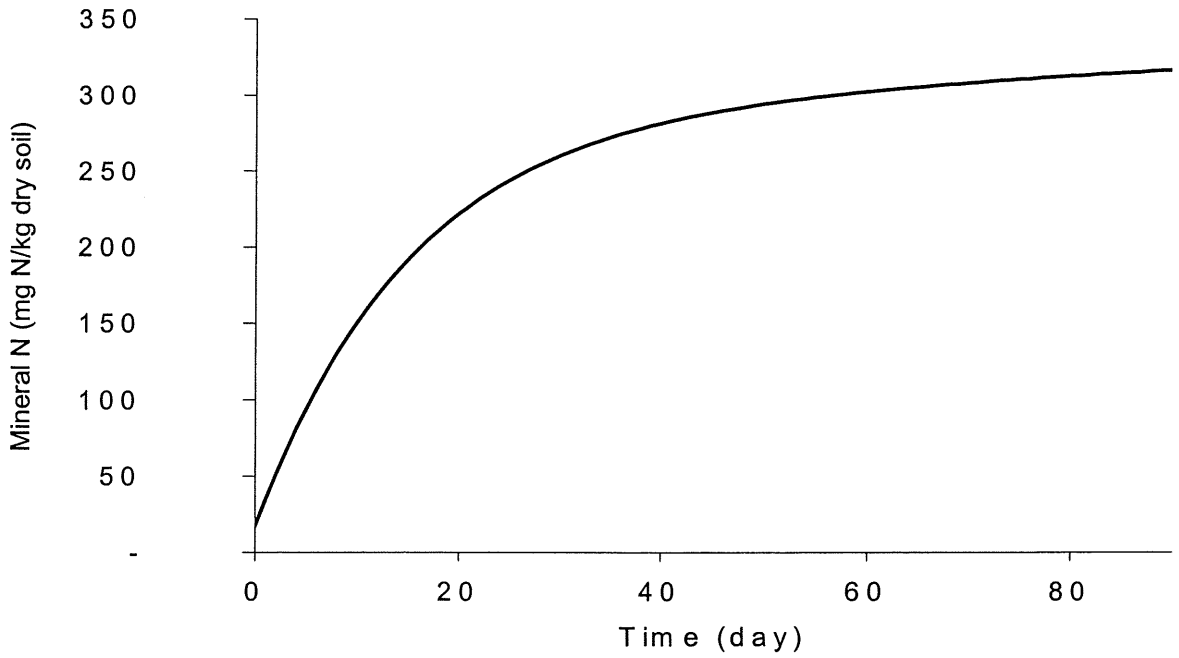


Figure 4.9: Simulation with MINMOD of nitrogen mineralization of incorporated subterranean clover at 24.5°C for 90 days. Parameters derived from experiment discussed in this report.

Calculation of B

In MINMOD the effect of temperature on the rate constant k is described by a modified Arrhenius equation. The k values as presented in table 4.7 were used to find the constants of this equation. Plotting ln(k) against T^{-1} yielded a linear curve with slope B and intercept with y-axis at ln(A). Figure 4.8 shows this linear curve, with slope B = 5321, ln(A) = 17.27 and R^2 is 0.93.

4.4 Discussion

4.4.1 Evaluation of tower

When the tower was placed in a sufficiently large room with a well-working air circulation, the tower-method satisfied the provisions. The occurrence of anaerobe conditions was not detected and therefore assumed not to have taken place. No significant block effects were found in the data of the 15-20-25°C treatments. The measured deviations in the tower from the programmed temperatures could be due to imperfect fine tuning of the temperature adjustment in the climate room. Another explanation would be that the heat was not distributed evenly within the climate chambers. The 10°C tower was placed in a refrigerator. The cooling system of the refrigerator was situated at the bottom. Therefore the water in the crate underneath was frozen. The room was also too small to provide a good air circulation. Besides, the relative humidity was far lower than in the other towers. Based on the later observation, one can conclude that the tower is better not used for 10°C treatments when a high relative humidity is desired. The data of mineral nitrogen at 17°C show a block effect. Due to the fact that no temperature and humidity measurements were executed, an explanation can only be guessed for . Although smaller than the chambers of the 15-20-25°C treatments, the 17°C tower was placed in a climate room. The data were not included for the determination of B and no simulations were executed for this treatment as the actual temperature in the tower was unknown. Experiments lasting a longer time (more than 5 weeks) may encounter problems. The soil moisture content might become too low for optimal mineralization and a significant systematic mistake is made in later calculations linked to the soil moisture content. Regularly spraying with water in the tower is suggested to prevent the drying out of the soil in the long term.

4.4.2 Experiment

No loss of N from the samples was detected but, as can be seen in figure 4.5 and table 4.5, at the start of the experiment an accumulation of nitrite occurred. The first days the transfer from nitrite to nitrate was hampered; normally this process proceeds five times as fast as nitrite is formed (Heller, pers. com). After the first week the occurrence of nitrite was negligible and the nitrite disappeared faster with increasing temperature. This finding suggests that the Nitrobacter group, which oxidises nitrite to nitrate, is more sensitive to low temperature than the Nitrosomonas group responsible for the first step in nitrification. This hypothesis is supported by Tyler *et al.* (1959) who concluded based on experiments with temperatures lower than 10°C that low temperature and alkaline soil reaction appear to favour nitrite accumulation from ammoniacal fertilisers even at low levels of addition. Van Schöll (1995) executed a similar experiment as ours, but at lower (0-15°C) temperatures. The soil used in that experiment had a much lower pH than the one used in this research. The worker did not measure nitrite separately and the first samples were taken one week after the incorporation of the plant material while the nitrite and the accompanying delay of mineralization in this experiment was mainly found in the first week. Therefore it is impossible to make a hypothesis about the contribution of pH and the low temperatures to the development of nitrite from the reviewed literature.

The low residual values for the fits on rate constant *k* can be contributed to incubation of soil three days before the start of experiment (de Neve *et al.*, 1996). No flush due to sudden change in temperature of soil was found for the first week of the experiment. Only the 25°C treatment showed a relative high mineralization rate in the beginning in comparison with the later course at this temperature. This flush at 25°C was observed by Addiscott (1983) as well. The finding was not elaborated.

It is questioned whether the incubation period was long enough to define the rate of mineralization at the different temperatures correctly. A simulation made with MINMOD at 24.5 degrees, based on data of the experiment, suggests that still a rather large quantity of mineral N is released between day 35 and 55 after incorporation of clover (figure 4.9). As net mineralization at 24.5°C in the experiment proceeded fastest it is expected that for the other treatments even a larger part of the course of mineralization was not measured. As result of the rather short sampling

period the values of k might have been overestimated as the data of the second, slower part of the mineralization were not included for the fit of *k*.

4.4.3 Fitting the data with a first order decay function

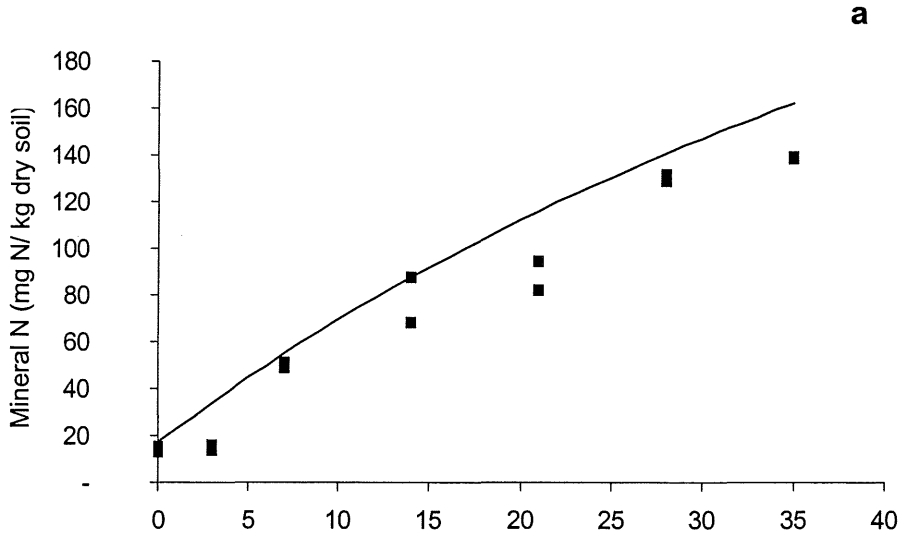
A high k for the 25°C treatment was determined in comparison with the ones of the other treatments. The steep course of the start of the mineralization curve is pointed out as cause of the remarkable value. The results of Stanford *et al.* (1973), Kladivko & Keeney (1987) and Tyler *et al.* (1959) indicate that *k* changes approximately twofold for each 10°C change in temperature (i.e. $Q_{10} = 2$). This is in accordance with the value found for Q_{10} in this research. The fitted B is rather low in comparison to the values found by Stanford *et al.* (1973) (6351 K^{-1}), Addiscott (1983) (6350 and 8313 K^{-1}) and van Schöll *et al.* (1997) (7161 K^{-1}). Addiscott (1983) and van Schöll *et al* (1997) executed experiments including temperatures lower than 10°C. Therefore it is suggested that the low temperatures at which the data were obtained offer an explanation for the differences in B's. The findings of Stanford *et al.* (1973) contradict this hypothesis. These workers found a higher B by excluding the value for the lowest temperature. The temperature regimes investigated did not contribute to the findings neither as both Addiscott (1983) and Stanford *et al.* (1973) worker with temperatures ranging from at least 5-25°C. Most research on the dependence of N mineralization on temperature has focused on mineralization from soil organic matter (Stanford *et al.,* 1973; Addiscott, 1983; Ellert & Bettany 1992; Kladivko & Keeney, 1987; Cassman & Munns, 1980; Kowalenko & Cameron, 1976). De Neve *et al. (*1996) found a strong interaction between temperature and resistance of crop residues to degradation in soil; N mineralization from resistant crop residues will be more enhanced by a rise in temperature than N mineralization from easily degradable residues. Soil organic matter has the highest resistance to degradation and thus high values for B were found by researchers working with just soil. Van Schöll *et al.* (1997) incorporated winter rye grown over winter while the current research worked with young clover. It is assumed that young clover is a far easier degradable substance than four-month old winter rye. The assumed low resistance of the subterranean clover is then the explanation for the low value of the fitted B.

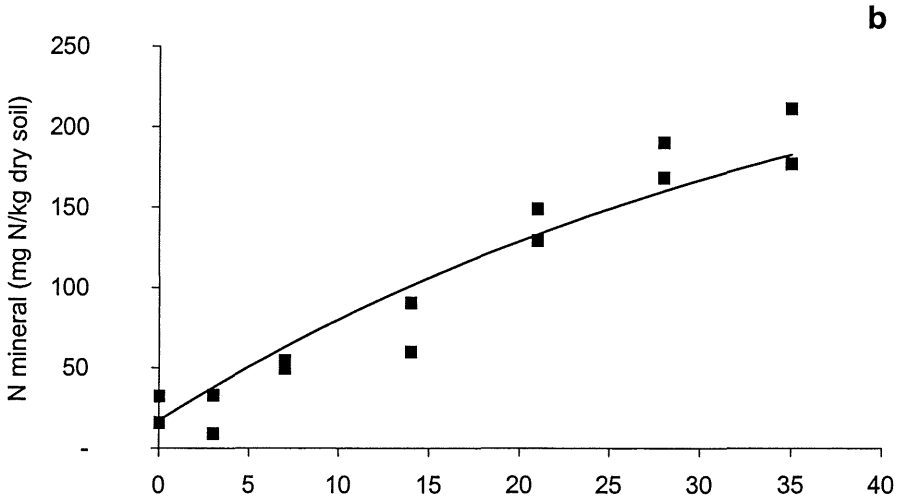
Chapter 5

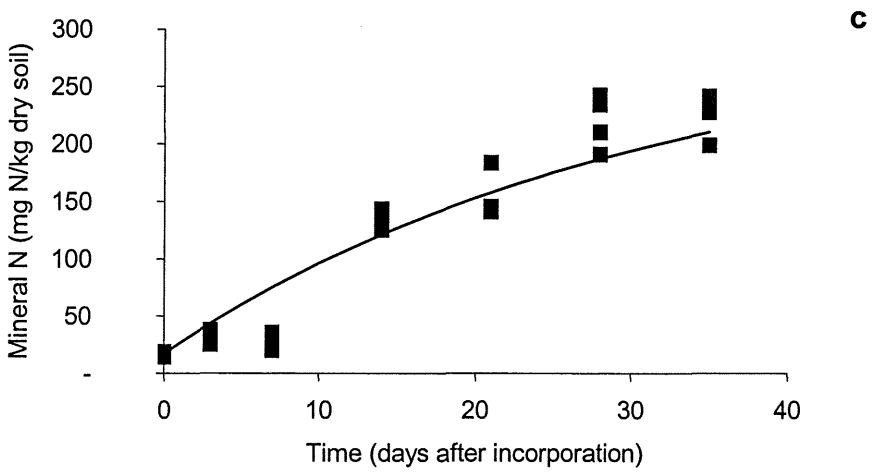
Performance of MINMOD

5.1 Comparison of the simulated and measured mineralization data

The Arrhenius equation is usually applied to model the temperature dependence of mineralization above 15°C. As van Schöll *et al.* (1997) found that the equation is also valid at lower temperatures, simulations were executed for the lowest temperature treatments. Simulation runs were made for the mineralization of N from subterranean clover at five temperatures: 6.8-9.8-13.8-18.8-24.5°C (see section 3.5 for the parameter values). Figure 5.1 a - e give the measured N mineral (dots) and the simulated N mineral (lines) in time at the different temperatures. Comparing these graphs shows that the mineralization is described well. The model responded correctly to the differences in temperature. This was expected, as the model was calibrated with the data from the clover incubations. The differing outcomes of the simulation are based only on changes in the temperature correction function as no other circumstances (e.g. soil moisture content) differed. The temperature correction function is based on an averaged B over the data of all temperature treatments. Mistakes in the sampling procedure have not been noticed, and can not be deduced from the results. Therefore deviations of the experimental results from the simulations had to be due to uncommon processes taking place in the samples during the experiment, which did not happen in all treatments. For the 10°C treatment an overestimation of the experimental results was found. The higher the temperature, the shorter the time period the model simulated too high values for the mineralization. This observation can be explained with the delay in activity of the nitrificating bacteria due to the low temperatures, as assumed on base of the results of the experiment. Comparison of the 20°C and 25°C treatments with simulations gave the best results. Probably the bacteria were better adapted to these temperature regimes due to the fact that soil samples were taken from the field in September when the soil is still relatively warm, between 15 and 20°C.


a
Mineral N (mg N/ kg dry soil)
180
160
140
120
100
80
60
40
20
-
0
5
10
15
20
25
30
35
40


b
N mineral (mg N/kg dry soil)
250
200
150
100
50
-
0
5
10
15
20
25
30
35
40


c
Mineral N (mg N/kg dry soil)
300
250
200
150
100
50
-
0
10
20
30
40
Time (days after incorporation)

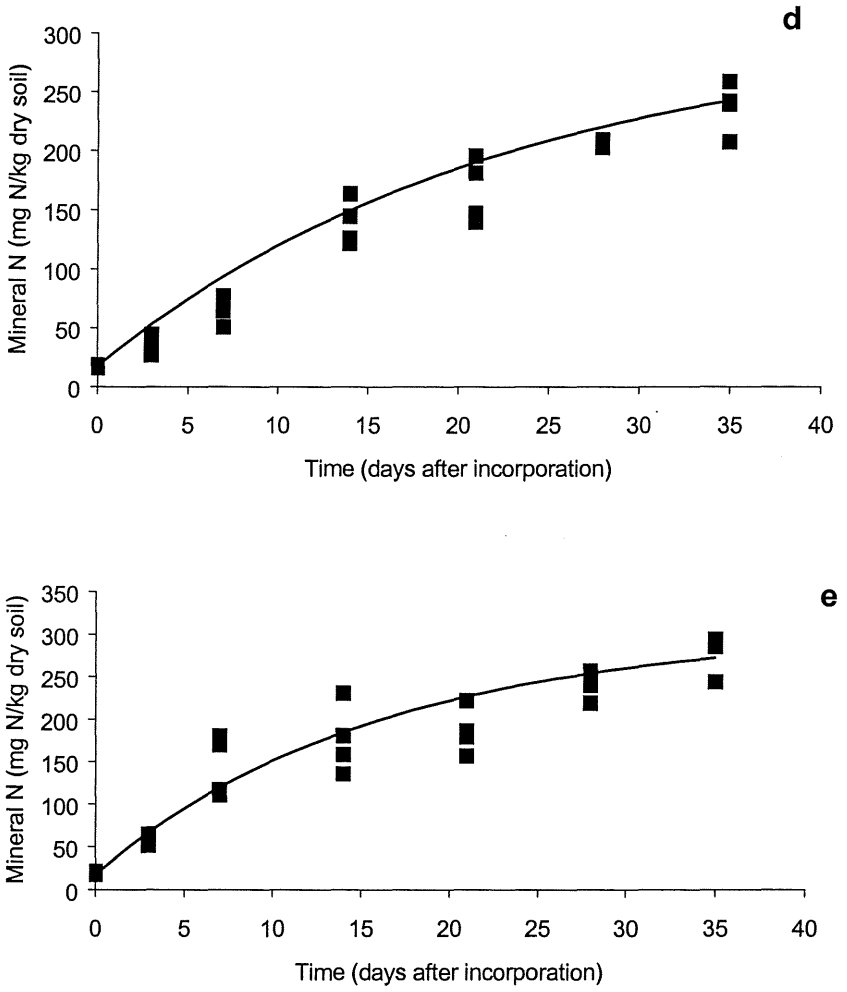


Figure 5.1: Simulation of net mineralization (line) with MINMOD at the constant temperatures of 6.8 (a), 9.8 (b), 13.8 (c), 18.8 (d) and 24.5°C (e) for 35 days. The points indicate the experimental values obtained at the same temperatures. Input values of the model can be found in section 3.5.

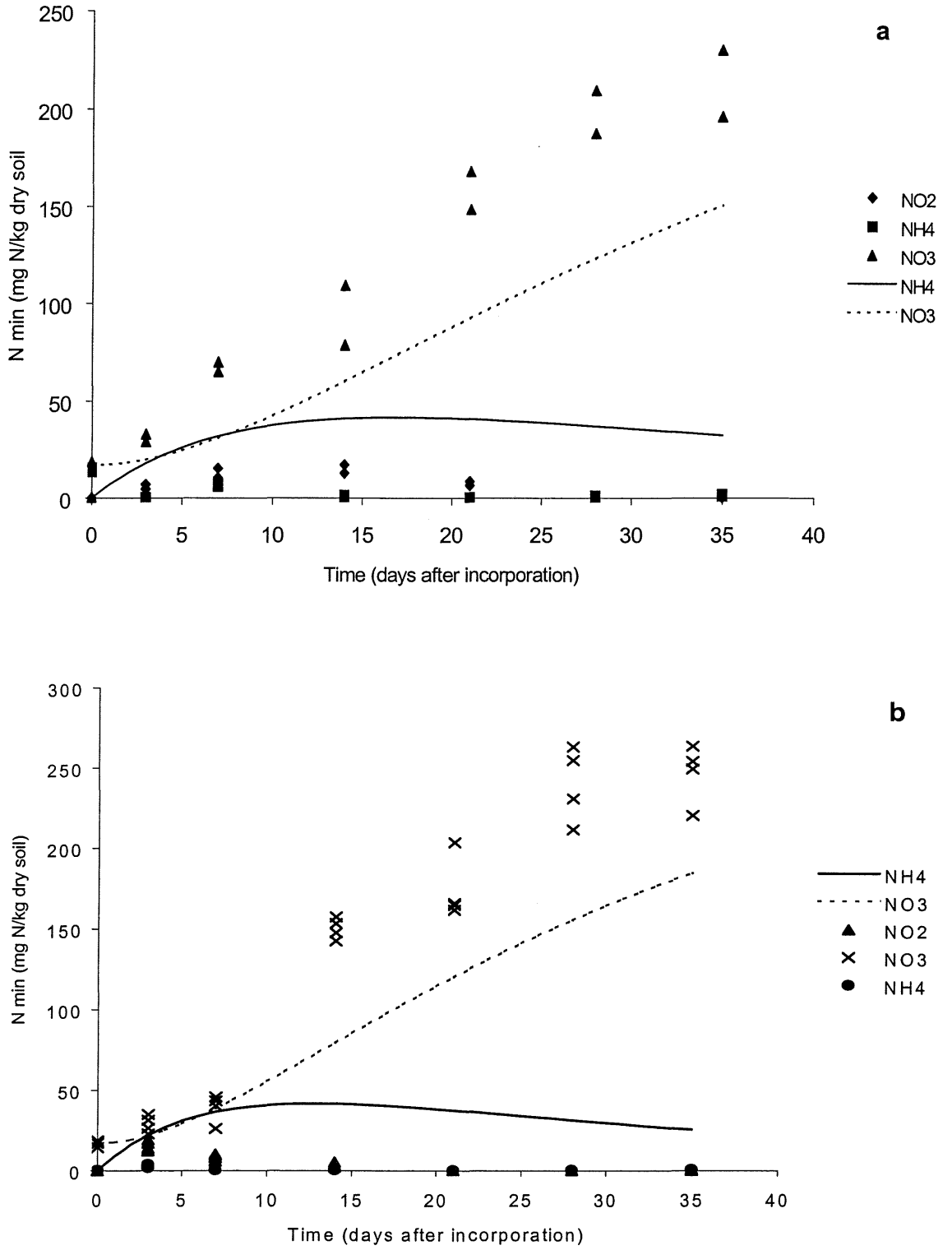


Figure 5.2: Simulation of cumulation of NO_3^- and NH_4^+ (lines) with MINMOD at the constant temperatures of 9.8 (a) and 24.5°C (b) for 35 days. The signs indicate the experimental values for NO_3^-, NH_4^+ and NO_2^- obtained at the same temperatures. Input values of the model can be found in section 3.5.

For the development of MINMOD the assumptions were made that plant material is chemically homogeneous and that the C/N ratio of the plant material and microbial biomass is constant. This led to a fixed rate constant for decomposition in the model. The assumptions proved to be valid for the circumstances the model was run for.
This might be due to the fact that very young material was incorporated. Probably the material did not contain much recalcitrant components yet and was still quite homogeneous in composition, even though roots and green plant material were incorporated together.

MINMOD does not model microbial biomass as a separate pool with an own specific activity. The activity is indirectly referred to in the descriptions of the rates of humification, ammonification and nitrification. From the comparison of experimental data and simulations no definite conclusion about the validity of this approach can be derived as there was too much noise found in the experimental data at lower temperatures. The occurrence of nitrite indicated abnormal development of the microbial activity, which makes it difficult to derive conclusions about the simulation of microbial activity development. At all temperatures expect 25°C, the simulation slightly overestimates net mineralization in the first week. This suggests that the neglect of the development of microbial activity results in a minor oversimplification of the rate of mineralization in time. In the version of MINMOD used for this research, the initial biomass is zero. Although it will not influence the outcome of the simulation of net mineralization, it seems from the biological point of view more logical to start with some biomass already present. From the figures presented by Janssen (1992) a biomass equal to 0.03 mg N was calculated which could be used as input value.

The formula, which was used to define the k`s at the different temperatures on basis of the experimental data, is only valid when no growth of the microbial biomass takes place. Though, MINMOD calculates a pool of 0.2 g immobilised nitrogen after 35 days (not shown) while the pool does not contain any at the beginning of the simulation. It means that about 40% of the nitrogen of the humificated plant material ends up in the microbial biomass. The overestimation of the growth of the pool of immobilised N can be caused by an underestimation of the dissimilation to assimilation ratio for soil biomass or/and an underestimation of the relative mineralization rate of microbial biomass. Whatever the cause is, in this case it has

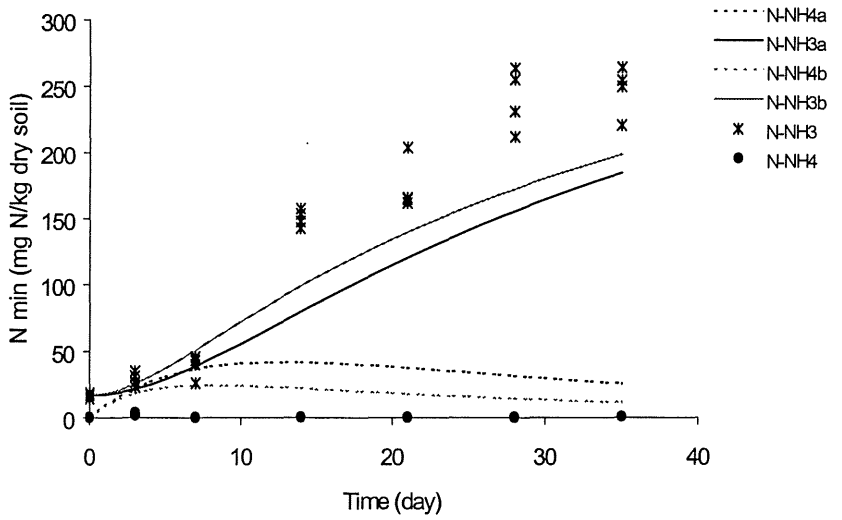


Figure 5.3: Simulation runs made with MINMOD at 13.8°C for 35 days with two rate constants of nitrification, 0.2 (a in legend) and 0.5 (b in legend). The dots and crosses indicate the measured values.

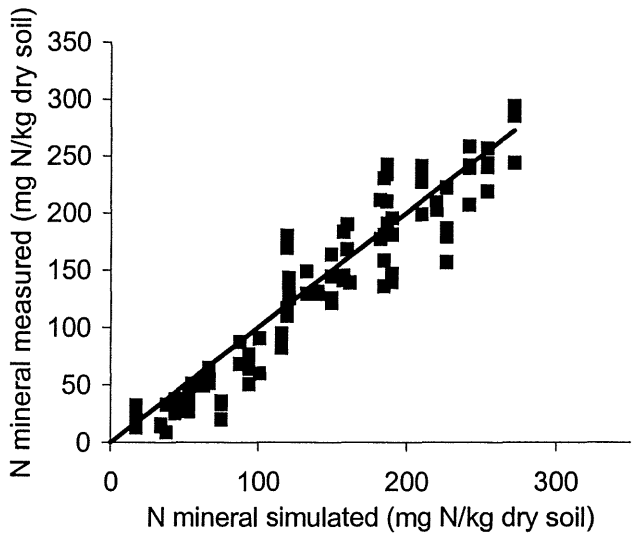


Figure 5.4: The 1:1 plot of simulated values for net mineralization against the measured values from incubated subterranean clover at 6.8 – 9.8 – 13.8 – 18.8 – 24.5°C for 35 days.

resulted in a fitted *k* which is too high. Besides, it can be concluded that the description of the kinetics of the pool of immobilised nitrogen is inadequate.

From figure 5.2 it can be concluded that the descriptions of ammonification and nitrification are inadequate. Nitrite is not simulated at all. The rate of nitrification is calculated based on a rate constant of nitrification, a correction factor for temperature and one for soil moisture content and the amount of ammonium present. Also the description of the rate of ammonification includes the two correction factors. There is no indication that temperature or soil moisture content would have an opposite effect on the two rates. Therefore the only parameter left in the description of the rate of nitrification that could be changed to obtain better simulation results, is the rate constant of nitrification. In the model the value 0.2 was given as rate constant of nitrification. This value was derived from Johnsson *et al.* (1987) and applied by van Schöll (1995) with satisfying results. No explanation for the meaning of this constant could be derived from the literature. Both workers incubated old material, barley stubble and over winter grown winter rye respectively. Moreover the concentrations of NH_4^+ and NO_3^- in time are interdependent. A larger nitrate production corresponds to a lower ammonium production if calculated with the same overall mineral N production. In other words, if the simulation of nitrate production is improved, it is expected that the simulation of the rate of ammonification deviates less from experimental results as well. It is therefore suggested that a too low value for the rate constant was defined in MINMOD due to the fact that very young and easy decomposable material was applied in the experiment. Figure 5.3 shows a comparison of the simulation results for ammonification and nitrification with rate constants 0.2, official input value for MINMOD, and 0.4. The simulation with the higher rate constant clearly gains a better result. The inadequate prediction of ammonification and nitrification and the significant improvement made with a simple adaptation of one value, call for a more thorough investigation of the value of the rate constant of nitrification as used in MINMOD.

To evaluate the overall performance of the model all simulated values were plotted against their corresponding measured value (figure 5.4). A perfect simulation of the experimental values would yield the line y = x. A paired t-test between this line and plotted values offers an indication of the quality of the simulation. The test showed

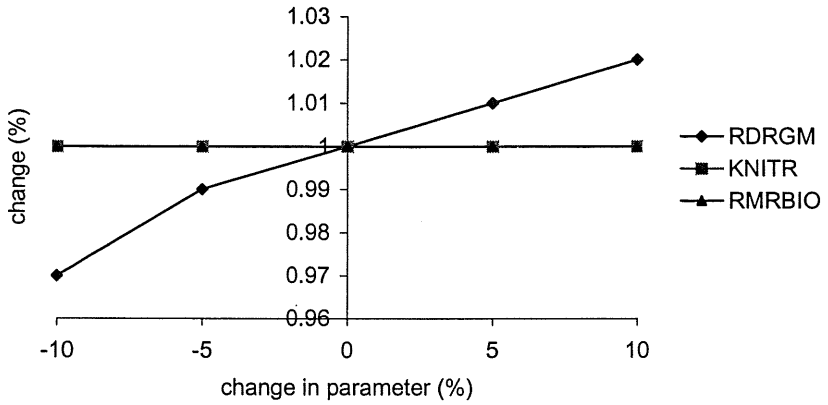


Figure 5.5: Comparison of simulation outcomes for net mineralization at day 35 and 24.5°C when the value of one of the following three parameters is changed: the rate constant of mineralization (RDRGM); the rate constant of nitrification (KNITR); the relative mineralization rate of microbial biomass (RMRBIO).

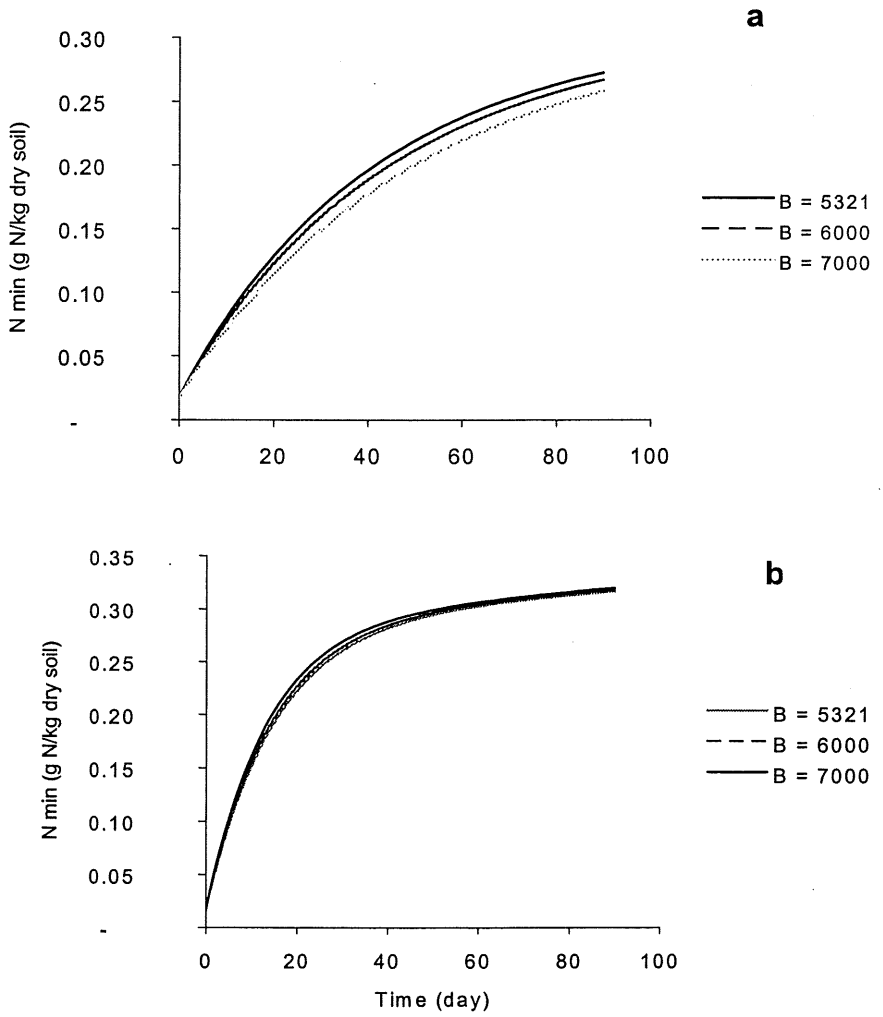


Figure 5.6: Simulations of net mineralization made with MINMOD at 9.8 (a) and 24.5°C (b) for 90 days with three different B's. The other input values for the model can be found in section 5.4.

that the plotted values did not deviate significantly (p = 0.0018) from the line y = x. The conclusion is therefore that the differences between the measured and simulated values are negligible.

5.2 Sensitivity analysis

The simulation results were evaluated for smaller time steps of integration than 0.4 day and for the application of the Euler and Runge-Kutta driver. No different results were found.

Figure 5.5 presents the results of the sensitivity analysis of three important parameters of MINMOD: the rate constant of mineralization (derived from experiment), the rate constant of nitrification and the relative mineralization rate of the microbial biomass (both derived from literature). One parameter value was changed at a time. The other parameters had the values as stated in section 3.5. In this way it becomes very clear which parameter influences the outcome of the simulation of net mineralization most. Besides, one can figure out which parameters have to be determined most accurately. From figure 5.5 can be deduced that only a change of value of the rate constant of mineralization makes a difference in the outcome of the simulation.

Figure 5.6 shows the results of simulation at 24.5 and 9.8°C with three different B's: 5321, 6000, and 7000. Although the lines divert more at the lowest temperature, at both temperatures the distances between the lines are minor. These simulation results strongly suggest that a fixed B, and thus a general temperature correction factor can be incorporated in MINMOD if the main interest is to model mineralization for temperatures between 10 and 25°C. One might wonder why the mineralization curves and thus the temperature correction factor diverse more at lower temperatures. The temperature correction function consists of the ratio of k at a defined temperature and the k at the reference temperature. When this ratio slightly changes with differing B`s, this becomes only clear when the distance between the reference and selected temperature is bigger. For the simulations from which the results are shown in figure 5.5, a reference temperature of 18.8°C was defined. The difference between 18.8 and 9.8 is larger than the distance between 18.8 and 24.5.

Van Schöll (1995) has already investigated the sensitivity for the C/N ratio of the input. It was found that the model adapts correctly to differences in C/N ratio of the applied plant material.

5.3 Concluding remarks

The simulation results suggest that MINMOD predicts adequately the net mineralization rate required for a model of the total intercropping system as described in chapter one. But it is questionable whether MINMOD is suitable for general use. Although the model will become easier to extrapolate when the temperature correction factor is assumed to be independent of site specific circumstances, the conditions for which the model is valid are very limited. This research worked with very specific material, young, very easily decomposable and probably quite homogeneous in contents. It is doubtful whether the model simulates the mineralization course correctly if the incorporated material is more heterogeneous or/and older (Whitmore & Groot, 1994). Besides, it appears that larger deviations from the experimental results are encountered when simulations are made with temperatures below 10°C. If the interest of the research is focused on the course in time of the different components of mineral N, the model is not suitable to use in the present shape. Figure 5.3 suggests that the performance of the model with respect to the simulation of ammonification and nitrification can be considerably improved with an adaptation of the rate constant of nitrification as used in MINMOD. The occurrence of nitrite has not been included in the model till now. The goal of this research is to present a reliable simulation of net mineralization in time. The model is able to do so even though nitrite is not modelled. Consequently, there is no urgent need to investigate the possibilities to include the occurence of nitrite in MINMOD.

Chapter 6

The fate of mineralized nitrogen in the leek intercropping system

A model was developed to describe the influence of the competition for light on yield, taking place in a leek (inter)cropping system at the FAW. In this research project, the mineralization subroutine of NWHEAT was adapted to describe the decomposition of undersown material. To complete the circle, the step from mineralization of N to uptake and utilisation by the crop has to be included to link the below- and aboveground interactions in the studied intercropping system. The first steps towards such a subroutine were made with an investigation of available modules in the literature.

Eight models, describing one or more steps on the way from mineralized nitrogen until crop growth influenced by nitrogen, were compared to obtain an overview of the main components of the models and the way the different steps are handled. Furthermore, features that specifically apply to the fate of nitrogen released from a green manure in a leek (inter)cropping system, are identified in a second section. The knowledge acquired former two sections is combined and on basis of the new insights recommendations are made for the development of a subroutine modelling the fate of mineral nitrogen in the leek intercropping system described in this thesis.

6.1 Review of role of nitrogen in crop growth models

6.1.1 General

In this paragraph eight models, or rather parts of the models, are studied; Authors are Groot (1987), Johnsson (1991), Kersebaum & Richter (1991), Van Keulen (1992), Veen & Frissel (s.a), Parton *et al.,* (1997), Habets & Oomen (1993) and van Keulen & Seligman (1987). Only Veen & Frissel (s.a) did not include the uptake of nitrogen by a crop. The main distinguishing characteristics are classified in a scheme (table 6.1). *Scale*, in *time* and *space*, and the *crop* which is described in the model, are included in the table as background information.

Attainability of soil nitrogen

A simple approach is to regard the rooted zone of the profile as a single compartment in which all mineral nitrogen is potentially available for uptake by the plant (Parton *et al.,* 1987). In more detailed models (e.g. Groot, 1987; Kersebaum & Richter, 1991) different horizontal layers in the root zone are distinguished, each with its own root density and N concentration. Availability of N to plant then depends both on the amount of N present in the soil and the extent and density of the root system of the plant. In the classification scheme under de heading *Rooting zone* a distinction is made between layered rooting zone (*L*) or no specific description (*none*).

The fate of nitrogen in soil

The number of processes influencing the fate of mineral nitrogen in the soil that is accounted for differs among the models. Not all mineral nitrogen in the soil is available for the plant. There is a strong competition for mineral nitrogen in the soil. Nitrate and ammonium can be subject to denitrification, leaching, ammonium

Table 6.1: Basic characteristics of eight models which contain descriptions for processes involved in the uptake of mineral nitrogen by crops.

Model	Scale		Crop	N availability		Growth reduction
	Time	*Space*		*Processes*	*Rooting zone*	
Groot (1987)	Growing season	Field	Winter wheat	L	L	Detailed
Johnsson (1991)	Three years	Field	Barley	D; L	L	-
Kersebaum & Richter (1991)	Growing season	Field	Winter wheat	L	L	Simple
Van Keulen (1982)	Growing season	Field	Natural vegetation	-	L	Simple
Veen & Frissel (s.a.)[a]	Month-2 years	Field	-	D; V; F; L	-	-
CENTURY (Parton *et al.,* 1997)	50 – 2000 years	Regional	Grassland	V	None	Simple
Habets & Oomen (1993)	Decade	Field	Diverse	L	L	Simple
van Keulen & Seligman (1987)	Growing season	Field	Spring wheat	V	L	Detailed

L = leaching; D = denitrification; V = volatilisation of ammonia; F = Fixation of ammonia to clay; L = division in layers.

[a] Model does not contain a subroutine for nitrogen uptake by crop

volatilisation and fixation of ammonium on clay minerals. These processes are referred to under the heading *N availability* in the classification scheme. The potential uptake of nitrogen depends on the demand of the crop. The basic assumption in all models was that the uptake rate is governed by the demand as long as the transport rate of nitrogen from bulk soil to the root surface exceeds the required rate to meet plant demand. In all models further uptake of N is inhibited if demand is satisfied. The nitrogen demand of any plant part at any point in time can be defined as the difference between the maximum amount attained under optimum N-supply and the actual amount in the tissue at that moment (van Keulen & Seligman, 1987). Kropff (1993) formulated the demand (D_N) as follows:

$$D_N = (N_{c,m} - N_{c,a})/T_c = (W*NC_m - N_{c,a})/T_c$$

Where $N_{c,a}$ = actual amount of N in the crop (kg N ha^{-1}); $N_{c,m}$ = maximum amount of N in the crop (kg N ha^{-1}); T_c = time coefficient (d); NC_m = N concentration of the crop (kg N kg^{-1} dry matter) and W = biomass (kg dry matter ha^{-1}). The time coefficient accounts for a delay in uptake, which is in the order of 2 days (van Keulen, 1982). In most models the maximum value changes during crop growth depending on the stage of development. Parton *et al.* (1987 defined the demand on basis of the C/N ratio of the plant. As the C content of plants does not fluctuate much normally, this approach yields similar results to the one based on N content of the crop. Johnsson (1991) developed a model to study the leaching of nitrogen in soil, therefore the definition of potential nitrogen uptake is very summier. He used a logistic uptake curve to define the cumulative potential of N demand during a growing season. The shape of the curve depends on the potential annual uptake of the crop growth, and some other (undefined) shape parameters.

Actual nitrogen uptake

The uptake rate is determined by the development of the crop nitrogen demand during crop growth (Booij *et al.*, 1995) The actual uptake of N by the crop is equal to the minimum of the demand of the plant and the maximum supply by the soil. The actual uptake rate proceeds at a maximum rate until crop demand is satisfied. In the model of Groot (1987) the effect that some roots grow in more favourable conditions than others is accounted for. If total uptake is lower in a layer than the nitrogen demand, the model checks whether uptake from the layers where affluent N is

present can be raised sufficiently to meet the total need. This calculation implies that roots growing under favourable conditions will compensate as much as possible for roots growing under less favourable conditions.

Growth reduction

In the models studied a meteorological condition, mostly radiation, determines the potential growth. Other involved variables like supply of macro nutrients limit the growth of the crop or vegetation. The approach referred to as *simple* in the classification scheme assumes that the growth rate is reduced if the N content of the vegetation decreases below a certain defined level. The threshold value can depend on the phenological stage of the plant. The reduction is linear with the N content of the plant and can be written as:

$$G_a/G_p = (N_{ca}-NC_{mn})/(N_{cr}-NC_{mn})$$

G_a = actual growth rate; G_p = potential growth rate; N_{ca} = actual N concentration of the crop; N_{cr} = critical nitrogen concentration of the crop; NC_{mn} = the minimum nitrogen concentration of a crop (van Keulen, 1982). In the *detailed* description nitrogen status affects the potential assimilation rate of individual leaves under saturating light conditions as the maximum rate of leaf photosynthesis is closely related to the nitrogen content of the leaf material (van Keulen & Seligman, 1987). Besides, nitrogen deficiency can influence allocation of assimilates in plants as nitrogen is a mobile element. Both the simple and detailed approach are based on the same assumption; A nitrogen deficiency affects only the light use efficiency. There are indications (pers. comm. Bastiaans) that other parameters of crop growth are influenced as well, the dry matter distribution and the morphology of the plant.

6.2 The fate of nitrogen in a leek cropping system

6.2.1 Root system of leek

At the Research Institute for Agrobiology and Soil Fertility in the Netherlands investigations were done to determine the differences in biomass production at recommended fertiliser rates between Brussels sprouts (*Brassica oleraceae* var. *gemmifera*) and leeks. Brussels sprouts produce approximately 16-17 tons of dry matter per ha, whereas leeks only produce 9-11 tons. Smit *et al.* (1996) showed in

studies conducted in the Wageningen Rhizolab that roots of leeks penetrated the soil profile slower than Brussels sprouts. Apart from that the volumetric rooting density of Brussels sprouts in the greatest part of the soil profile was higher than that of leeks. The question was whether the lower rooting density of leeks compared to that of Brussels sprouts is responsible for its relatively low growth rate or whether its low root density is merely a consequence of a lower potential growth rate. The results of the experiments of Smit *et al.* strongly suggest that the relatively low biomass production of leek compared to that of Brussels sprouts is not limited by an inferior root system. The first indication is that reducing the fertiliser application rate of nitrogen from over 200 kg to approximately 100 kg ha^{-1} hardly influenced biomass production or nitrogen uptake in leek, whereas that of Brussels sprouts was significantly reduced. The second indication were the results of simulation studies of de Willigen & van Noordwijk (1987). The studies have shown that root densities of approximately 1 cm cm^{-3} can sustain an uptake rate of nitrogen of 0.2-0.3 g m^{-2} d^{-1}, even at very low concentrations of nitrogen in the soil. Smit *et al* (1996) found the following maximum root number in their Rhizolab experiments: 1.1 cm cm^{-3} at a depth of 15-25 cm. They concluded that the accompanying uptake rate of nitrogen is sufficient to support the maximum demand of leek at recommended fertiliser rates (Smit *et al.*, 1996). This suggests that the morphology of the root system of leeks is in general not limiting nitrogen uptake and thus biomass production under ample supply of nitrogen. However, the 1 cm cm^{-3} criteria can not be fulfilled in the period immediately after planting and at lower depths (Smit *et al.*, 1996). An interesting phenomenon with leeks is moreover the distribution of roots in the profile. Maximum root intensity was not found in the upper soil layers, as is the case for most crops, but between 15-25 cm depth.

6.2.2 Synchronisation of availability of nitrogen with demand of leek

Improvements in nitrogen utilisation are expected for leeks if the availability of N is synchronised with the crop's specific pattern of N demand (Smit *et al.* 1996). To define demand the relation between N uptake and dry matter weight has to be known and connected to time. Booij *et al.* (1995) found in their experiments with leek and Brussels sprouts an asymptotic function (Landsberg, 1977) the most appropriate to describe the relationship between dry matter weight and nitrogen uptake if all nitrogen was applied before transplanting:

$$W = W_m (1 - e^{-kNu}) \quad (6.1)$$

Where W = dry matter weight, W_m = maximum dry matter weight for each harvest date, Nu = nitrogen uptake and k = constant. Plasticity of the plants allowed luxury consumption of nitrogen taking place when the availability was ample and dilution of nitrogen when shortage of nitrogen developed during later growth stages. Booij *et al.* (1995) found that the nitrogen concentration in the dry matter should be kept on 2.8 – 3.1% during the whole growing period to achieve a near-maximum dry matter production at any time. Near maximum yield was defined as 95% of the asymptotic value (W_m) of the function. In contrary to Booij *et al.* (1995) Greenwood *et al.* (1990) and Justes *et al.* (1994) found a decreasing nitrogen percentage needed to obtain maximum dry matter yield with increasing crop weight. Greenwood *et al.* proposed a general relationship for the minimum nitrogen concentration, which is required at any time during crop growth to obtain maximum dry matter production at that time:

$$\ln(\%N) = 5.697 - 0.5\ln(W)$$

where %N = N concentration in the dry matter, W = crop dry weight (kg ha^{-1}). Most of the data presented by these authors are restricted to dry matter yields lower than 8 t ha^{-1}. The strongest dilution of N in the plant tissue occurred in the range 1.5 – 4 t ha^{-1}. Booij *et al.* (1995) did not have data within this range. They concluded that the concept of the constant nitrogen concentration is likely to hold for higher dry matter yields, while the non-linear relationship holds for lower dry matter yields (< 4 t ha^{-1}).

To obtain a rough idea whether it would be possible to synchronise the mineralization of incorporated clover and the demand of leek, calculations can be made for nitrogen demand of leek, based on estimations and figures from literature, and compared with output of simulations with MINMOD. Extending the samples of the experiment to the field size, based on the surface area of the sample boxes, which was 0.012 m^2 , 27.75 kg N ha^{-1} was incorporated. Binkley &Vitousek (1992) found that more than 100 kg N ha^{-1} can be bound from the air with the cropping of clover species for one year. Bokhorst (1992) and Barney (1987) mention an amount of incorporated nitrogen of about 50 kg ha^{-1}. Clearly the amount of nitrogen incorporated for the

experiment is below real figures. In the experiment executed by Barney (1987) subterranean clover was used; the obtained 50 kg corresponded to a dry matter yield of 1600 kg ha^{-1}. The experiment was executed in winter so the yield was rather low. As the clover in the intercropping situation will not be on the field for a full growing season, the measured yield by Barney might be a good starting point for our calculations as well. In intercropping experiments with leek and different clover species, executed at the FAW the cover percentage of clover was 60% (unpublished data, Imhof). Combining the figures of Barney and Imhof with the N concentration of the subterranean clover used for the experiment (35,59 g N kg^{-1} dry weight) leads to a yield of 34 kg N ha^{-1} for the intercropping situation.

The calculation of the nitrogen demand of leek is based on research done in the Netherlands at the DLO Research Institute for Agrobiology and Soil Fertility. The different articles discussing the research project contain lots of useful data to base our calculations on. The main difference between the Dutch and Swiss situation appears in the yield data; leek in Switzerland is harvested one month earlier than in the Netherlands due to the earlier start of the winter. On top of that, yields are lower in Switzerland compared at the same point in time, also when just data of research stations are considered. To approach the Swiss situation, Dutch yields obtained from plots without nitrogen fertiliser application were used for the calculations. Hereby it should be kept in mind that the deposition of N from the air is far higher in the Netherlands as in Switzerland. In other words, a certain amount of nitrogen has actually been applied although not visible in the data. The yield figures have been adjusted for the leek density in the intercropping situation according to Imhof (unpublished data) and
van de Poll (1998). All yield data of Booij *et al.* (1996) were decreased with 23% (figure 6.1). The calculation was made on basis of the assumption that the within-row competition is larger than the between-row competition for light. In other words, that the changed spacing of the leek plants did not influence the competition for light or yield substantially. From the relationship between nitrogen uptake and dry matter production at each harvest date as defined in equation 6.1 the required nitrogen uptake to achieve near maximum yield, was calculated.

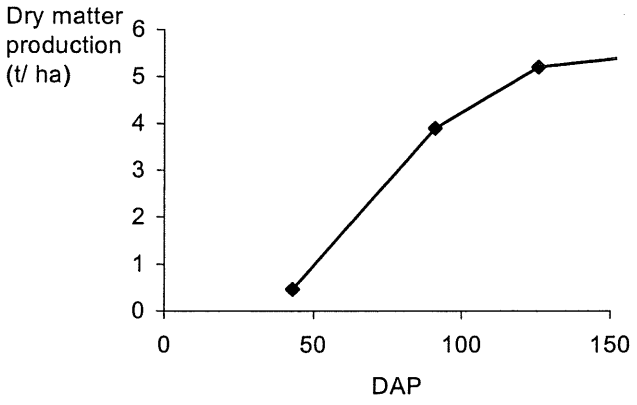


Figure 6.1: Dry matter production of leeks in 1991. No nitrogen application(revised from Booij et al., 1996). DAP = days after

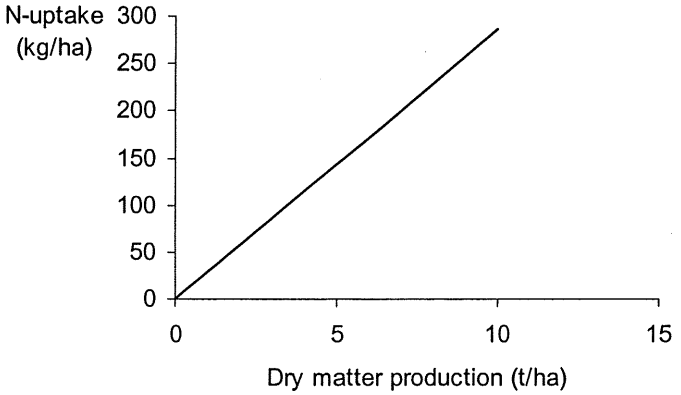


Figure 6.2: The linear regression line calculated for the relationship between nitrogen uptake and near maximum dry matter production of leek based on equation 6.1. Data obtained from an experiment with different N application rates. (revised from Booij et al., 1996). Regression equation: Y = 28.57x (r^2 = 0.88).

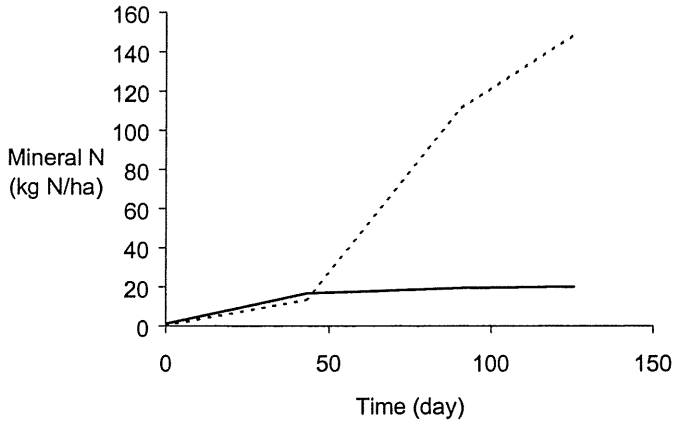


Figure 6.3: Nitrogen uptake of leek needed to achieve 95% of maximum yield (broken line) and net nitrogen mineralization of subterranean clover at 18.8°C simulated with MINMOD (continuous line). Figures based on yields as expected in the leek intercropping system.

Figure 6.2 shows the linear relationship that was found by Booij *et al.* ($p < 0.001$) when plotting the combination of near maximum dry matter (0.95 W_m) and the corresponding N-uptake for all nitrogen application levels. The combination of the data presented in figure 6.1 and figure 6.2 results in an estimation of the nitrogen demand of leek in time. Figure 6.3 shows both the course of nitrogen mineralization simulated for incorporated subterranean clover with a N-input of 34 kg ha^{-1} at a constant temperature of 18.8°C and the estimation of the nitrogen demand of leek in time. With the course of nitrogen mineralization, the time refers to days after incorporation in the soil and for the nitrogen demand of the leek the time refers to days after transplanting. Although the figures used for this part of the research are estimations it is believed that they give a reasonable impression of the quantitative relation between the processes taking place. The clover was incorporated at zero days after transplanting of the leek as a first try. Clearly the mineralization of the subterranean clover can only supply a very limited part of the nitrogen needed by the leek over the growing season. The well synchronised provision and demand of N in the first part of the growing season can be achieved only if the clover is incorporated at the same time as the leek is transplanted. This procedure would encounter quite some objections. First of all, the subterranean clover will have to be sown before the leek is transplanted but it is the question whether the subterranean clover would be able to produce a reasonable amount of biomass in the time between the start of the growing season, and the point in time of transplanting of the leek. Secondly, the weed and pest suppressing effect of the clover will not be utilised. Furthermore it is questionable whether the leek roots are developed well enough directly after transplanting to be able to take up the available nitrogen. These are rather ponderous arguments pleading for an alternative approach. One could incorporate the undersow later. The selected point in time should then be based on a economical and ecological weighting of the importance of the three functions of the undersow: the pest reducing, the weed suppressing and the nitrogen providing effect. Besides, the fact that a bigger percentage of the clover will be mineralized after the growth season when incorporated later, should be taken into account as well. Also with this approach all N supplied by the undersow is taken up by the crop as the supply by the green manure does not exceed the demand of leek at any point in time. It also for this reason that it did not seem useful to simulate incorporation of the undersow at a later point in time as the final conclusion would be the same.

6.3 Concluding remarks

The literature revealed that quite some subroutines were developed, which would fit into the intercropping model in order to bridge the gap between mineralization of nitrogen and functioning of crop. Actually, from table 6.1 it can be concluded that reasonable agreement exists upon the processes that have to be included in such a subroutine and on the way they interact. Almost all workers mention layered soils, volatilisation and/or leaching and describe the influence of nitrogen supply on crop growth in a simple way. Referring to the last point, it can be questioned though whether the simple approach was followed voluntarily or just because of lack of a better description. It is of uttermost importance to truly understand the mechanism behind the processes involved in the transfer of nitrogen from soil to plant. As the intercropping model works on a higher integration level the underlying processes, subroutines in the model, have to be described in the simplest way to be able to understand the results on the higher integration level. In our view the best approach to reach a simplified but correct description of a process is to start with a complete overview of the system and select the mechanisms that seem to have most influence on the output of the system. Based on the comparison of simulation and experimental results effort has to be made to stick to the smallest possible deviation from the experimental results in a biologically sound way.

Many crop growth models in the literature largely neglect root growth and functioning, as far as uptake of nutrients is concerned. The most detailed description found in the studied models was the layering of the soil to simulate the fact that roots are unable to reach nutrients from just everywhere but are bound to a limited volume for uptake. As long as these models are meant to be used on high fertility soils, such as found in Western Europe there is some justification for this neglect, as even here a sparse root system may suffice to take up a nutrient for an adequately long period at the required rate (De Willigen & Noordwijk, 1987). Moreover, the results of the experiments of Smit *et al.* (1996) strongly suggest that the relatively low biomass production of leeks compared to that of Brussels sprouts is not limited by an inferior root system. It does not seem worthwhile to speculate on root growth and uptake of leek to a great detail while the main interest is in prediction of above-ground dry matter production and the root system does not seem to be a determining factor for the growth of the crop.

Significant influence on the availability of N for the crop is expected from other processes related to the fate of nitrogen besides uptake by plant. Four processes were described in the studied models. Leaching and volatilisation were included most often. The relative importance of the different processes will have to be defined in field experiments. The influence on the fate of mineral N in the soil of Switzerland, which tend to have rather high pH might need to be investigated. Mills *et al.* (1974) present averaged nitrogen losses of 47% in soils with a pH range of 7.5-8.0. Veen and Frissel (1981) report even a higher value. A loss of 80% of the formed NH3-N 15 days after application of N fertiliser.

The selection of soil processes that should be included, just like the choice for a certain definition of nitrogen demand, actually all depend on the objective(s) of the model. It should be very clearly stated what questions the final model should help to answer. If there is for example, no interest in the effect of leached N on the concentration of N in the groundwater, there is no need to include all kinds of soil processes in far detail. Another question which should be answered before starting to develop the next part of the leek intercropping model is to what extent it should be possible to extrapolate the model to other crops or other environmental circumstances. For the correct simulation of growth of certain crops, development stages are of great importance while this growth process can be neglected in the case of leeks. To conclude, it is of uttermost importance to constantly keep the goal of the complete system in mind while developing the subroutine that will bridge the gap between the mineralization of green manure and crop growth.

The comparison of nitrogen supply by subterranean clover and the N demand of the leek in time showed that not the synchronisation of the two processes in time is the weakest point of the system. The N provision by the undersow is clearly insufficient for optimal growth of the leek. As the values used for the calculations were taken from a few literature sources and are not specific for the Swiss production situation, next step should be to present preciser figures. It is expected that the nitrogen shortage in the system is actually smaller as leek yields are generally lower in Switzerland than in the Netherlands. Literature suggested that the yield of a specific clover species seems to be a very site-specific question. Quantitative data on the production of clover in terms of biomass and nitrogen content should be produced

production of clover in terms of biomass and nitrogen content should be produced and the qualities of the different clover species for incorporation in the leek intercropping system should be evaluated. When quantitative data about the three main effects of the undersow, weed suppression, pest control and nitrogen supply are known, a sound analysis of the influence of undersown subterranean clover on the production of leek can be made.

Chapter 7
Epilogue

In chapter one, the general introduction, three research questions were posed. In this chapter answers are formulated and the complete executed research is evaluated.

What is the quantitative relation between temperature and rate of mineralization for incorporated Trifolium subterraneum?

The quantitative relation between temperature and rate of mineralization for incorporated *Trifolium subterraneum* was defined on basis of the Arrhenius equation With increasing temperature in the range from 6.8 until 24.5°C the rate of mineralization increased. The equipment used for the incubation experiment, referred to as `the tower` was found to be suitable to for short time experiments (5 weeks) when the moisture content of the soil is allowed to fluctuate slightly. This conclusion is based on measurements made in the tower and the samples. During the incubation period the moisture content of the soil declined slowly. For this experiment it did not cause problems as the difference between the start and final situation of soil moisture content was not too large and mineralization was optimal during the complete period. However, for longer incubation experiments , which demand a stabile soil moisture content, the tower is not the appropriate equipment. In all treatments nitrite was detected during the first week of the incubation period. The occurrence of nitrite was not expected on basis of findings from earlier research and the literature review. It was obvious that the concentration nitrite increased with decreasing temperature. This indicates that temperature was a determining factor for the occurrence of nitrite in this research.

Is it possible to predict the course of nitrogen mineralization from the incorporated crop with the mineralization subroutine in the model NWHEAT as transformed by Laura van Schöll?

The construction of MINMOD was meant to predict the amount of mineralized nitrogen in the soil solution in time as a result of the decomposition of added plant material, using only easily obtainable input parameters. The different steps in the process of decomposition were modelled following first order kinetics. Differences in the plant material were accounted for via the N content of the crop. The influence of temperature was described by an empirically derived correction function. Comparison

of the values obtained via simulation and experiment showed that the model was able to accurately describe net mineralization in the soil solution during five weeks of incubation at constant temperatures ranging from 6.8 until 24.5°C. The course of the NH_4^+ and NO_3^- concentrations was however not simulated properly. Model analysis indicates that most likely the rate constant of mineralization was underestimated. Investigation of the value of the rate constant of nitrification is therefore advised.

In constructing MINMOD, assumptions were made. It can thus be incomplete or oversimplified. The model functions properly for the rather specific conditions of the experiment: incorporation of homogeneous material and at higher temperatures. It is expected that the neglect of the heterogeneity of plant material in MINMOD will result in worse simulation results in case plant material with a higher lignin content than the clover used for the experiment, is incorporated (Whitmore & Groot, 1994). The performance of the model was weakest at the lower temperatures (<10°C), although the simulation of net mineralization in time was still satisfactorily. It is unclear whether the model does not suffice or that the larger deviations between the simulations and the experiment were due to experimental conditions, which were not optimal for the 10°C treatment.

The model was compared to results of the experiment that lasted for five weeks. At the end of the experiment up to 58% of the applied organic N had been mineralized. In order to find out whether the model can predict the mineralization of the remaining part it should be compared to the outcomes of an experiment that covers a longer period of incubation.

Neither the denitrification part nor the moisture correction function in the model were validated. The temperature correction function appeared to be rather insensitive for differences in the main determining parameter, B. Simulation were made with B values ranging from 5321 till 7000 K^{-1} at 9.8 and 24.5°C. No different values were obtained. These results suggest that a fixed temperature correction function can be used in the model, which makes it unnecessary to determine the k and B from an experiment. Next step in the optimisation of MINMOD should be a check of the performance of the model with field data. The influence of the aboveground crop growth on the rate of mineralization has not been discussed in this thesis. As

MINMOD will be incorporated in an intercropping model the influence of this factor should be investigated as well.

Which processes should be taken into account for the development of a link between below- and aboveground interactions specifically important for the studied leek intercropping system?

The comparison of nitrogen supply by subterranean clover and the N demand of the leek in time showed that not the synchronisation of the two processes in time is the weakest point of the system. The N provision by the undersow seems to be highly insufficient for optimal growth of the leek. As this part of the complete intercropping system was only investigated superficially, a more thorough research has to be executed to confirm the findings. However, the calculations strongly suggest that the gap between nitrogen supply and demand is so big that a precise prediction of mineralization in time does not contribute to the overall performance of the total leek intercropping model. In other words, a much simpler description of mineralization in time will suffice for incorporation in the leek intercropping model. Sowing clover twice a year -one or two months before, and at the moment the leek is planted- could be a possibility to increase the overall production of clover biomass. Only when quantitative data about the three main effects of the undersow, weed suppression, pest control and nitrogen supply, are known, a sound analysis of the relative importance of the three factors for the production of leek can be made. Other issues like the improvement of overall soil fertility on the long term and increased diversity on the field are not taken into account as weighting factors at the moment. The relative importance of these factors might increase in the future together with the changing function of agriculture in Europe.

A model was developed for aboveground competition for light taking place in a leek intercropping system, by Daniel Baumann at the FAW. In this research project, MINMOD was constructed to describe the decomposition of undersown material. To complete the circle, the step from mineralization of N to uptake and processing by the crop has to be modelled to link the below ground and above ground interactions in the studied intercropping system. Several examples of descriptions of this part of the system were found in the literature. Reasonable agreement exists about the processes a model should account for but the influence of the in general rather high pH of soils in Switzerland on the fate of mineral N needs attention. The selection of

soil processes that should be included, just like all other choices that have to be made to develop a model, actually all depend on the objective(s) of the model and to what extent it should be possible to extrapolate the model to for example other main crops. During this research project we became aware of the continuous discussion about dilemmas like the proper way to develop a model; how to define the level of detail needed to achieve adequate results; the scientific appreciation of the results of simulations and the performance of a model. Personally we felt a lack of critical mass to approach this issues professionally. During the course of the research we developed certain ideas about this kind of dilemmas but to obtain in general a certain level of quality as theoretical production ecologists, we think it is highly advisable to include a broad discussion on the quality aspect of models in the teaching programme.

To conclude, in this study the relation between the relative mineralization rate and temperature was quantified. MINMOD was able to simulate mineralization accurately under conditions as defined by the experimental set up. Yet the model performance will have to be evaluated for field conditions and the impact of the incorporation of clover on the yield of leek will have to be investigated more thoroughly.

Chapter 8

References

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

Alexander, M. 1961. *Introduction to Soil Microbiology.* New York, USA, John Wiley & Sons.

Altieri, M.A. 1994. *Biodiversity and pest management in agroecosystems.* New York, FPP.

Association of Official Analytical Chemists, 1975, *Official methods of analysis.* PO box 540, Benjamin Franklin Station, Washington. USA.

Atkinson, D., C.A. Watson, J.E. Hooker and K.E. Black. 1995. Nutrient conservation in organic crop production systems: implications for root systems. pp. 59-60. In: Cook, H.F. and H.C. Lee (eds). *Soil management in sustainable agriculture.* Proc. Of the third international conference on sustainable agriculture, 31 August till 4 September 1993, Wye college, University of London, Wye College Press.

Azam, F., F.W. Simmons and R.L. Mulvaney. 1993. Mineralization of N from plant residues and its interaction with native soil N. *Soil Biol. Biochem.* 25;12, 1787-1793.

Baumann, D.T., M.J. Kropff and L. Bastiaans. In press. *Intercropping leek to suppress weeds.*

Barney, P.A. 1987. The use of *Trifolium repens*, *Trifolium subterraneum* and *Medicago lupulina* as overwintering leguminous green manures. *Biological Agriculture and Horticulture* 4, 225-234.

Binkley, D. and P. Vitousek. 1992. Soil nutrient availability. pp. 75-92. In: Pearcy, R.W., J.R. Ehleringer, H.A. Mooney and P.W. Rundel (eds.), *Plant physiological Ecology; filed methods and instrumentation.* London, Chapman & Hall.

Bokhorst, J.G., J. Bloksma and E.T. Lammerts van Bueren. 1992. *Biologische teelt van prei.* The Netherlands, Louis Bolk Instituut.

Booij, C.J.H., J. Noorlander and J. Theunissen. 1997. Intercropping cabbage with clover: effects on ground beetles. *Entomological Research in Organic Agriculture* 1997, 261-268.

Booij, R., A.D.H. Kreuzer, A.L. Smit and A. van der Werf. 1996. Effect of nitrogen availability on dry matter production, nitrogen uptake and light interception of Brussels sprouts and leeks. *Netherlands Journal of Agricultural Science* 44, 3-19.

Bosatta, E., I.K. Iskandar, N.G. Juma, G. Kruh, J.O. Reuss, K.K. Tanji and J.A. van Veen. 1980. Soil Microbiology. pp. 38-44. In: Frissel, M.J. and J.A. van Veen. *Simulation of nitrogen behaviour of soil-plant systems:* Papers of a workshop: Models for the behaviour of nitrogen in soil and uptake by plant: comparison between different approaches; Wageningen, the Netherlands, January 28 - February 1, 1980, Pudoc.

Bosch, H. 1993. *Teelt van prei.* Teelthandleiding nr. 56, Proefstation voor de akkerbouw en de groenteteelt in de vollegrond, Lelystad.

Bradbury, N.J.A.P., P.B. Whitmore, S. Hart, and D.S. Jenkinson. 1993. Modelling the fate of nitrogen in crop and soil in the years following the application of ^{15}N labelled fertiliser to winter-wheat. *J. Agric.Sci. Camb.* 121, 363-379.

Breland, T.A. 1994a. Enhanced mineralization and denitrification as a result of heterogeneous distribution of clover residues in soil. *Plant and Soil* 166, 1-12.

Breland, T.A. 1994b. Measured and predicted mineralization of clover green manure at low temperatures at different depths in two soils. *Plant and Soil* 166, 13-20.

Breland, T.A. 1996. Green manuring with clover and ryegrass catch crops undersown in small grains: effects on soil mineral nitrogen in field and laboratory experiments. *Acta Agric. Scand. B, Soil and Plant Sci.* 46, 178-185.

Bremner, J.M. and K. Shaw. 1958. Denitrification in soil; II. Factors affecting denitrification. *J. Agric. Sci.* 51, 40-52.

Brewster, J.L. 1994. *Onions and other vegetable alliums.* Cambridge, CAB International, Crop production science in horticulture 3.

Brussaard, L. 1991. The roles of various functional groups of organisms. pp 27-34. In: Breemen, N. van (ed). Decomposition and accumulation of organic matter in terrestrial ecosystems: Research priorities and approaches. Proc. Workshop Doorwerth, the Netherlands.

Burford, J.R. and J.M. Bremner. 1975. Relationships between the denitrification capacities of soils and total, watersoluble and readily decomposable soil organic matter. *Soil Biol. Biochem.* 7, 389-394.

Burns, B.R. 1969. Influence of soil moisture tension on nitrate accumulation in soils. *Soil Sci. Soc. Amer. Proc.* 33, 263-266.

Cassman, K.G. and D.N. Munns. 1980. Nitrogen mineralization as affected by soil moisture, temperature, and depth. *Soil Sci. Soc. Am. J.* 44, 1233-1237.

Dourleijn, C.J. 1994. *Ontwerp en analyse van proeven voor plantenteelt en rassenonderzoek.* Lecture notes. Landbouwuniversiteit Wageningen, the Netherlands.

Ellert, B.H. and J.R. Bettany. 1988. Comparison of kinetic models for describing net sulfur and nitrogen mineralization. *Soil Sci. Soc. Am. J.* 52, 1692-1702.

Ellert, B.H. and J.R. Bettany. 1992. Temperature dependence of net nitrogen and sulfur mineralization. *Soil Sci. Soc. Am. J.* 56, 1133-1141.

Fox, R.H. and V.P. Piekielek. 1984. Relationships among anaerobically mineralized nitrogen, chemical indexes and nitrogen availability to corn. *Soil Sci. Soc. Am. J.* 48, 1087-1090.

Frankenberger, W.T. and H.M. Abdelmagid. 1985. Kinetic parameters of nitrogen mineralization rates of leguminous crops incorporated into soil. *Plant and Soil* 87, 257-271.

Gilmour, J.T., M.D. Clark and G.C. Sigua. 1985. Estimating net nitrogen mineralization from carbon dioxide evolution. *Soil Sci. Soc. Am. J.* 49, 1398-1402.

Greenwood, D.J., Ken-inchi Kubo, J.G. Burns and A. Draycott. 1989. Apparent recovery of fertilizer N by vegetable crops. *Soil Sc. and Plant Nutrition* 35, 367-381.

Greenwood, D.J., G. Lemaire, G. Gosse, A. Draycott and J.J. Neeteson. 1990. Decline in percentage N of C3 and C4 crops with increasing plant mass. *Annals of botany* 66, 425-436.

Griffiths, B. and D. Robinson. 1992. Root-induced nitrogen mineralisation: A nitrogen balance model. *Plant and Soil* 139, 253-263.

Groot, J.J.R. 1987. *Simulation of nitrogen balance in a system of winter wheat and soil.* Simulation report CABO-TT nr. 13, Centre for Agrobiological Research and Departement of Theoretical Production Ecology, Wageningen Agricultural University, the Netherlands.

Groot, J.J.R. and P. de Willigen. 1991. Simulation of the nitrogen balance in the soil and a winter wheat crop. pp. 261-272. In: J.J.R. Groot, P. de Willigen and E.L.J. Verberne (eds). *Nitrogen turnover in the soil-crop system: Modelling of biological transformations, transport of nitrogen and nitrogen efficiency.* Proceedings of a workshop held at the institute for Soil Fertility research, 5-6 June 1990, Haren, the Netherlands. Developments in plant and soil sciences vol. 44, Dordrecht, the Netherlands, Kluwer Academic Publishers.

Habets, A.S.J. and G.J.M. Oomen. 1994. Modelling nitrogen dynamics in crop rotations in ecological agriculture. pp. 269-274. In: J.J. Neeteson and J. Hassink (eds). *Nitrogen mineralization in agricultural soils. P*roc. symp. held at the institute for Soil Fertility research, 19-20 April 1993, Haren, NL.

Handbuch Gemüse-Manuel des légumes. 1999. Eidgenossische Forschungsanstalt Wädenswil; Station Fédérale de Recherches Agronomique; Forschungsinstitut für Biologischen Landbau. Switzerland.

Hansen, S., H.E. Jensen, N.E. Nielsen and H. Svensen. 1990. *DAISY – Soil plant atmosphere system model.* Npo-forskning fra Miljo/sttyrelsen, nr. A10, Denmark, National agency of environmental protection.

Harmsen, G.W. and D.A. van Schreven. 1955. Mineralization of organic nitrogen in soil. *Adv. Agron.* 7, 229-398.

Heller, W.E. 1999. Stickstoff-Mineralisierung aus Komposten im Brutversuch. *Agrarforschung* 6;2, 75-77.

Heß, H.E., E. Landolt and R. Hirzel. 1970. Band 2: Nymphaeaceae bis Primulaceae. In: *Flora der Schweiz und angrenzender Gebiete.* Basel und Stuttgart, Birkhäuser Verlag.

Houba, V.J.G., I. Novozamsky, A.W.M. Huybregts and J.J. van der Lee. 1986. Comparison of soil extractions by 0.01 *M* $CaCl_2$, by EUF and by some conventional extraction procedures. *Plant and Soil* 96, 433-437.

Hovenkamp, K. 1996. *Intercropping leek with subterraneum clover: effects on thrips population, leek rust, leek moth, yield and quality.* Msc thesis, Wageningen Agricultural University, the Netherlands, Department of Ecological Agriculture.

Ilnicki, R.D. and A.J. Enache. 1992. Subterranean clover living mulch: an alternative method of weed control. pp. 249-264. In: Paoletti, M.G. and D. Pimentel (eds.). *Special Issue Biotic Diversity in Agroecosystems.* Proceedings from a symposium on agroecology and conservation issues in tropical and temperate regions, 26-29 September 1990, Padova, Italy.

Imhof, T., D.T. Baumann, and I. Wayser-Hammel. 1996a. Herbstlauch: Einfluss von Untersaaten und Beregnung auf Trips tabaci. *Der Gemüsebau/Le Maraîcher* 5, 14-15.

Imhof, T., D.T. Baumann, E. Städtler and I. Wyser-Hammel. 1996b: Undersaat in Herbstlauch reduziert die Thripspopulation. *Agrarforschung* 3;7, 337-340.

Janssen, B.H. 1992. *Organic matter and soil fertility.* Lecture notes. Department of Soil Science and Plant nutrition. Wageningen Agricultural University, the Netherlands.

Jenkinson, D.S. 1990. The turnover of organic carbon and nitrogen in soil. *Phil. Trans. R.Soc. Lond.* 329, 361-368.

Johnsson, H., L. Bergström, P.-E. Jansson and K. Paustian. 1987. Simulated nitrogen dynamics and losses in a layered agricultural soil. *Agric. Ecosystems Environ.* 18, 333-356.

Justes, E., B. Mary, J.-M. Meynard, J.-M. Machet, and L. Thelier-Huches. 1994, Determination of a critical nitrigen dilution curve for winter wheat crops. *Annals of Botany* 74, 397-407.

Kersebaum, K.C. and J. Richter. 1991. Modelling nitrogen dynamics in a plant-soil system with simple model for advisory purposes. pp 273-282. In: J.J.R. Groot, P. de Willigen and E.L.J. Verberne (eds). *Nitrogen turnover in the soil-crop system: Modelling of biological transformations, transport of nitrogen and nitrogen efficiency.* Proceedings of a workshop held at the institute for Soil Fertility research, 5-6 June 1990, Haren, the Netherlands. Developments in plant and soil sciences vol. 44, Dordrecht, the Netherlands, Kluwer Academic Publishers.

Kesper, C. and T. Imhof. 1998. Anbauprobleme im Feldgemüsebau. *Der Gemüsebau* 5, 17-20.

Keulen, H. van. 1982. Crop production under semi-arid conditions as determined by nitrogen and moisture availability. pp 234-249. In: Penning de Vries, F.W.T. and Laar, H.H. van. *Simulation of plant growth and crop production.* Wageningen centre for agricultural publishing and documentation, the Netherlands.

Keulen, H. van and N.G. Seligman. 1987. *Simulation of water use, nitrogen nutrition and growth of a spring wheat crop.* Simulation monographs, Wageningen, the Netherlands, Pudoc.

Kladivko, E.J. and D.R. Keeney. 1987. Soil nitrogen mineralization as affected by water and temperature interactions. *Biol. Fertil. Soils* 5: 248-252.

Knowles, R. 1981. Denitrification. pp 315-329. In: F.E. Clark and T. Rosswall (eds). *Terrestrial nitrogen cycles.* Ecological bulletin 33, Stockholm, Sweden, Swedish Natural Science Research Council.

Kohl, D.H., F. Vithayathil, P. Whitlow, G. Shearer and S.H. Chien. 1976. Denitrification kinetics in soil systems: The significance of good fits of data to mathematical forms. *Soil Sci. Soc. Am. J.* 40, 248-253.

Kowalenko, C.G. and D.R. Cameron. 1976. Nitrogen transformations in an incubated soil as affected by combinations of moisture content and temperature and adsorption-fixation of ammonium. *Can. J. Soil Sci.* 56. 63-70.

Kraalingen, D.G.W. van, C. Rappoldt and H.H. van Laar. 1994. The FORTRAN Simulator Translator (FST), a simulation language. pp 219-230. In: Goudriaan, J. and van Laar, H.H., *Modelling Potential Crop Growth Processes.* Current issues in production ecology. volume 2. Kluwer academic publishers. Dordrecht, the Netherlands.

Kropff, M.J. 1993a. Eco-physiological models for crop-weed competition. pp. 25-32. In: Kropff, M.J. and H.H.van Laar. *Modelling crop-weed interactions.* Wallingford, UK, CAB International in association with International Rice Research Institute.

Kropff, M.J. 1993b. Mechanisms of competition for nitrogen. pp. 77-82. In: Kropff, M.J. and H.H.van Laar. *Modelling crop-weed interactions.* Wallingford, UK, CAB International in association with International Rice Research Institute.

Kropff, M.J., Lotz, L.A.P. and Weaver, S.E. 1993. Practical applications. pp. 149-168. In: Kropff, M.J. and H.H.van Laar. *Modelling crop-weed interactions.* Wallingford, UK, CAB International in association with International Rice Research Institute.

Landsberg, J.J. 1977. Some useful equations for biological studies. *Experimental agriculture* 13, 273-286.

Laura, R.D. 1976. Effects of alkali salts on carbon and nitrogen mineralization of organic matter in soil. *Plant and Soil* 44, 597-605.

Leffelaar, P.A. 1995, Basic elements of dynamic simulation. pp 11-28. In: Leffelaar, P.A. (ed). *On system analysis and simulation of ecological processes.* 2nd ed., Current issues in production ecology, vol 1., Dordrecht, the Netherlands, Kluwer Academic publishers.

Linn, D.M. and J.W. Doran. 1984. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. *Soil Sci. Soc. Am. J.* 48, 1267- 1272.

Maag, S. 1999. *Untersuchungen zur Transferdynamik und Pflanzenverfügbarkeit von Stickstoff nach Einarbeitung verschiedener Zwischenreihenbegrünungen.* MSc thesis, ETH Zürich, Switzerland.

Marshman, N.A. and K.C. Marshall. 1981. Bacterial growth on proteins in the presence of clay minerals. *Soil Biol. Biochem.* 31, 127-134.

Marstorp, H. and H. Kirchmann. 1991. Carbon and nitrogen mineralization and crop uptake of nitrogen from six green manures legumes decomposing in soil. *Acta Agric. Scand.* 41, 243-252.

Mary, B. and S. Recous. 1994. Measurement of nitrogen mineralization and immobilization fluxes in soil as a means of predicting net mineralization. *Eur. J. Agron.* 3;4, 291-300.

McGill, W.B. 1996. Review and classification of ten soil organic matter (SOM) models. pp 11-132. In: D.S. Powlson, P. Smith and J.U. Smith (eds). *Evaluation of Soil Organic Matter Models.* NATO ASI Series 138, Berlin Heidelberg, Springer Verlag.

Mills, H.A., A. V. Barker and D.N. Maynard. 1974. Ammonia volatilization from soils. *Agronomy Journal* 66, 355-358.

Molina, J.A.E. 1996. Description of the model NCSOIL. In: D.S. Powlson, P. Smith and J.U. Smith (eds). *Evaluation of Soil Organic Matter Models.* NATO ASI Series 138, Berlin Heidelberg, Springer Verlag.

Molina, J.A.E., C.E. Clapp, M.J. Schaffer, F.W. Chichester, and W.E. Larson. 1983. NCSOIL, a model of N and C transformations in soil: description, calibration and behaviour. *Soil Sci. Soc. Am. J.* 47, 85-91.

Müller-Schärer, H. and C.A. Potter. 1991. Cover plants in field grown vegetables: prospects and limitations. pp. 599-604. In: *Brighton crop protection conference – weeds.*

Müller-Schärer, H., C.A. Potter and F. Grab. 1992a. Zeitbezogene Schadenwelle im Lauch. *Der Gemüsebau* 6, 7-9.

Müller-Schärer, H., C.A. Potter and B. Hurni. 1992b. Untersaaten bei Herbstlauch. *Der Gemüsebau* 10, 4-7.

Müller-Schärer, H., C.A. Potter and B. Hurni. 1992c. Cover plants in field planted leek: impact on yield, insect pests and nitrogen availability in the soil. pp. 353-355. In: *Proc. 1st International Weed Control Congress,* Monash University, Melbourne, Australia, 17-21 Feb. 1992, Weed Science Society of Victoria Inc. Melbourne, Australia

Müller-Schärer, H. and D.T. Baumann. 1993a. Unkrautregulierung im Gemüsebau: Konzepte zur Reduktion des Herbizideneinsatzes. *Landwirtschaft Schweiz Band* 6, 401-412.

Müller-Schärer, H. and D.T. Baumann. 1993b. Untersaaten im Feldgemüsebau: Möglichkeiten und Begrenzungen. pp 371-372. *Proc. Of 8th EWRS Symposium: Quantitative approaches in weed and herbicide research and their practical application.* Braunschweig, Germany.

Myers, R.J.K., C.A. Campbell and K.L. Weier. 1982. Quantitative relationship between net nitrogen mineralization and moisture content of soils. *Can. J. Soil Sci.* 62, 111-124.

Németh, T. El-Galil, L.A., Radimzky, L. and Baczo, G. 1996. Effect of plant residues on ammonium and nitrate content of soils during incubation. pp109-114. In: Cleemput, O. van *et al. Progress in nitrogen cycling studies.* Kluwer Academic Publishers. The Netherlands.

Neve, S.de and G. Hofman. 1996. Modelling N mineralization of vegetable crop residues during laboratory incubations. *Soil Biol. Biochem.* 28, 1451-1457.

Neve, S. de, J. Pannier and G. Hofman. 1996. Temperature effects on C- and N mineralization from vegetable crop residues. *Plant and soil* 181, 25-30.

Nguyen, M.L., R.J. Haynes and K.M. Goh. 1994. Nutrient budgets and status in three pairs of conventional and alternative mixed cropping farms in Canterbury, New Zealand. *Agriculture, Ecosystems and Environment* 52, 149-162.

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

Oglesby, K.A. and J.H. Fownes. 1992. Effects of chemical composition on nitrogen mineralization from green manures of seven tropical leguminous trees. *Plant and Soil* 143, 127-132.

Oude Voshaar, J.H. 1995. *Statistiek voor onderzoekers met voorbeelden uit de landbouw- en milieuwetenschappen.* 2nd ed.,Wageningen, the Netherlands, Wageningen Pers.

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.

Pathak, H. and D.L.N. Rao. 1998. Carbon and nitrogen mineralization from added organic matter in saline and alkali soils. *Soil Biol. Biochem.* 30;6, 695-702

Paul, E.A. 1984. Dynamics of organic matter in soils. *Plant and Soil* 76, 275-285.

Poll, W. van de. 1998. *Intercropping leek with celery; effects on yield, nitrogen uptake and root dynamics.* Msc thesis, Wageningen Agricultural University, the Netherlands, Department of Theoretical Production Ecology.

Reber, H. and A. Schara. 1971. Degradation sequences in wheat straw extract inoculated with soil suspentions. *Soil Biol. Biochem.* 3, 381-383.

Rees, R.M. 1989. Measurement of mineralisation. In: Hansen, J.A. and Hendriksen, K. *Nitrogen in organic wastes applied to soils.*

Reinertsen, S.A., L.F. Elliott, V.L. Cochran and G.S. Campbell. 1984. Role of available carbon and nitrogen in determining the rate of wheat straw decomposition. *Soil Biol. Biochem.* 16;5, 459-464.

Robinson, D., B. Griffiths, K. Ritz and R. Wheatley. 1989. Root-induced nitrogen mineralisation: A theoretical analysis. *Plant and Soil* 117, 185-193.

Ruiter, P.C. de and H.G. van Faassen. 1994. A comparison between an organic matter dynamics model and a food web model simulating nitrogen mineralization in agro-ecosystems. pp 207-219. In: J.J. Neeteson and J. Hassink (eds). *Nitrogen mineralization in agricultural soils.* Proc. symp. held at the institute for Soil Fertility research, 19-20 April 1993, Haren, the Netherlands.

Ruiter, P.C. de, A.M. Neutel and J.C. Moore. 1994. Modelling food webs and nutrient cycling in agro-ecosystems. *Tree* 9;10, 378-383.

Ruiter, P.C. de, J.A. van Veen, J.C. Moore, L. Brussaard and H.W. Hunt. 1993a. Calculation of nitrogen mineralization in soil food webs. *Plant and Soil* 157, 263-273.

Ruiter, P.C. de, J.C. Moore, K.B. Zwart, L.A. Bouwman, J. Hassink, J.A. de Vos, J.C.Y. Marinissen, W.A.M. Didden, G. Lebbink and L. Brussaard. 1993b. Simulation of nitrogen mineralization in the below ground food webs of two winter wheat fields. *Journal of Applied Ecology* 30, 95-106.

Russel, E.W. 1973. *Soil conditions and plant growth.* 10th edition. Longman London. pp 150-260.

Sarathchandra, S.U., K.W. Perrott, M.R. Boase and J.E. Waller. 1988. Seasonal changes and the effects of fertiliser on some chemical, biochemical and microbiological characteristics of high-producing pastoral soil. *Biol. Fertil. Soils* 6, 328-335.

Schnitzer, M. 1977. Recent findings on characterisation of humic substances extracted from soils from widely differing climatic zones. pp 117-132. In: I.A.E.A.E. *Soil organic matter studies.* Volume 2. Vienna.

Schöll, L. van. 1995. *Mineralization of nitrogen from incorporated catch crops: Experiment and simulation.* MSc thesis, Wageningen Agricultural University, the Netherlands.

Schöll, L. van, A.M. van Dam and P.A. Leffelaar. 1997 Mineralisation of nitrogen from an incorporated catch crop at low temperatures: experiment and simulation. *Plant and Soil* 188, 211-219.

Schweizer, C. 1997. *Untersuchungen zur Stickstoffdynamik, der damit verbunddenen Pflanzenverträglichkeit und zur N-Verfügbarkeit von Komposten aus unterschiedlichen Herstellungsverfahren.* Msc thesis, Eidgenössische Technische Hochschule Zürich, Switserland, Institut für Pflanzenwissenschaften, Gruppe Pflanzenernährung.

Schweizerische Referenzmethoden der Eidgenössischen Landwirtschaftlichen Forschungsanstalten. 1996a, revised 1998. Volumenextraktion mit Wasser. Band 1: Bodenuntersuchungen zur Düngeberatung. Eidg. Forschungsanstalt für Agrarökologie und Landbau (FAL), Reckenholz, Eidg. Forschungsanstalt für Pflanzenbau (RAC), Changings, Eidg. Forschungsanstalt für Wein-, Obst_ und Gemüsebau (FAW).

Schweizerische Referenzmethoden der Eidgenössischen Landwirtschaftlichen Forschungsanstalten. 1996b, revised 1998. Untersuchung der N-dynamik von Böden und Substraten im aeroben Brutversuch. Band 2: Bodenuntersuchung zur Standort-Charakterisierung. Eidg. Forschungsanstalt für Agrarökologie und Landbau (FAL), Reckenholz, Eidg. Forschungsanstalt für Pflanzenbau (RAC), Changings, Eidg. Forschungsanstalt für Wein-, Obst_ und Gemüsebau (FAW).

Smit, A.L. and Van der Werf, A. 1992. Physiology of nitrogen uptake and utilisation (in Dutch). pp 51-69. In: Van der Meer, H.G. and Spiertz, J.H.J. *Stikstofstromen in agroecosystemen.* Agrobiologische thema's no 6. DLO-centrum voor Agrobiologisch onderzoek (CABO-DLO). Wageningen, the Netherlands.

Smit, A.L., R. Booij, and A. van der Werf. 1996. The spatial and temporal rooting pattern of Brussels sprouts and leeks. *Netherlands Journal of Agricultural Science* 44, 57-72.

Smith, J.L., R.R. Schnabel, B.L. McNeal, and G.S. Campbell. 1980. Potential errors in the first-order model for estimating soil nitrogen mineralization potentials. *Soil Sci. Soc. Am. J.* 44, 996-1000.

Solomon, D. 1994. *Mineralization, immobilization and uptake of N from N-poor and N-rich organic mixtures.* MSc thesis, Wageningen Agricultural University, the Netherlands, Department of Soil Science and Plant Nutrition.

Stanford, G., S. Dzienia and R.A. Vanderpol. 1975b. Effect of temperature on denitrification rate in soils. *Soil Sci. Soc. Amer. Proc.* 39, 866-870.

Stanford, G. and E. Epstein. 1974. Nitrogen mineralization-water relations in soils. *Soil Sci. Soc. Am. J.* 38, 103-107.

Stanford, G., M.H. Frere and D.H. Schwaninger. 1973. Temperature coefficient of soil nitrogen mineralization. *Soil Science* 154-4, 321-323.

Stanford, G. and S.J. Smith. 1972. Nitrogen mineralization potentials of soils. *Soil Sci. Soc. Am. Proc.* 36, 465-472.

Theunissen, J. 1997. Application of intercropping in organic agriculture. *Entomological research in organic farming,* 251-259.

Theunissen, J. and G. Schelling. 1996. Pest and disease management by intercropping: suppression of thrips and rust in leek. *International Journal of pest management* 42;4, 227-234.

Thorup-Kristensen, K. 1994. An easy pot incubation method for measuring nitrogen mineralization from easily decomposable organic material under well defined conditions. *Fertilizer research* 38, 239-247.

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

Tyler, K.B., F.E. Broadbent, and G.N. Hill. 1959. Low temperature effects on nitrification on four California soils. *Soil Sci.* 87, 123.129.

Vandermeer, J.H. 1989. *The ecology of intercropping*. Cambridge University Press. Great Brittain.

Veen, J.A. van and M.J. Frissel. s.a. Mathematical modelling of nitrogen transformations in soil. pp 133-156 In: J.K.R. Gasser (ed). *Modelling nitrogen from farm wastes.* London, Applied Science, Publishers LTD.

Veen, J.A. van, W.B. McGill, H.W. Hunt, M.J. Frissel and C.V. Cole. 1981. Simulation models of the terrestrial nitrogen cycle. pp. 25-48. In: Clark, F.E. and T. Rosswall (eds). *Terrestrial nitrogen cycles,* Ecological bulletin 33, Stockholm, Sweden, Swedish Natural Science Research Council.

Velthof, G.L. 1997. *Nitrous oxide emissions from intensively managed grasslands*. PhD thesis, Wageningen Agricultural University.

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

Vigil, M.F. and D.E. Kissel. 1991. Equations for estimating the amount of nitrogen mineralized from crop residues. *Soil Sci. Soc. Am. J.* 55, 757-761.

Weeda, E.J., R. Westra, C. Westra, T. Westra. 1987. *Ecologische flora van Nederland*, deel 4. Uitgave IVN in samenwerking met VARA en VEWIN. De Lange/Van Leer, Deventer.

Weingartner, U. 1996. *Untersuchungen zur Nitrifikationsleistung von Böden bei unterschiedlicher Belastung mit Schwermetallen*. MSc thesis, Institut for Pflanzennahrung, ETH Zürich.

Werf, A. van der, C.T. Enserink, A.L. Smit and R. Booij. 1996. Components of relative growth rate and nitrogen productivity of Brussels sprouts and leeks grown at two widely differing light intensities. *Netherlands Journal of Agricultural Science* 44, 21-29.

Whitmore, A.P and J.J.R. Groot. 1994. The mineralization of N from finely or coarsely chopped crop residues: measurement and modelling. pp 245-254. In: Neeteson, J.J. and Hassink, J. (eds). *Nitrogen mineralization in agricultural soils*. Proc. af symposium held at the Institute for Soil Fertility Research, Haren, the Netherlands. 19-20 april 1993.

Wijnja, H. and M.L. van Beusichem. 1998. *Inleiding bodemkunde (A), Inleiding Bodemkunde (B) en geologie.* Lecture notes. Department of Soil Science and Geology, Department of Soil Science and Plant Nutrition and department of Water conservation. Wageningen Agricultural University, the Netherlands

Willigen, P. de. 1991. Nitrogen turnover in the soil-crop system; comparison of fourteen simulation models. pp. 141-151. In: J.J.R. Groot, P. de Willigen and E.L.J. Verberne (eds). *Nitrogen turnover in the soil-crop system: Modelling of biological transformations, transport of nitrogen and nitrogen efficiency.* Proceedings of a workshop held at the institute for Soil Fertility research, 5-6 June 1990, Haren, the Netherlands. Developments in plant and soil sciences vol. 44, Dordrecht, the Netherlands, Kluwer Academic Publishers.

Willigen, P. de and J.J. Neeteson. 1985. A comparison of six simulation models on the N cycle. *Fertilizer Research* 8, 157-171.

Yang, H.S. 1996. *Modelling organic matter mineralization and exploring options for organic matter management in arable farming in northern China.* Phd thesis, Wageningen Agricultural University, the Netherlands.

Appendices

Appendix 1: Definition of state and rate constants and parameters

AMMO = amount of ammonium [g N/kg dry soil]
ANLAY = amount of mineral N [g N/kg dry soil]
B = constant used for calculation temperature correction factor [°K]
CCGM = carbon content of green manure [g C/g dry weight]
DECDEF = value of temperature correction factor for humification at 0°C [-]
DELT = time step of integration [day]
DENI = amount of N denitrified [g N/kg dry soil]
DISASS = dissimilation to assimilation ratio for soil biomass [-]
DRGM = decomposition rate of green matter [g dry weight/kg dry soil day]
FINTIM = end time of simulation [day]
GMLAY = amount of green manure applied [g dry weight/kg dry soil]
FRZPNT = temperature at which soil is actually frozen [°C]
IAMMO = initial amount of ammonium N [g N/kg dry soil]
IGMLAY = initial amount of green manure [g dry weight/kg dry soil]
IIMMOB = initial amount of N immobilized by soil micro-organisms [g N/kg dry soil]
IMINGM = initial amount of N mineralized from the green manure [g N/kg dry soil]
INDENI = initial amount of N denitrified [g N/kg dry soil]
INITR = initial amount of N nitrified [g N/kg dry soil]
IMMOB = amount of N immobilized by soil micro-organisms [g N/kg dry soil]
KDENI = rate constant of denitrification [l/day]
KNITR = rate constant of nitrification [1/day]
MINGM = amount of N mineralized from the green manure [g N/kg dry soil]
MRBIO = mineralization rate microbial biomass [g N/kg dry soil day]
NCGM = nitrogen content of the green manure [g N/g dry weight]
NCRB = nitrogen to carbon ratio of the microbial biomass
NDEMB = nitrogen demand for assimilation biomass upon humification of green manure [g N/kg dry soil day]
NDEMBB = nitrogen demand for assimilation from decomposition of microbial biomass [g N/kg dry soil day]
NDEMBS = nitrogen requirement of the microbial biomass that has to be covered by the mineral N in the soil solution [g N/kg dry soil day]
NITDEF = value of temperature correction factor for nitrification at 0°C [-]
NITR = amount of nitrate [g N/kg dry soil]
NLOSS = amount of mineral nitrogen lost from the soil solution by assimilation [g N/kg dry soil day]
PDRGM = potential humification rate of green manure [g dry weight/kg dry soil day]
PMINN = potential rate of ammonium release from green manure [g N/kg dry soil day]
PNDEMB = potential rate of nitrogen demand for assimilation from decomposition of green manure [g N/kg dry soil day]
PNDEMS = potential rate of nitrogen demand for assimilation, that has to be supplied by th mineral nitrogen in the soil solution [g N/kg dry soil day]
PRAMMO = potential rate of ammonification
RAMMO = rate of net increase of ammonium [g N/kg dry soil day]
RAMMOB = rate of increase of ammonium released by dissimilation of the microbial biomass
RDENI = rate of denitrification [g N/kg dry soil day]
RDRGM = relative humification rate of humification [l/day]

REFT = reference temperature [°C]
RIMMO = rate of N immobilization [N/kg dry soil day]
RMINN = rate of mineralization of N from green manure [g N/kg dry soil day]
RMRBIO = relative mineralization rate of microbial biomass [1/day]
RNIT = net rate of nitrification [g N/kg dry soil day]
RNITR = rate of nitrification from the green manure [g N/kg dry soil]
T = soil temperature [°C]
TRFDEC = temperature reduction factor of humification [-]
TRFNIT = temperature reduction factor of nitrification [-]
WCACT = actual water content [cm3/cm3]
WCFLDC = water content at field capacity [cm3/cm3]
WCMAX = water content at saturation point [cm3/cm3]
WCPF24 = water content at pF 2.4 [cm3/cm3]
WCWILT = water content at wilting point [cm3/cm3]
WRFDEC = soil moisture correction factor of decomposition [-]
WRFDEN = soil moisture correction factor of denitrification [-]

Appendix 2: The FST listing of MINMOD

```
DECLARATIONS
DEFINE_CALL MINR(INPUT,INPUT,INPUT,INPUT,INPUT,INPUT,INPUT,INPUT,...
                 INPUT,INPUT,INPUT,INPUT,INPUT,INPUT,INPUT,INPUT,...
                 INPUT,INPUT,INPUT,INPUT,INPUT,INPUT,OUTPUT,OUTPUT,...
                 OUTPUT,OUTPUT,OUTPUT,OUTPUT,OUTPUT,OUTPUT)

DEFINE_CALL DENITR(INPUT,INPUT,INPUT,INPUT,INPUT,INPUT,OUTPUT,OUTPUT)

INITIAL

      INCON IGMLAY=13.5, IMINGM=0.
*     [g d.w./kg soil]
      INCON IIMMOB=0.,IAMMO=0.0001, INITR=0.017, INDENI=0.
*     [g N/kg soil]
      PARAMETER RDRGM=0.046, RMRBIO=0.0018, KDENI=0.00017, KNITR=0.2
*     [day-1]
      PARAMETER CCGM=0.47, NCGM=0.036,
*     [g C/g d.w.] and [g N/g d.w.]
      PARAMETER NCRB=0.1, DISASS=2.
*     [g N/g C], [g C diss/g C ass]
      PARAMETER B=5321.
*     [K-1]
      PARAMETER WCWILT=0.03,WCPF24=0.172,WCACT=0.245,...
                WCFLDC=0.254, WCMAX=0.428
*     [m3 water/m3 soil]
      PARAMETER T=6.8, REFT=20., FRZPNT=-2.
*     [deg C]

      TIMER  STTIME=0., FINTIM=125., DELT=0.5, PRDEL=1.0

      PRINT  T, ANLAY, AMMO, NITR, IMMOB, NDEMBS, NDEMB, DENI, MINGM

      TRANSLATION_GENERAL DRIVER = 'EUDRIV'

*     Reading of the temperature data in file "weather\nld1.954"
*      WEATHER WTRDIR='C:\SYS\WEATHER\', CNTR='NLD', ISTN=1, IYEAR=1954
```

```
DYNAMIC
      GMLAY=INTGRL(IGMLAY,DRGM)
*     [g d.w./kg soil]

     MINGM=INTGRL(IMINGM,RMINN)
      IMMOB=INTGRL(IIMMOB,RIMMO)
      AMMO=INTGRL(IAMMO,RAMMO)
      NITR=INTGRL(INITR,RNIT)
      ANLAY=NITR+AMMO
      DENI=INTGRL(INDENI,RDENI)
*     [g N/kg soil]

*As simulations only took place with constant temperatures the next part
was not *mobilized in this research
*      AVTEMP= 0.5* (TMMX+TMMN)
*      T=AVTEMP

      CALL MINR (T,FRZPNT,B,REFT,WCACT,WCWILT,WCPF24,WCFLDC,...
                 WCMAX,RDRGM,RMRBIO,GMLAY,IMMOB,NCGM,NCRB,CCGM,...
                 DISASS,ANLAY,KNITR,DELT,AMMO,NITR,TRFNIT,DRGM,...
                 RMINN,RIMMO,RAMMO,RNITR,NDEMB,NDEMBS)

      CALL DENITR (WCACT,WCMAX,NITR,KDENI,TRFNIT,RNITR,RNIT,RDENI)

END
STOP
************************************************************************
****
      SUBROUTINE MINR (T,FRZPNT,B,REFT,WCACT,WCWILT,WCPF24,WCFLDC,
     $                 WCMAX,RDRGM,RMRBIO,GMLAY,IMMOB,NCGM,NCRB,CCGM,
     $                 DISASS,ANLAY,KNITR,DELT,AMMO,NITR,TRFNIT,DRGM,
     $                 RMINN,RIMMO,RAMMO,RNITR,NDEMB,NDEMBS)


      IMPLICIT REAL (A-Z)


********* Calculation of the temperature correction factor for
********* decomposition rate constant.

*     If temperature comes below actual freezing point, decomposition
*     halts, the temperature correction factor is 0. Between actual
```

```
*     soil freezing point and 0°C the correction factor is linear from 0
*     to the intercept of the Arrhenius function with Yaxis. At tempera-
tures
*     above 0C the temperature effect is described by a modified Arrhenius
func
*     tion.

      IF (T.LE.FRZPNT) THEN
           TRFDEC=0.
      ELSEIF (T.LT.0.) THEN
            DECDEF= EXP(-B/273.)/EXP(-B/(REFT+273.))
                TRFDEC=(DECDEF/FRZPNT)*(FRZPNT-T)
      ELSE
                TRFDEC=EXP(-B/(T+273.))/EXP(-B/(REFT+273.))
      ENDIF

********* Calculation of the soil moisture correction factor
********* for the decomposition rate constant.

*     At soil water contents below wilting point (pF 4.2), decomposition
*     halts, the correction factor is 0.

      IF (WCACT.LE.WCWILT) THEN
        WRFDEC=0.
      ELSEIF (WCACT.LE.WCPF24) THEN
        WRFDEC=( WCACT/(WCPF24-WCWILT) - WCWILT/(WCPF24-WCWILT) )
      ELSEIF (WCACT.LE.WCFLDC) THEN
        WRFDEC=1.
      ELSE
        WRFDEC=( 0.8*WCACT/(WCFLDC-WCMAX) - 0.8*WCMAX/(WCFLDC-WCMAX) )
      ENDIF

********* Calcualtion of the temperature correction factor for
********* nitrification.
*      Nitrification is corrected for temperature by a temperature
*      correction factor. The correction factor is linear from
*      0 at -1C to 1 at 5C. At temperatures above 5C the rela-
*     tive nitrification rate is not influenced by temperature.

      IF (T.LE.FRZPNT) THEN
        TRFNIT= 0.
      ELSEIF (T.LE.5.) THEN
```

```
        NITDEF=EXP(-B/278.)/EXP(-B/(REFT+273))
        TRFNIT=(NITDEF/(5.-FRZPNT))*(T-FRZPNT)
      ELSE
        TRFNIT=TRFDEC
      ENDIF

********* Calculation of the potential and actual decomposition
********* rate constants.

      PDRGM=-RDRGM*TRFDEC*WRFDEC*GMLAY
*     pot. Decomposition [g d.w./kg soil/day]
      PMINN=-PDRGM*NCGM
*     pot. Mineralisation released as NH4 [g N/kg soil/day]
      PNDEMB=-NCRB*PDRGM*CCGM/(DISASS+1.)
*     pot. Microbial consumption of N


*     The nitrogen demand of the microbial biomass has to be
*     satisfied.
*     If the potential rate of mineralization is bigger than the
*     demand by the biomass, potential rates are actual rates and
*     ammonium will be released. Nitrification will take place.

      IF (PNDEMB.LE.PMINN) THEN
        DRGM=PDRGM
        RMINN=PMINN
        NDEMB=PNDEMB
        PRAMMO=RMINN-NDEMB
        NLOSS=0.
        RNITR=KNITR*TRFNIT*WRFDEC*AMMO

*     If the nitrogen demand by the biomass is bigger than the
*     potential rate of mineralization, no ammonium will be released
*     and soil mineral nitrogen will be taken up. No nitrification will
*     take place.
*     In case soil nitrogen covers the demand not supplied by
*     nitrogen from fresh material, potential rates are actual rates.
*     First all ammonium will be immobilized, if the amount of ammonium
*     is not sufficient, nitrate is consequently immobilized.
```

```
      ELSE
        PNDEMS=AMAX1(0.,PNDEMB-PMINN)
        IF (ANLAY.GE.PNDEMS*DELT) THEN
          DRGM=PDRGM
          RMINN=PMINN
          NDEMB=PNDEMB
*         Total microbial consumption of N
          NDEMBS=PNDEMS
*         Microbial consumption of N from the soil solution
          IF (AMMO.GE.PNDEMS*DELT) THEN
             PRAMMO=-NDEMBS
             RNITR=0.
             NLOSS=NDEMBS
          ELSE
             PRAMMO=-AMMO/DELT
             RNITR=-NDEMBS-PRAMMO
             NLOSS=NDEMBS
          ENDIF

*     If soil mineral nitrogen is smaller than demand made by the
*     biomass, potential rates are reduced.

        ELSE
          DRGM=PDRGM*(ANLAY/(PNDEMS*DELT))
          RMINN=-DRGM*NCGM
          NDEMB=-NCRB*DRGM*CCGM/(DISASS+1.)
          NDEMBS=NDEMB-RMINN
*         From these eq. It can also be shown that NDEMBS=-(NITR+AMMO)/DELT
          PRAMMO=-AMMO/DELT
          RNITR=-NITR/DELT
          NLOSS=NDEMBS
        ENDIF
      ENDIF


********** Calculation of the mineralization of N from the microbial
********** biomass.
*      Mineralization of biomass N is corrected for temperature and soil
*      moisture. Part of N will be assimilated, the remaining released as
*      ammonium.
```

```
      MRBIO=RMRBIO*TRFDEC*WRFDEC*IMMOB
      NDEMBB=MRBIO/(DISASS+1.)
      RAMMOB=MRBIO*DISASS/(DISASS+1.)



********** Calculation of the nitrification rate and net ammonifi-
********** cation rate.
*       The correction factor for water content is the same as for decompo-
*       sition. Net ammonification is potential ammonification rate from
*       fresh matter plus ammonification rate from microbial biomass
*       minus nitrification rate.


      RAMMO=PRAMMO+RAMMOB-RNITR
      RIMMO=NDEMB-MRBIO+NDEMBB

      RETURN
      END

************************************************************************
****
      SUBROUTINE DENITR (WCACT,WCMAX,NITR,KDENI,TRFNIT,RNITR,RNIT,RDENI)

      IMPLICIT REAL (A-Z)


      IF (WCACT.GE.(0.8*WCMAX)) THEN
         WRFDEN=(WCACT-0.8*WCMAX)/(0.2*WCMAX)
         RDENI=KDENI*TRFNIT*WRFDEN*NITR
      ELSE
         RDENI =0.
      ENDIF

      RNIT=RNITR-RDENI

      RETURN
      END
************************************************************************
***
ENDJOB
```

Appendix 3: Equations belonging to chapter 4

- *Conversion of data from micro M N (X_1) to mg N kg^{-1} dry soil (X_3).*

Micro M N → mg N l^{-1} solution:
$X_1 : 1000 * 14.007 = X_2$

The 234 ml soil solution was incorporated in 116 g dry soil:
$X_2 * 0.234 * (1000/116) = X_3$

- *To fit the curve $N_{min} = N_{max} *(1-e^{-kt})$ in order to obtain the rate constant k (d^{-1}), the maximum amount of organic N that can be mineralised (N_{max})(mg N kg^{-1} dry soil) has to be calculated from supplementary data. N_{min} is the measured value of total mineral N (NH_4+NO_2+ NO_3)(mg N kg^{-1} dry soil) and t the days after incorporation of the clover (d).*

N_{max} can be calculated from the following formula (Schöll *et al.*, 1997):

$$N_{max} = G_m * (N_{contGm} - C_{contGm} * ({}^{N}/_{C})_{microo} *({}^{ass}/_{diss+ass}))$$

G_m = the amount of incorporated green matter (g dry weight kg^{-1} dry soil); N_{contGm} = the N content of the green manure (g N kg^{-1} dry weight); C_{contGm} = the C content of the green manure (g C kg^{-1} dry weight); $({}^{N}/_{C})_{microo}$ = the N to C ratio of the microbial biomass, taken as (1:10) (-) (Groot, 1987); $_{diss}$ and $_{ass}$ the dissimilation and assimilation fraction respectively of the microbial biomass, with value of 2 and 1(-) (Groot, 1987).

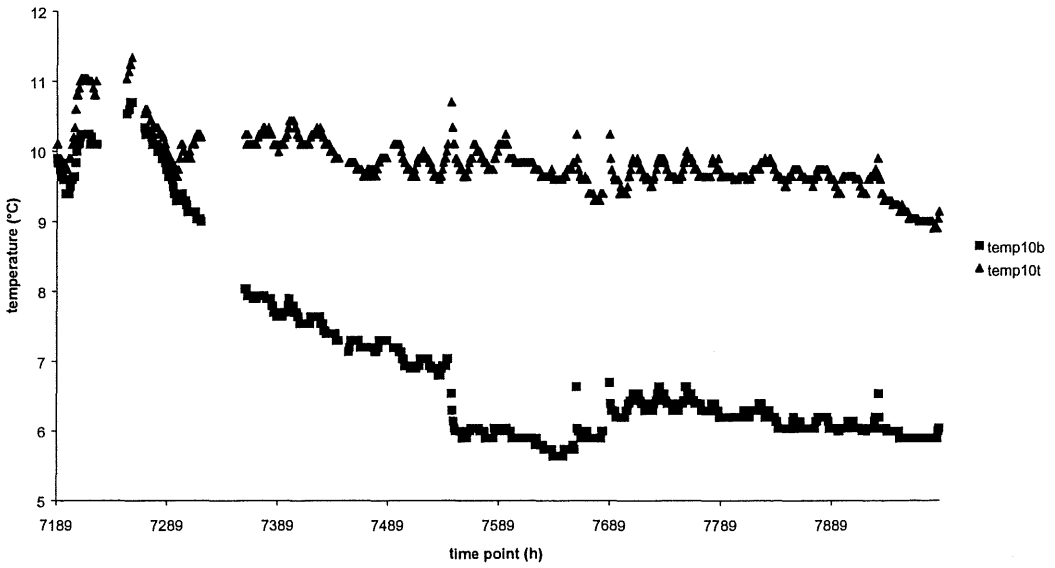
$N_{max} = (1.6 * 1000/116) * (0.036 - 0.47 * 0.1 * 0.3333) = 0.28$

- *Calculation of Q_{10}*

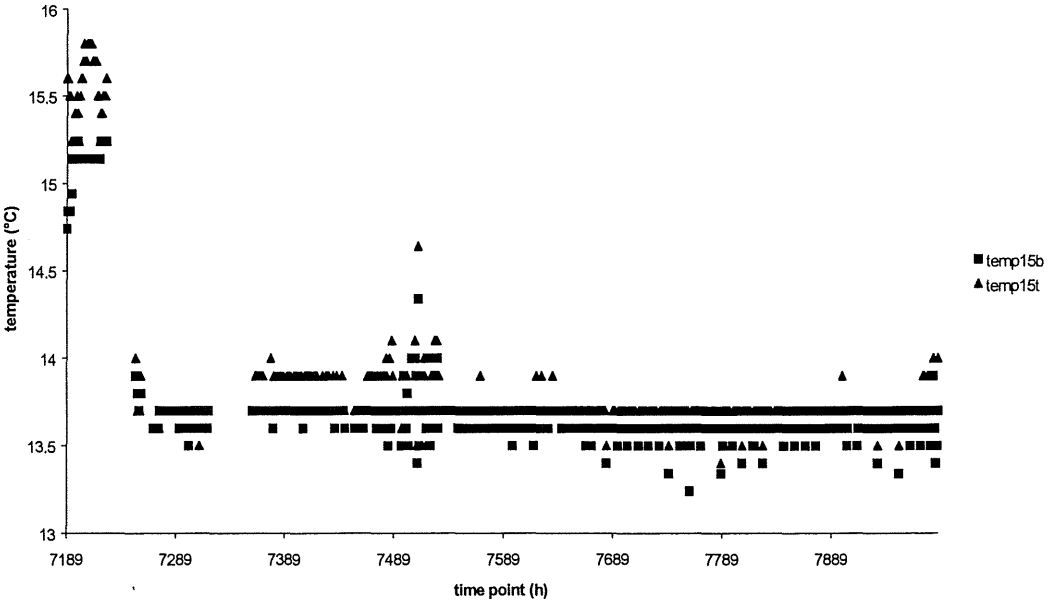
$k/k_0 = Q_{10}^{(\Delta T/10)}$
$k/k_0 = e^{(\ln Q10)\ (\Delta T/10)}$
$\ln k/k_0 = \ln e^{(\ln Q10)\ (\Delta T/10)}$
$\ln k/k_0 = (\ln Q_{10})/10\ (\Delta T)$

Appendix 4: Measured temperatures and relative humidity in towers of the 10 – 15 – 20 - 25°C treatments.

a



b



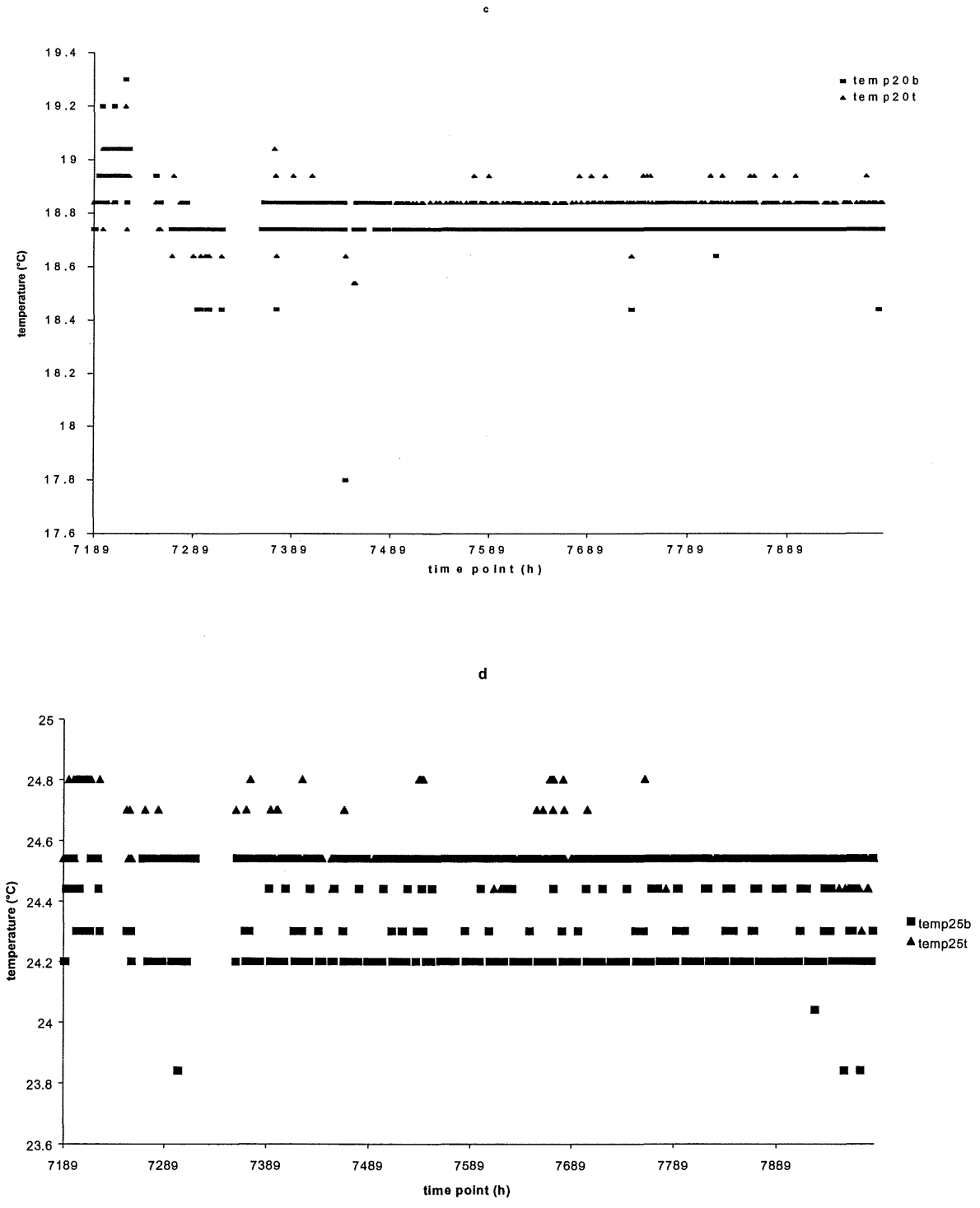


Figure a-d: Temperature course in the top (t) and bottom (b)crate of the tower at 10-15-20-25°C during the incubation period of the experiment.

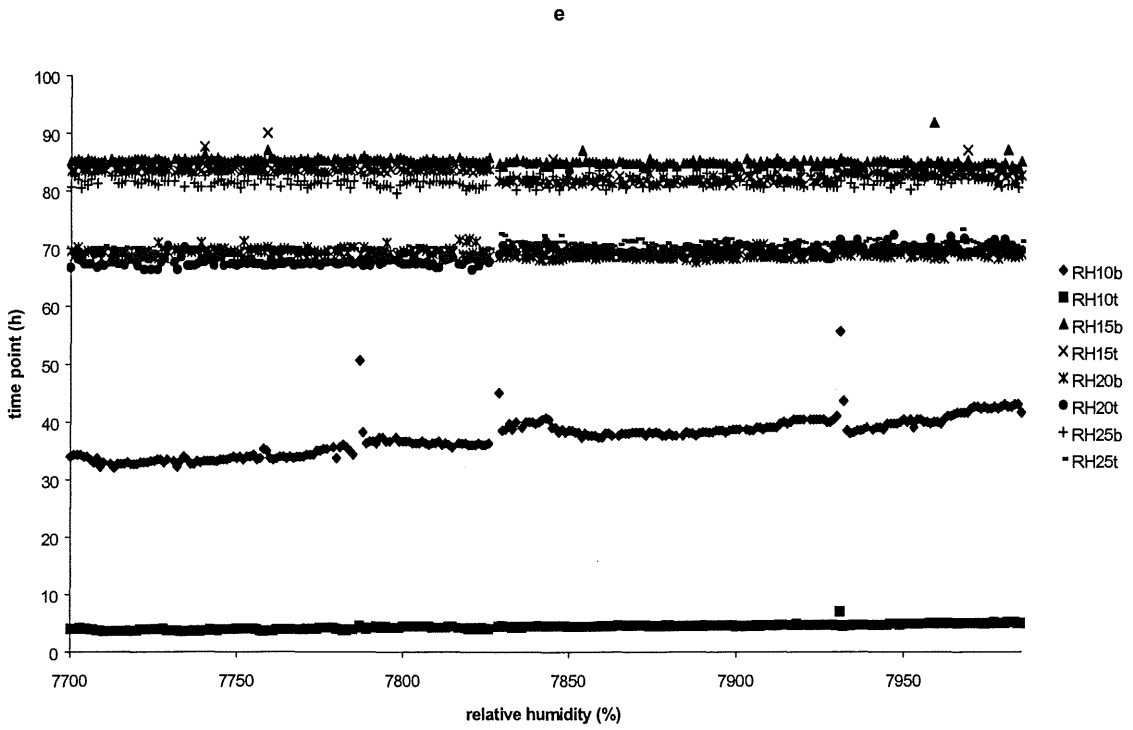


Figure e: Course of relative humidity in the two crates (t = top, b = bottom) of the tower at 10-15-20-25°C during the incubation period of the experiment.

Appendix 5: Measured values of experiment

Values soil:clover samples already corrected with values blank soil samples. Under de heading clover: Y = incubated with clover; N = blank soil sample. Under the heading block:1 = top crate; 2 = bottom crate. Nmin = NO_2^- + NO_3^- + NH_4^+

Days after incubation	Temperature	Clover	Block	NO_2^- (mg N kg-1 dry soil)	NO_3^- (mgN kg-1 dry soil)	NH_4^+ (mgN kg-1 dry soil)	Nmin (mgN kg-1 dry soil)
3	25	Y	1	17.1	65.8	3.1	85.9
3	25	Y	2	17.1	60.3	3.3	80.7
3	25	Y	1	10.1	61.7	0.7	72.6
3	25	Y	2	15.3	58.1	0.6	74.0
7	25	Y	1	0.3	130.9	0.3	131.5
7	25	Y	2	0.7	137.1	0.2	138.0
7	25	Y	1	0.2	190.1	0.3	190.6
7	25	Y	2	0.4	200.5	0.2	201.1
14	25	Y	1	0.1	209.1	0.2	209.4
14	25	Y	2	0.2	163.3	1.0	164.6
14	25	Y	1	0.4	186.5	0.2	187.1
14	25	Y	2	1.0	257.9	0.3	259.2
21	25	Y	1	0.1	213.5	0.2	213.9
21	25	Y	2	0.1	191.1	0.2	191.4
21	25	Y	1	0.1	220.6	0.4	221.1
21	25	Y	2	0.1	256.3	0.1	256.5
28	25	Y	1	0.2	272.0	0.2	272.5
28	25	Y	2	0.1	285.4	0.2	285.7
28	25	Y	1	0.1	247.7	0.2	248.0
28	25	Y	2	0.2	268.5	0.4	269.1
35	25	Y	1	0.1	318.8	1.0	319.9
35	25	Y	2	0.1	278.0	0.6	278.8
35	25	Y	1	0.2	325.5	0.5	326.2
35	25	Y	2	0.2	327.7	0.6	328.5
0	25	N	1	0	19.5	0.1	19.6
0	25	N	2	0	20.8	0.2	20.9
0	25	N	1	0	17.5	0.1	17.5
0	25	N	2	0	17.9	0.1	17.9
3	25	N	1	0	19.3	0.7	20.0
3	25	N	2	0	20.5	0.3	20.8
3	25	N	1	0	21.8	0.4	22.1
3	25	N	2	0	21.5	0.3	21.9
7	25	N	1	0	22.0	1.2	23.2
7	25	N	2	0	21.1	0.2	21.3
7	25	N	1	0	21.0	0.2	21.2
7	25	N	2	0	18.9	0.2	19.0
14	25	N	1	0	28.5	2.3	30.8
14	25	N	2	0	29.0	1.5	30.5
14	25	N	1	0	24.7	0.3	25.1
14	25	N	2	0	28.4	0.3	28.7
21	25	N	1	0	43.6	0.3	43.9
21	25	N	2	0	28.5	0.4	28.9
21	25	N	1	0	31.4	0.3	31.7
21	25	N	2	0	32.0	1.7	33.6
28	25	N	1	0	31.6	0.6	32.2
28	25	N	2	0	31.0	1.4	32.4
28	25	N	1	0	30.7	0.8	31.5
28	25	N	2	0	19.3	0.5	19.8
35	25	N	1	0	35.5	0.3	35.8
35	25	N	2	0	36.0	0.4	36.3
35	25	N	1	0	32.7	0.6	33.3
35	25	N	2	0	33.6	0.7	34.3

Days after incubation	Temperature	Clover	Block	NO_2^- (mgN kg-1 dry soil)	NO_3^- (mgN kg-1 dry soil)	NH_4^+ (mgN kg-1 dry soil)	Nmin (mgN kg-1 dry soil)
3	20	Y	1	16.5	33.9	1.2	51.7
3	20	Y	2	16.5	41.8	4.8	63.1
3	20	Y	1	21.5	23.5	4.2	49.3
3	20	Y	2	27.0	12.2	6.8	46.0
7	20	Y	1	4.1	78.5	0.2	82.8
7	20	Y	2	3.8	91.3	0.2	95.3
7	20	Y	1	3.3	65.7	0.2	69.2
7	20	Y	2	5.0	79.1	0.7	84.8
14	20	Y	1	0.4	149.9	0.2	150.5
14	20	Y	2	0.4	145.5	0.3	146.3
14	20	Y	1	0.3	168.7	0.2	169.2
14	20	Y	2	0.2	187.5	0.3	188.1
21	20	Y	1	0.2	224.7	0.2	225.2
21	20	Y	2	0.1	210.4	0.2	210.7
21	20	Y	1	0.1	169.3	0.2	169.5
21	20	Y	2	0.1	176.6	0.2	176.9
28	20	Y	1	0.1	232.0	0.2	232.3
28	20	Y	2	0.1	236.7	0.3	237.1
28	20	Y	1	0.2	230.0	0.2	230.4
28	20	Y	2	0.1	232.3	0.2	232.6
35	20	Y	1	0.1	235.6	0.4	236.0
35	20	Y	2	0.1	267.9	0.3	268.3
35	20	Y	1	0.2	269.9	0.4	270.5
35	20	Y	2	0.2	286.4	0.5	287.2
0	20	N	1	0	15.9	0.0	15.9
0	20	N	2	0	18.1	0.0	18.1
0	20	N	1	0	18.3	0.1	18.5
0	20	N	2	0	17.7	0.4	18.1
3	20	N	1	0	18.6	0.1	18.7
3	20	N	2	0	17.3	0.2	17.5
3	20	N	1	0	19.6	0.3	19.9
3	20	N	2	0	19.2	0.2	19.4
7	20	N	1	0	18.5	0.2	18.6
7	20	N	2	0	18.8	0.2	19.0
7	20	N	1	0	17.4	0.3	17.6
7	20	N	2	0	18.5	0.1	18.6
14	20	N	1	0	22.6	0.3	22.9
14	20	N	2	0	22.7	0.4	23.2
14	20	N	1	0	21.7	0.8	22.5
14	20	N	2	0	29.2	0.5	29.7
21	20	N	1	0	28.8	0.5	29.3
21	20	N	2	0	27.7	0.3	28.0
21	20	N	1	0	30.9	0.4	31.3
21	20	N	2	0	30.5	0.4	31.0
28	20	N	1	0	26.2	0.8	27.1
28	20	N	2	0	26.8	0.3	27.1
28	20	N	1	0	26.4	0.4	26.8
28	20	N	2	0	30.8	0.4	31.2
35	20	N	1	0	26.1	0.3	26.4
35	20	N	2	0	28.7	0.4	29.1
35	20	N	1	0	23.7	0.6	24.3
35	20	N	2	0	35.4	0.2	35.6

Days after incubation	Temperature	Clover	Block	NO_2^- (mgN kg-1 dry soil)	NO_3^- (mgN kg-1 dry soil)	NH_4^+ (mgN kg-1 dry soil)	Nmin (mgN kg-1 dry soil)
3	17	Y	1	24.5	32.7	5.0	62.2
3	17	Y	2	23.1	19.5	5.6	48.1
3	17	Y	1	22.6	37.0	6.6	66.2
3	17	Y	2	25.5	29.1	6.9	61.5
7	17	Y	1	5.9	85.2	0.2	91.3
7	17	Y	2	4.6	76.8	0.3	81.7
7	17	Y	1	2.9	63.3	0.3	66.5
7	17	Y	2	3.3	52.9	0.2	56.3
14	17	Y	1	0.6	148.7	0.2	149.5
14	17	Y	2	0.4	152.6	0.2	153.2
14	17	Y	1	0.6	175.4	0.2	176.1
14	17	Y	2	0.2	143.4	0.2	143.9
21	17	Y	1	0.2	179.0	0.2	179.4
21	17	Y	2	0.1	153.4	0.2	153.7
21	17	Y	1	0.2	196.0	0.2	196.5
21	17	Y	2	0.2	192.1	0.2	192.6
28	17	Y	1	0.2	277.6	0.2	278.0
28	17	Y	2	0.1	215.2	0.2	215.5
28	17	Y	1	0.2	238.4	0.2	238.9
28	17	Y	2	0.1	238.3	0.2	238.7
35	17	Y	1	0.1	261.7	0.5	262.3
35	17	Y	2	0.1	254.2	0.5	254.8
35	17	Y	1	0.2	274.3	0.5	275.0
35	17	Y	2	0.1	260.4	0.4	261.0
0	17	N	1	0	15.6	1.1	16.7
0	17	N	2	0	16.2	0.1	16.3
0	17	N	1	0	16.4	0.0	16.4
0	17	N	2	0	16.1	0.0	16.1
3	17	N	1	0	17.3	0.5	17.8
3	17	N	2	0	17.9	0.3	18.1
3	17	N	1	0	17.1	0.2	17.3
3	17	N	2	0	18.1	0.1	18.2
7	17	N	1	0	18.1	0.1	18.2
7	17	N	2	0	17.5	0.2	17.7
7	17	N	1	0	16.3	0.3	16.6
7	17	N	2	0	17.2	0.2	17.4
14	17	N	1	0	16.3	0.4	16.6
14	17	N	2	0	16.6	0.8	17.4
14	17	N	1	0	19.7	1.6	21.3
14	17	N	2	0	20.2	0.6	20.8
21	17	N	1	0	24.9	0.2	25.2
21	17	N	2	0	26.0	0.8	26.8
21	17	N	1	0	24.9	0.6	25.6
21	17	N	2	0	27.5	0.4	27.8
28	17	N	1	0	26.1	0.5	26.6
28	17	N	2	0	18.4	0.5	18.8
28	17	N	1	0	26.6	0.4	27.0
28	17	N	2	0	31.6	0.7	32.2
35	17	N	1	0	26.4	0.9	27.3
35	17	N	2	0	24.4	0.5	24.9
35	17	N	1	0	25.2	0.7	25.9
35	17	N	2	0	26.1	0.3	26.5

Days after incubation	Temperature	Clover	Block	NO_2^- (mgN kg-1 dry soil)	NO_3^- (mgN kg-1 dry soil)	NH_4^+ (mgN kg-1 dry soil)	Nmin (mgN kg-1 dry soil)
3	15	Y	1	12.2	34.9	3.8	50.9
3	15	Y	2	13.9	26.3	3.3	43.5
3	15	Y	1	19.1	31.2	3.7	54.0
3	15	Y	2	16.9	22.9	1.8	41.6
7	15	Y	1	9.3	40.0	0.5	49.9
7	15	Y	2	8.0	43.2	0.3	51.4
7	15	Y	1	6.0	45.7	0.2	52.0
7	15	Y	2	10.1	26.0	0.2	36.4
14	15	Y	1	5.2	157.4	0.3	162.9
14	15	Y	2	2.3	142.4	0.2	144.8
14	15	Y	1	2.2	153.2	0.2	155.6
14	15	Y	2	3.0	147.5	0.2	150.8
21	15	Y	1	0.2	161.8	0.3	162.3
21	15	Y	2	0.3	165.8	0.3	166.5
21	15	Y	1	0.2	164.7	0.2	165.2
21	15	Y	2	0.4	203.6	0.3	204.4
28	15	Y	1	0.2	263.3	0.3	263.8
28	15	Y	2	0.2	254.9	0.3	255.4
28	15	Y	1	0.2	211.7	0.2	212.1
28	15	Y	2	0.2	230.9	0.5	231.6
35	15	Y	1	0.2	249.4	0.7	250.4
35	15	Y	2	0.2	263.7	0.6	264.5
35	15	Y	1	0.7	220.5	0.7	221.9
35	15	Y	2	0.1	254.3	0.6	255.0
0	15	N	1	0	16.8	0.4	17.2
0	15	N	2	0	18.5	0.0	18.5
0	15	N	1	0	17.7	0.0	17.7
0	15	N	2	0	14.3	0.0	14.3
3	15	N	1	0	17.5	0.1	17.6
3	15	N	2	0	15.7	0.2	15.9
3	15	N	1	0	16.3	0.1	16.4
3	15	N	2	0	14.9	0.1	15.0
7	15	N	1	0	16.5	0.1	16.6
7	15	N	2	0	15.6	0.6	16.2
7	15	N	1	0	16.3	0.4	16.7
7	15	N	2	0	16.0	0.2	16.2
14	15	N	1	0	19.4	0.5	20.0
14	15	N	2	0	19.1	0.2	19.3
14	15	N	1	0	20.4	0.4	20.8
14	15	N	2	0	17.6	0.5	18.1
21	15	N	1	0	20.5	0.5	21.1
21	15	N	2	0	20.9	0.2	21.2
21	15	N	1	0	19.6	0.5	20.1
21	15	N	2	0	20.7	1.5	22.2
28	15	N	1	0	20.4	0.4	20.7
28	15	N	2	0	21.2	0.5	21.6
28	15	N	1	0	21.9	0.7	22.6
28	15	N	2	0	20.7	0.3	21.0
35	15	N	1	0	22.7	0.3	23.1
35	15	N	2	0	20.7	0.4	21.1
35	15	N	1	0	21.9	0.5	22.4
35	15	N	2	0	25.6	0.4	26.0

Days after incubation	Temperature	Clover	Block	NO_2^- (mgN kg-1 dry soil)	NO_3^- (mgN kg-1 dry soil)	NH_4^+ (mgN kg-1 dry soil)	Nmin (mgN kg-1 dry soil)
3	10	Y	1	6.9	22.0	0.1	29.1
3	10	Y	2	6.8	23.1	0.1	29.9
3	10	Y	1	4.6	28.1	0.2	32.9
3	10	Y	2	7.2	24.5	0.2	31.9
7	10	Y	1	15.2	47.3	7.3	69.7
7	10	Y	2	15.6	42.1	6.5	64.1
7	10	Y	1	10.3	48.5	6.0	64.8
7	10	Y	2	11.9	49.0	5.3	66.2
14	10	Y	1	17.0	90.7	1.4	109.0
14	10	Y	2	18.4	76.0	7.4	101.8
14	10	Y	1	12.7	65.1	0.7	78.4
14	10	Y	2	16.5	63.6	2.6	82.7
21	10	Y	1	6.3	161.2	0.3	167.7
21	10	Y	2	16.9	94.2	1.3	112.4
21	10	Y	1	8.3	139.6	0.2	148.2
21	10	Y	2	19.1	76.5	4.3	99.9
28	10	Y	1	1.1	207.0	1.0	209.1
28	10	Y	2	10.3	134.5	5.0	149.9
28	10	Y	1	0.8	186.3	0.2	187.2
28	10	Y	2	4.8	147.3	0.2	152.3
35	10	Y	1	0.2	227.7	1.8	229.7
35	10	Y	2	6.0	155.9	1.2	163.1
35	10	Y	1	0.4	194.2	1.0	195.6
35	10	Y	2	4.7	156.7	1.3	162.7
0	10	N	1	0	15.5	0.4	15.9
0	10	N	2	0	13.0	0.0	13.0
0	10	N	1	0	13.6	18.7	32.3
0	10	N	2	0	15.1	0.0	15.2
3	10	N	1	0	15.3	0.2	15.5
3	10	N	2	0	15.9	0.2	16.1
3	10	N	1	0	15.0	9.4	24.4
3	10	N	2	0	15.2	0.3	15.6
7	10	N	1	0	14.9	0.9	15.9
7	10	N	2	0	15.3	0.3	15.6
7	10	N	1	0	14.6	0.3	14.9
7	10	N	2	0	14.3	0.1	14.5
14	10	N	1	0	18.3	1.3	19.6
14	10	N	2	0	15.1	0.6	15.7
14	10	N	1	0	17.3	0.2	17.5
14	10	N	2	0	12.7	0.3	13.0
21	10	N	1	0	19.1	0.6	19.7
21	10	N	2	0	17.4	0.5	17.8
21	10	N	1	0	17.6	0.3	17.9
21	10	N	2	0	17.2	0.2	17.4
28	10	N	1	0	18.1	0.3	18.4
28	10	N	2	0	23.2	0.3	23.4
28	10	N	1	0	19.2	0.3	19.5
28	10	N	2	0	17.7	0.3	18.0
35	10	N	1	0	17.6	0.7	18.3
35	10	N	2	0	20.2	0.8	21.0
35	10	N	1	0	18.4	0.4	18.8
35	10	N	2	0	26.0	0.5	26.6