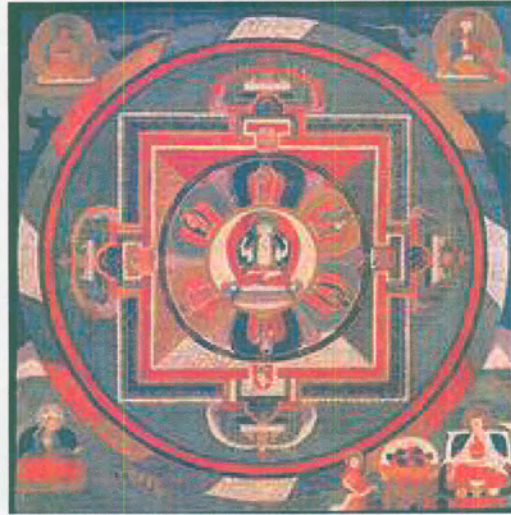




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# **NITROGEN TRANSFORMATIONS IN SOUTH AFRICAN SOILS**

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## DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is entirely my own original research, except where acknowledged, and that it has not at any time, either partly or fully, been submitted to any other university for the purpose of obtaining a degree.

A handwritten signature in black ink, appearing to read 'Agrella'.

Karen Agrella

16 Februarie 2001

Date





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## TABLE OF CONTENTS

### Chapter 1

Literature study – Soil nitrogen transformations.....1

### Chapter 2

Extended abstract

Research experiment 1 – Influence of moisture and substrate on nitrogen transformations.....1

Research experiment 2 – Mineralisation and nitrification in soil in the region of Pretoria (South Africa).....11

Research experiment 3 – Nitrous oxide production from South African soils.....23

General conclusions

### Chapter 3

Glossary of Soil and Soil microbiological terms

List of abbreviations

Appendix I

Appendix II

Appendix III

Nitrogen transformations in South African Soils



## Chapter 1

Literature study -

Soil nitrogen transformations

# CHAPTER 1

## LITERATURE STUDY

### TABLE OF CONTENTS

|  |           |
|--|-----------|
| <b>1. INTRODUCTION .....</b>   | <b>1</b>  |
| <b>2. ORIGIN, DISTRIBUTION AND CYCLING OF NITROGEN .....</b>                             | <b>2</b>  |
| 2.1 <i>THE NITROGEN CYCLE .....</i>  | 4         |
| <b>3. THE DECOMPOSITION PROCESS: MINERALISATION, IMMOBILISATION AND DEGRADATION.....</b> | <b>7</b>  |
| 3.1 <i>FACTORS INFLUENCING MINERALISATION AND IMMOBILISATION .....</i>                   | 10        |
| 3.1.1 <i>Temperature and humidity .....</i>  | 10        |
| 3.1.2 <i>Substrate .....</i>   | 11        |
| 3.1.3 <i>Moisture and oxygen .....</i>   | 12        |
| 3.1.4 <i>Acidity.....</i>  | 14        |
| <b>4. NITRIFICATION .....</b>  | <b>14</b> |
| 4.1 <i>PROCESSES OF NITRIFICATION.....</i>   | 15        |
| 4.1.1 <i>Chemoautotrophic nitrification.....</i>   | 15        |
| 4.1.2 <i>Heterotrophic nitrification.....</i>  | 15        |
| 4.2 <i>FACTORS REGULATING NITRIFICATION.....</i>   | 16        |
| 4.2.1 <i>Substrates and products .....</i>   | 16        |
| 4.2.2 <i>Substrate repression.....</i>   | 17        |
| 4.2.3 <i>End product repression .....</i>  | 17        |
| 4.2.4 <i>Soil pH.....</i>  | 18        |
| 4.2.5 <i>Aeration and moisture.....</i>  | 18        |
| 4.2.6 <i>Temperature.....</i>  | 18        |
| <b>5. MOVEMENT OF NITROGEN IN SOILS .....</b>  | <b>19</b> |
| <b>6. GASEOUS LOSSES OF NITROGEN .....</b>   | <b>20</b> |
| 6.1 <i>DENITRIFICATION.....</i>  | 21        |
| 6.2 <i>NITRIFICATION .....</i>   | 21        |
| 6.3 <i>CHEMODENITRIFICATION.....</i>   | 23        |
| 6.4 <i>FACTORS CONTROLLING N<sub>2</sub>O EMISSIONS.....</i>                             | 24        |
| 6.4.1 <i>Soil aeration and moisture.....</i>   | 24        |
| 6.4.2 <i>Temperature.....</i>  | 25        |
| 6.4.3 <i>pH.....</i>   | 25        |
| 6.4.4 <i>Source of carbon .....</i>  | 26        |
| <b>7. REFERENCES .....</b>   | <b>26</b> |

## LIST OF FIGURES

|   |    |
|---|----|
| <b>Figure 1.</b> Presentation of a typical nitrogen cycle.....  | 6  |
| <b>Figure 2.</b> Schematic representation of the turnover of nitrogen in relation to the priming effect of fertiliser nitrogen.....   | 9  |
| <b>Figure 3.</b> Contour map of O <sub>2</sub> concentrations in a clay soil particle.....  | 13 |
| <b>Figure 4.</b> A conceptual model of the two levels of regulation of N trace gas production via nitrification and denitrification: (a) flux of N through the process “pipes” and (b) holes in the pipes through which trace N-gases “leak”..... | 22 |
| <b>Figure 5.</b> Nitrogen emissions from grassland soils.....   | 23 |



## 1. INTRODUCTION

As one of the essential elements for plant growth, nitrogen (N) is required in large quantities by most agricultural crops. In the past, biological N<sub>2</sub> fixation was the main source of N for cultivated crops. In Europe, since World War II, N fertilisers have become increasingly available, which have led to increasing yields and improved crop quality. The additional nitrogen has to be managed effectively to ensure maximum efficiency and minimum pollution (Stevenson, 1982). Extensive research has been done in many developed countries concerning nitrogen cycles, transformations and pollution.

In developing countries like South Africa, the little research (compared to Europe and North America) that has been done mainly focussed on fertiliser management but with little emphasis on pollution. Compared to Europe nitrogen pollution in South Africa is limited, except for certain intensively cultivated areas because the use of N fertilisers per hectare is much less. The production is low overall and the losses of nitrogen are expected to be much less. It is also very important that the N present during the season and the residual N at the end of the season do not contaminate our scarce water resources.

Agricultural practices in South Africa are largely extensive and a more important challenge for agriculture is to minimise fertiliser input and cost by managing the natural N in the soil efficiently. At present cultivated areas cover about 10 million ha, or 8.2% of South Africa. The importance of agriculture in South Africa may, however, be gauged by the fact that while only covering 4% of the African continent it produces 30.4% of the continent's maize and 29.5% of its wheat (Vlassak & Agenbag, 2000).

According to Venter (1982) an average of 60 kg N ha<sup>-1</sup> is applied to maize, indicating that approximately 270 000 tons N is added to the more than 4 million hectares of maize fields every year. Domestic N fertiliser use in South Africa increased from 348 000 tons in 1992 to 415 000 tons in 1996 (Vlassak & Agenbag, 2000). Since 1982, when Venter stated those numbers, fertiliser use has increased significantly because of the increased demand for food security, not only in South Africa but especially in the rest of Africa that import our crops. Until recently, residual and organic nitrogen present in the soil during the growing season



has not been taken into account in nitrogen fertiliser recommendations. Fundamentally, the contribution of residual nitrogen could have a large impact on the management of N fertilisation in South Africa. It has been found that after a poor harvest, large amounts of inorganic N, especially  $\text{NO}_3^-$ -N, is carried over to the next season that should be considered in the nitrogen fertiliser programme of maize in the following season (Van der Walt & Du Preez, 1991).

## 2. ORIGIN, DISTRIBUTION AND CYCLING OF NITROGEN

In a natural undisturbed ecosystem, large quantities of N are cycled within the system and both the input and loss rates are low. On a global scale, 90 to 97% of the production of plant biomass is derived from recycled N within the biosphere, with 3 - 10% fixed annually (Haynes, 1986).

Mineral N in the soil represents a very small and usually transient pool of N in terms of the total N stored in any ecosystem. The major forms of mineral N ( $\text{NH}_4^+$ , ammonium and  $\text{NO}_3^-$ , nitrate) usually account for less than 2% of the total N content of soils (Stevenson, 1982). It is nonetheless this N that is available for direct uptake by plants (Haynes, 1986). When land is first placed under cultivation, the total N content of the soil usually declines and a new equilibrium level that is characteristic of the climate, cultural practice, and soil type is established. At equilibrium, any N removed by harvested crops must be compensated for by the addition of an equivalent amount of newly fixed N to maintain crop production (Stevenson, 1982; Sprent, 1987).

In most developing countries agricultural systems rely heavily on soil reserves to meet the N requirements of plants that cannot sustain consistent high yields in the long term (Stevenson, 1982). The high-producing agricultural systems of the western world that are characterised by intensive production methods rely on large fertiliser inputs to sustain productivity (Haynes, 1986). Large quantities of the produced organic matter is removed from the cultivated fields and sometimes very little are returned to the soil.

Nitrate N is easily lost from soils through leaching to groundwater and through denitrification and volatilisation. The rate of nitrification is regulated by the availability of  $\text{NH}_4^+$ -N within the soil. A low rate of nitrification (which itself results in gaseous losses of N as  $\text{NH}_4^+$ -N) is therefore essential in N conservation in most natural ecosystems (Verstraete, 1981). Thus, the internal cycling processes within terrestrial ecosystems that prevent accumulation of  $\text{NH}_4^+$ -N and therefore nitrification in the soil are particularly important in N conservation (Haynes, 1986).

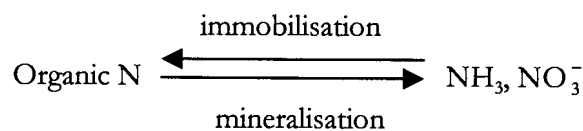
Because of the importance of the nitrogen supply in crop production and soil fertility, a deficiency markedly reduces yield as well as crop quality. Immobilisation by microorganisms can prevent or retard loss of nitrate from the soil leading to improved economy of N fertiliser and decreasing the risk of contaminating groundwater. Excessive immobilisation competes by microorganisms with plants for N and leads to temporary deficiencies in crops. Therefore it is agronomically important to maintain a balance between mineralisation and immobilisation (Cheshire *et al.*, 1999).

Nitrogen is known as the major plant nutrient to be the most susceptible to microbial transformation. Nitrogen undergoes a number of transformations, which involve organic and inorganic compounds. These transformations occur simultaneously, and individual steps result in different products of mineralisation and immobilisation. The reactions may be viewed in terms of a cycle in which the element is shuttled back and forth due to the influence of microflora. A small part of the large reservoir of  $\text{N}_2$  in the atmosphere is converted to organic compounds by certain free-living microorganisms or by a symbiotic microbial-plant association that makes the element directly available to the plant (Alexander, 1961).

## 2.1 *The nitrogen cycle*

Nitrogen cycles are very dynamic. Depending on the need there can be discerned between a range of nitrogen cycles. N cycles differ as ecosystems differ and that influences certain state variables in the different environments, like soil fertility and environmental quality. The components of the nitrogen cycle governed by microbial metabolism<sup>a</sup> consist of several distinct individual chemical transformations.

A representation of a typical N cycle is given in Figure 1. Conversions of organic N to plant available mineral forms ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ) occur through biochemical transformations mediated by microorganisms and are influenced by those factors that affect microbial activity (temperature, moisture, pH, etc.). The first step, ammonification, denotes the processes by which organic nitrogenous compounds (substrate) are transformed by hydrolysis to yield  $\text{NH}_4^+$ . Heterotrophic microorganisms carry out these N mineralisation reactions. A key feature of the internal cycle exist in the overall N cycle - the biological turnover of N through mineralisation-immobilisation (Stevenson, 1982):



In nitrogen mineralisation, part of the large reservoir of organic complexes in the soil, namely proteins, polypeptides, nucleoproteins, nucleic acids, and aromatic compounds are decomposed and converted to ammonium and nitrate that can be used by plants.  $\text{NH}_4^+$  can be lost through volatilisation under certain conditions. Nitrogen immobilisation or assimilation is the opposite of mineralisation and leads to the biosynthesis of complex molecules of microbial protoplasm using ammonium and nitrate. The mineralisation of organic nitrogen and the micro floral assimilation of inorganic ions proceed simultaneously (Alexander, 1961).

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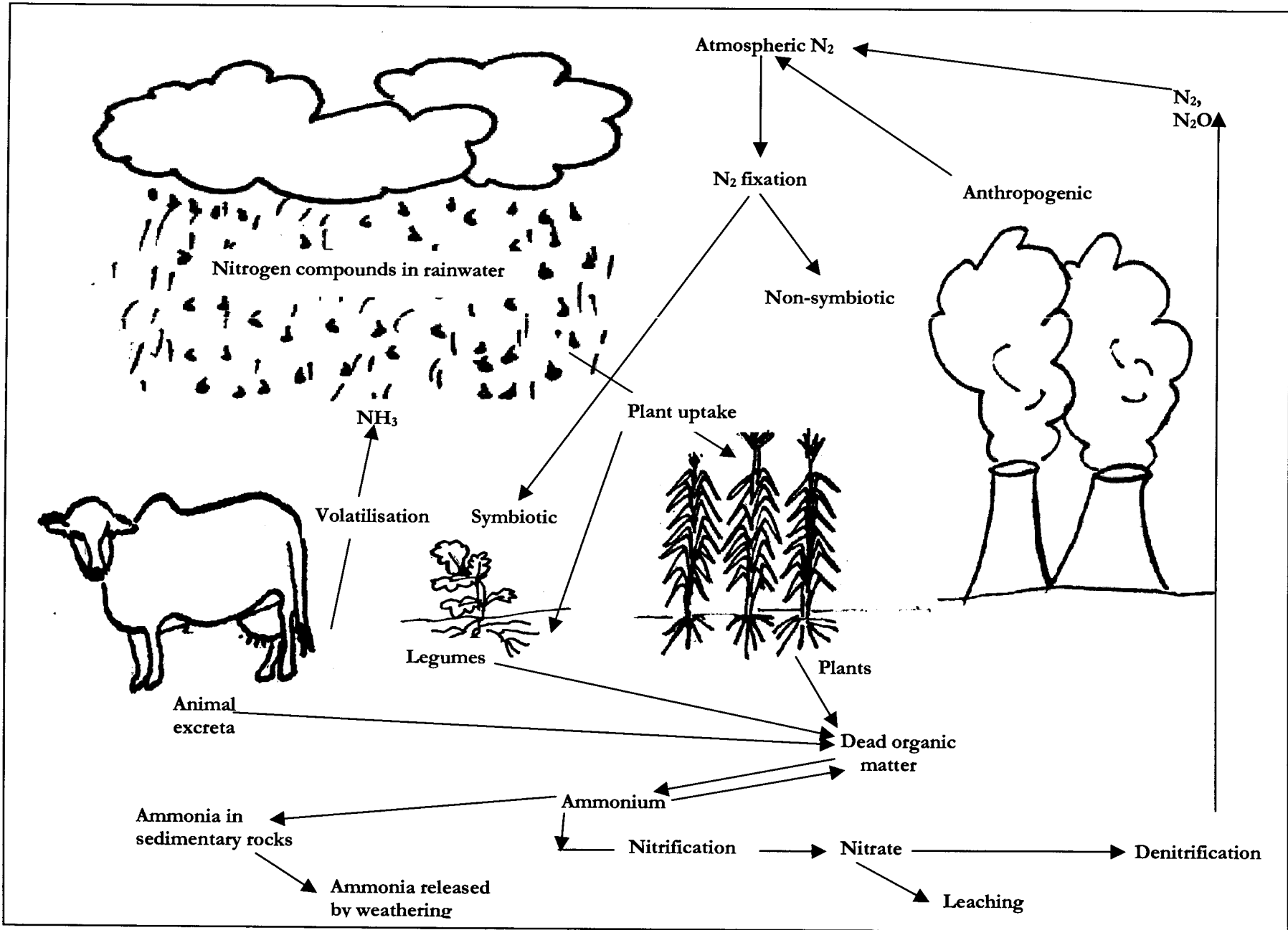
<sup>a</sup> Underlined words are explained in the Glossary (Chapter 3)

A specialised group of bacteria, the nitrifying bacteria (autotrophes), are able to oxidise ammonia to nitrate in a process called nitrification. Nitrogen, once in the nitrate form, can be taken up by plants or may be lost from the soil in several ways, namely leaching to ground water or gaseous losses through denitrification to NO (nitric oxide), N<sub>2</sub>O (dinitrogen oxide) and N<sub>2</sub> (dinitrogen).

Any leaks in the N cycle will deplete the soil's nitrogen reserve, resulting in less plant growth. In nature a reverse process exist in N-dynamics to amend these losses; otherwise the world's nitrogen reservoir would be diminishing continuously. Although inert as far as plants, animals, and most microorganisms are concerned, N<sub>2</sub> is acted upon by certain microorganisms, sometimes in symbiosis with a higher plant, which can use it as a nitrogen source for growth. This process, nitrogen fixation, results in the accumulation of new N-containing organic compounds in the cells of the responsible organisms. The N<sub>2</sub> thus fixed re-enters the N cycle when the newly formed tissues are in turn mineralised.

By means of these reactions, the soil micro flora regulates the supply and governs the availability and chemical nature of the nitrogen in soil (Alexander, 1961).





6

Figure 1. Representation of a typical nitrogen cycle

### 3. THE DECOMPOSITION PROCESS: MINERALISATION, IMMOBILISATION AND DEGRADATION

The release of carbon (C) and N from organic matter differs in that C is generally volatilised as carbon dioxide (CO<sub>2</sub>) or methane (CH<sub>4</sub>) while N tends to be conserved. Part of this conversion is caused by the demand for N by the decomposer microorganisms since nitrogen is an essential nutrient element for them.

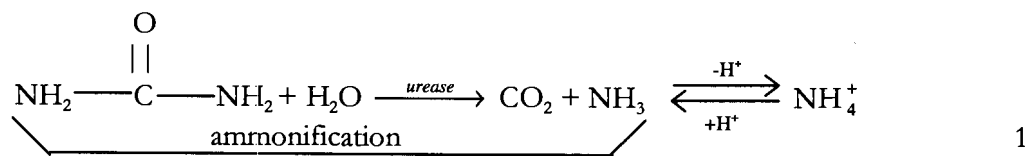
Most of the nitrogen found in surface soil horizons is in organic combination. The nitrogenous compounds present in the soil organic fraction are readily metabolised by microorganisms in culture solution. In a natural system however, the mineralisation rate of N compounds by microorganisms is slower. Some constituents are more resistant to microbial attack, and they tend to accumulate in the soil where they are still far from fully mineralised or transformed into biomass (Stevenson, 1982). Under European conditions, due to the cooler climate, the chemical composition of organic matter is such that the resistance to decomposition is appreciable so that only a small proportion of the nitrogen reservoir of the soil is mineralised in each growing season (Alexander, 1961). Under South Africa conditions overall warmer temperatures speed up the mineralisation process and larger quantities of nitrogen is mineralised.

N mineralisation is defined as the transformation of N from the organic state into the inorganic forms of NH<sub>4</sub><sup>+</sup> or NH<sub>3</sub>. The process of mineralisation plays a key role in this universal cycle, being responsible for the fundamental transformation of organic N in plant residues back into simple inorganic forms originally used by the plants in their photosynthetic activities (Stevenson, 1982).

The process is performed by heterotrophic soil organisms that utilise nitrogenous organic substances as an energy source. In this enzymically catalysed reactions NH<sub>4</sub><sup>+</sup> is produced by hydrolysis of mineral nitrogen. Organisms responsible for these reactions that have been isolated include members of the genera *Bacterium*, *Corynebacterium*, *Mycobacterium*, and *Nocardia* (Ladd & Jackson, 1982).

A major feature of the universal cycle is the interaction between autotrophic and heterotrophic biological activity. Through photosynthesis, green plants trap and store solar energy in the form of plant tissue. On return to the soil, the plant material is used as a source of energy by heterotrophic microorganisms, and the organic N is transformed back into the simple inorganic compounds originally taken up by the plants. In some cases the microbial biomass may even incorporate mineral N from the surrounding soil or litter during the decomposition of organic residues with a wide C: N ratio (Haynes, 1986, Janssen, 1996).

In addition to organic N of cellular origin, soils may receive organic N as urea ( $\text{CO}(\text{NH}_2)_2$ ), a major nitrogenous constituent of the urine of grazing animals, and an applied fertiliser. Decomposition of urea by ureases is an important reaction (equation 1) in the ammonification process (Ladd & Jackson, 1982).



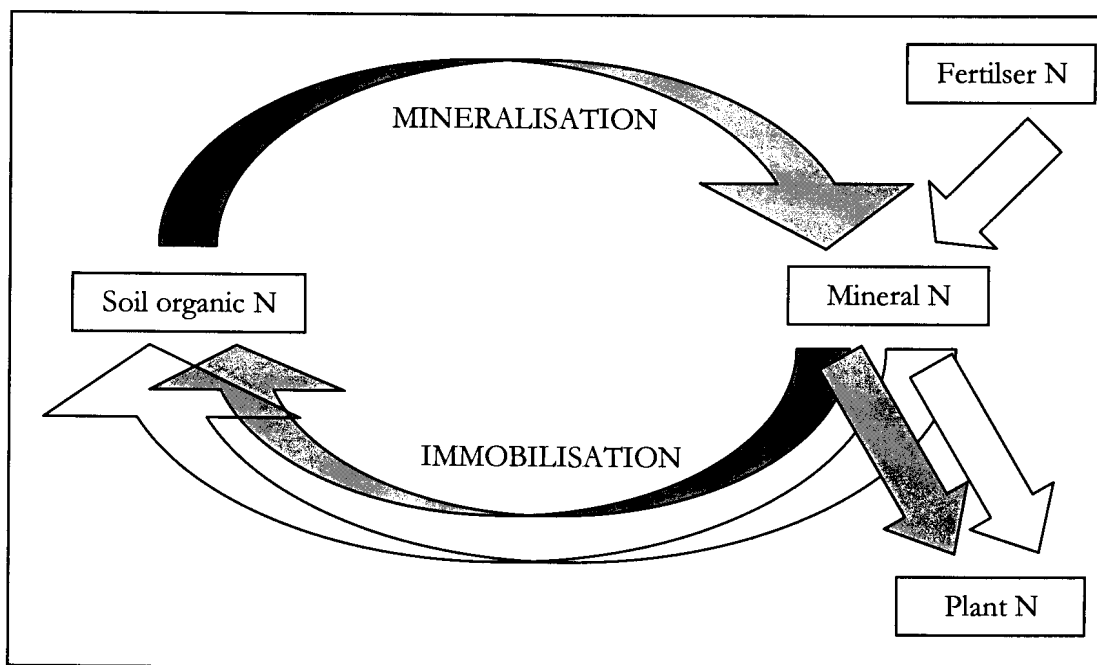
The process of continuous transfer of mineralised N into organic materials in soil microbial biomass (SMB) and the release of immobilised N back into inorganic pools is known as Mineralisation-Immobilisation-Turnover (MIT) (fig. 2).

MIT can be defined as the continuous and simultaneous transfer of mineralised N into organic products of synthesis and of immobilised N back into inorganic decay products – underlying the building up and dying away of the heterotrophic biomass. The added energy of the fertiliser material has a stimulating effect on soil organic matter transformations, called the “priming or preparing effect”.

It is assumed that all immobilisation occurs from the inorganic pool,  $\text{NH}_4^+$  or  $\text{NO}_3^-$  (Jarvis *et al.*, 1996). Part of the converted matter is used for assimilation in microbial tissue and part of oxidation to gain energy (dissimilation). Immobilisation predominantly occurs from the

$\text{NH}_4^+$ -N pool. However, where  $\text{NH}_4^+$  is not available  $\text{NO}_3^-$  is assimilated by SMB in the presence of readily available C (Jarvis *et al.*, 1996).

$\text{NH}_4^+$ -N produced in the mineralisation process or otherwise accumulated in a  $\text{NH}_4^+$ -N pool in the soil is markedly preferred over  $\text{NO}_3^-$ -N by heterotrophic microorganisms during immobilisation. When  $\text{NO}_3^-$  is available but  $\text{NH}_4^+$  missing, the former is utilised. Sudden changes in the chemical and physical conditions of the soil caused, for example, by wetting and drying, may kill microorganisms and may be followed by sudden and temporary flushes of energy stimulating MIT (Janssen & Persson, 1982).



**Figure 2.** Schematic representation of the turnover of nitrogen in relation to the priming effect of fertiliser nitrogen (Haynes, 1986).

As new cells or hyphae are formed, not only must carbon, hydrogen, and oxygen be combined into protoplasmic complexes but so must nitrogen, phosphorous, potassium, sulphur, magnesium, and iron. Each one of these elements is thus immobilised. Microorganisms cannot multiply nor can organic matter be decomposed unless nitrogen is available to be assimilated into microbial protoplasm, and assimilation will take place as long



as there is microbial activity. Whenever mineralisation occurs, part of the N will be immobilised. Thus, a determination of the quantity of inorganic nitrogen produced or lost during incubation does not measure one or the other process but rather the net release or tie-up of N. These amounts are greatly underestimated (Alexander, 1977; Stevenson, 1982). Preliminary incubation studies on South African soils have shown that when microorganisms utilise available N from the soil environment, when decomposing organic substrate which has a high C:N, N is immobilised for up to two months before an attempt at release by microbial colonisers (Vlassak *et al.*, 1999).

Immobilisation of at least some of the N released during mineralisation is an indispensable and integrated prerequisite for mineralisation. The heterotrophic phase of the ecosystems, carrying out mineralisation, is not only respiring and mineralising but is also a renewal of organic matter, and an assimilation of mineral nutrients providing the multiplication, growth, and maintenance of the living and active microbial flora or biomass (Stevenson, 1982).

### ***3.1 Factors influencing mineralisation and immobilisation***

#### **3.1.1 Temperature and humidity**

The mineralisation of soil organic matter depends on its composition and abundance, and on climatic factors, in particular soil and air temperature and humidity. Climate influence mineralisation processes through their effects on the microbial activity in the soil. Leiròs *et al* (1999) found that low humidity and temperatures limit mineralisation. The rate of mineralisation increase linearly with soil moisture content up to a certain extent. Above a mean annual temperature of approximately 25°C in aerobic soils, the rate of decomposition can be higher than that of organic matter accumulation (Prescott *et al.*, 1993). The latter is true for many parts of South Africa where, due to the relatively warm winters and hot summers in South Africa, the decomposition rate of organic matter is high compared to Central and Northern Europe and the northern parts of North America.

### 3.1.2 Substrate

N mineralisation depends on both decomposability and N concentration of the substrate. When the initial C:N of a substrate is higher than that of the microbes, the fraction of organic N that is mineralised is less than the fraction of organic C that is dissimilated. During mineralisation the C:N of the remaining substrate decreases until it has the same value as that of the microbes.

Stevenson (1982) describe the relationship between the C:N ratio of crop residues and mineral N levels is as follows:

|           | C:N   |                       |   |
|-----------|---|-----------------------|---|
|           | <20   | 20 – 30               | >30   |
| Mineral N | Net gain of $\text{NH}_4^+$ and $\text{NO}_3^-$ | Neither gain nor loss | Net uptake of $\text{NH}_4^+$ and $\text{NO}_3^-$ |

Residues that have C:N greater than about 30, equivalent to N contents of about 1.5% or less, result in lowering of mineral N reserves because of net immobilisation by microorganisms. That gives way for a nitrogen negative period. As the C:N lowers the nitrogen is released through mineralisation. On the other hand, residues with C:N below about 20, or N contents greater than about 2.5%, lead to an increase in mineral N levels through net mineralisation. Under conditions suitable for microbial activity, rapid decomposition occurs with the concurrent liberation of considerable quantities of C as  $\text{CO}_2$ . To meet the N requirements of microorganisms, mineral N is consumed. However, when the C:N ratio of the decomposing material has been lowered to about 20,  $\text{NO}_3^-$  levels once again increase because of net mineralisation (Stevenson, 1982).

The C:N in the A-horizon of cultivated soils in South Africa vary normally between 8:1 and 15:1. Most commonly it is between 10 - 12:1. The C:N tend to be lower in soils in drier areas as those in more humid regions where the annual temperatures do not vary much. It is also lower in warmer areas than cooler regions on the condition that the rainfall is about the

same for both regions. A survey conducted by Du Toit and Du Preez (1995) on 50 cultivated soils mostly in the summer rainfall areas of the Free State, showed that the organic carbon content of the largest portion of these soils was well below 1%. C:N of these soils varied between 7.0 and 15.56. Results also showed that cultivation had a significant negative effect on mineralisation characteristics.

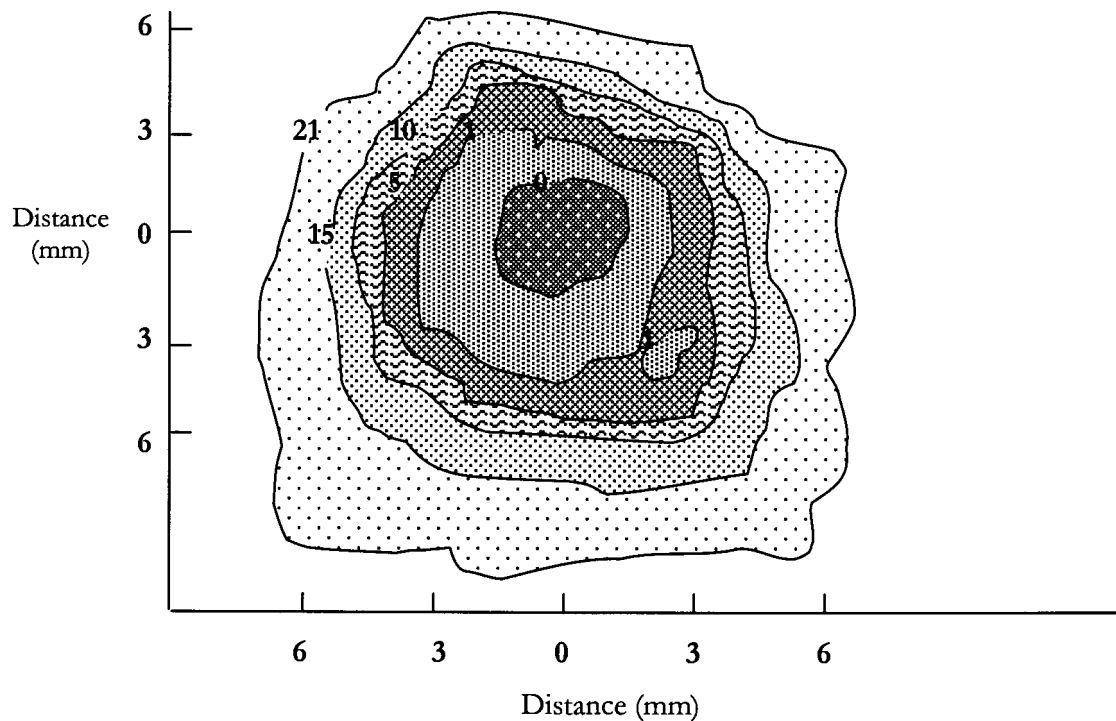
The narrower the C:N of added plant material, the faster the cycle will run down and the shorter the suppression of free nitrate forming. Under South African conditions legumes ploughed in the soil will have the smallest suppression of nitrate formation and nitrates will become available faster to the soil in contrast to when wheat straw or hay will be ploughed in.

Fresh additions of organic materials (with higher C:N) have the potential to mineralise at the highest rates. Depending on its composition, it has been shown that fresh residues were about seven times more decomposable than soil organic matter in arable soils. Biologically fixed N may also increase the N content of specific crop residues and, thus, potential mineralisation rates (Jarvis *et al.*, 1996). Russell (1973) described crop residues as comprising three separate groups of materials, (i) cell wall and structural materials, consisting of the skeletal framework (i.e. cellulose) and cementing/encrusting materials, (ii) reserve substrates including starches, fats, and proteins, and (iii) cell contents (i.e. proteins, sugars, unassimilated  $\text{NO}_3^-$ , and traces of  $\text{NO}_2^-$  and  $\text{NH}_4^+$ ). Once in the soil, the simpler N compounds mineralise more quickly than complex materials. Lignin, for example, is resilient to attack and it may also protect cellulose by encrustation.

### 3.1.3 Moisture and oxygen

Under South African conditions it was found that mineralisation increases with an increase in water content (Wiltshire, 1990). If soil is pre-incubated at 5% moisture during an incubation study, and the moisture increased to 15%, increased amounts of C and N will be mineralised during subsequent incubation, when compared with mineralisation from a soil that is kept at a lower moisture content (Van Gestel *et al.*, 1993). In terrestrial environments with high soil water contents, however, more organic matter may accumulate because

decomposition will be inhibited by lower O<sub>2</sub> availability. The soil may seem as a rather uniform environment but the micro-aggregates in soil have different concentration levels of oxygen. The outer zones of a small soil particle may be fully oxic while the centre remains completely anoxic (Fig. 3).



**Figure 3.** Contour map of O<sub>2</sub> concentrations in a clay soil particle. The axes show the dimensions of the particle. The numbers on the contours are O<sub>2</sub> concentrations (in percent; air is 21% O<sub>2</sub>). In terms of oxygen relationships for microorganisms, each zone can be considered a different microenvironment (reprinted from Brock *et al.*, 1994)

This suggests that various physiological types of microorganisms could coexist in such a soil particle. Anaerobic organisms could be active near the centre of the particle, microaerophiles could be active further out, and obligatory aerobic organisms could metabolise in the outer 2 - 3 mm of the particle. Microenvironments are heterogeneous and conditions in a given microenvironment can change rapidly (Brock *et al.*, 1994).



### 3.1.4 Acidity

It has been difficult to assess the agronomic significance of the effect of pH on mineralisation. Mineralisation of organic N occurs over the entire pH range but the rate decreases progressively below about 6 (Curtin *et al.*, 1998). Information on the pH-dependence of N mineralisation, which has mostly been obtained from liming studies on acid soils, is inconsistent. In a liming study in Wisconsin, Dancer *et al.* (1973) showed that mineralisation was not affected by pH in the range 4.7 - 6.6. However, nitrification decreased 3- to 5-fold as pH decreased. Weier & Gilliam (1986) found that liming had little influence on N mineralisation in acid (pH < 5) Histosols in North Carolina.

## 4. NITRIFICATION

Mineralisation and nitrification takes place simultaneously in the same soil. The termination of the reactions involved in organic nitrogen mineralisation occurs at the point where ammonium is formed.  $\text{NH}_4^+$ , the most reduced form of inorganic nitrogen, serves as the starting reagent for the nitrification process, the biological oxidation of  $\text{NH}_4^+$  to form nitrate or nitrite (Alexander, 1961).

The strictly biological nature of nitrification was firmly established with the isolation of nitrifying bacteria by Winogradsky in the period 1889 - 1890. He described representatives of two small groups of specialised chemoautotrophic (chemolithotrophic) bacteria and clearly related the metabolism of each of the two corresponding stages of nitrogen: the oxidation of  $\text{NH}_4^+$  to  $\text{NO}_2^-$ , and the subsequent of  $\text{NO}_2^-$  to  $\text{NO}_3^-$  (Schmidt, 1982). One group, the  $\text{NH}_4^+$  oxidisers, initiates the process with the formation of  $\text{NO}_2^-$ , while a second group, the  $\text{NO}_2^-$  oxidisers, completes the process by converting  $\text{NO}_2^-$  to  $\text{NO}_3^-$  as promptly as it is formed (Haynes, 1986).

Although the autotrophic nitrifiers are thought to be by far the most predominant agents of nitrification in the soil environment, several other minor pathways have been suggested.

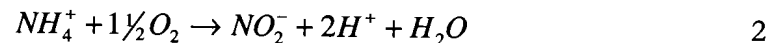
These include, for one,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  production mediated by heterotrophes (Focht & Verstraete, 1977).

#### 4.1 Processes of nitrification

##### 4.1.1 Chemoautotrophic nitrification

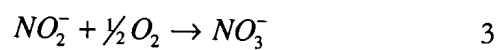
Autotrophic nitrification is carried out by bacteria of the family Nitrobacteraceae. All organisms of this family derive their energy from the oxidation of either  $\text{NH}_4^+$  or  $\text{NO}_2^-$ .

*Ammonium oxidation.* The reaction characterises the first step in nitrification by chemoautotrophic bacteria (Alexander, 1977):



The reaction is typically performed by *Nitrosomonas (europaea)*, *Nitrosococcus (mobilis, oceanus, nitrosus)*, *Nitrosovibrio (tenius)*, *Nitrosolobus (multiformes)* and *Nitrospira (briensis)*.

*Nitrite oxidation.* The oxidation of  $\text{NO}_2^-$  to  $\text{NO}_3^-$  is as follows (Schmidt, 1982):



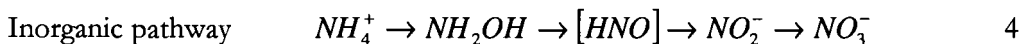
Organisms that perform the reaction include *Nitrobacter (winogradskyi, agilis)*, *Nitrospina (gracilis)* and *Nitrococcus (mobilis)* (Schmidt, 1982).

##### 4.1.2 Heterotrophic nitrification

Heterotrophic nitrification occurs when  $\text{NO}_2^-$  and/or  $\text{NO}_3^-$  are produced from organic or inorganic compounds by heterotrophic organisms through reactions that produce some energy but do not represent the sole sources of energy for the organisms.

Fungal species including *Aspergillus wentii*, *Aspergillus flavus* and *Penicillium* spp. are able to oxidise  $\text{NO}_2^-$  to  $\text{NO}_3^-$ . Bacterial species like *Arthrobacter* can produce  $\text{NO}_3^-$  from media containing  $\text{NH}_4^+$  only (Schmidt, 1982).

The biochemical pathway of heterotrophic nitrification might follow an organic, inorganic, or a combination of the two pathways.



Heterotrophic nitrification may take place under conditions apparently unsuitable for autotrophes. The pH optimum for nitrification in some acid soils where heterotrophic nitrification is suspected is approximately 4.5 and in such soils nitrification can continue at temperatures of 50 to 60°C. In contrast, autotrophic nitrification is generally considered to have a pH optimum of 6 to 7 and an upper temperature limit of about 40°C. However, unequivocal evidence of heterotrophic nitrification cannot be proved (Schmidt, 1982).

## 4.2 Factors regulating nitrification

In comparison with ammonification, which is mediated by the diverse heterotrophic biomass, nitrification is mediated predominantly by a small group of autotrophic bacteria. Thus, the latter process is generally influenced more strongly by external factors such as moisture, temperature and pH than the former.

### 4.2.1 Substrates and products

The autotrophic nitrifiers are dependent on either  $\text{NH}_4^+$  or  $\text{NO}_2^-$  as specific energy sources so that substrate concentration can be a very important factor influencing nitrifier activity. Populations and *in situ* activities of nitrifiers in soils are usually limited by the rate of production of  $\text{NH}_4^+$ . Soil conditions that favour the occurrence of  $\text{NH}_3$  (high pH and low

CEC) restrict nitrification because of the toxicity of free  $\text{NH}_3$ . The  $\text{NO}_2^-$  oxidation stage is the most susceptible to inhibition by molecular  $\text{NH}_3$ , so that applications of urea or anhydrous  $\text{NH}_3$  to calcareous soils have resulted in  $\text{NO}_2^-$  accumulation (Schmidt, 1982).

Several studies have shown that the addition of  $\text{NH}_4^+$  fertiliser to soils can increase populations of *Nitrosomonas* by several hundred million per gram of soil. Similar results for the population of *Nitrobacter*, following the addition of  $\text{NO}_2^-$  to soils have also been observed.

#### 4.2.2 Substrate repression

The  $\text{NH}_4^+$ -oxidising bacteria are characteristically less sensitive than *Nitrobacter* to high  $\text{NH}_4^+$  concentrations. It has been reported that the rate of  $\text{NO}_3^-$  production increased with increasing  $\text{NH}_4^+$  concentration from 50 to 800  $\mu\text{g N g}^{-1}$  but at higher  $\text{NH}_4^+$ -N concentrations  $\text{NO}_2^-$  accumulated.

#### 4.2.3 End product repression

End product repression of nitrification can also occur. High concentrations of  $\text{NO}_3^-$  (2500 to 4200 mg N liter<sup>-1</sup>) inhibits the production of  $\text{NO}_2^-$  by *Nitrosomonas* in the logarithmic phase of growth (Painter, 1977). High concentrations of  $\text{NO}_3^-$  are also known to noncompetitively inhibit oxidation of  $\text{NO}_2^-$  by *Nitrobacter*. End product repression can be important when experiments are carried out in closed containers, especially when high rates of  $\text{NH}_4^+$  are added and the reaction is allowed to proceed for a long period (i.e., weeks) (Haynes, 1986).



#### 4.2.4 Soil pH

Soil pH is well known to be a limiting factor for nitrification. Generally, in culture, the optimal pH for growth and metabolism of autotrophic nitrifiers is in the range pH 7 to 9. Nevertheless, in soils above pH 7.5, toxic levels of  $\text{NH}_3$  may result in the inhibition of the activity of *Nitrobacter* and in the accumulation of  $\text{NO}_2^-$ .

The lower limit for autotrophic nitrification is generally found to be around pH 4.5. It is commonly observed that nitrification can occur in soils of pH 4 to 5. It is possible that the sites at which bacteria perform their oxidation have a higher pH than that determined from bulk soil samples, while strains of nitrifiers may exist in acid soils that have adapted to acidic soil conditions.

#### 4.2.5 Aeration and moisture

In general, the maximum rate of nitrification occurs at soil moisture potentials in the range of -10 to -33 kPa (field capacity), presumably depending principally on soil physical properties. At 0 kPa (oversaturated), nitrification is either absent or occurs at a very slow rate because of the shortage of  $\text{O}_2$  in the soil system caused by excess water.

When dry soils are re-wetted, even by small amounts of precipitation, there is a characteristic flush of mineralisation of native soil organic N. This increase in mineralisation rate is accompanied by a flush of nitrification and the temporary accumulation of  $\text{NO}_3^-$  in the soil (Haynes, 1986; Van Gestel *et al.*, 1993)

#### 4.2.6 Temperature

The optimum temperature range for nitrification in soils varies between 25 and 35°C. It does, however, seem that indigenous nitrifiers have temperature optima adapted to their climatic regions. There are few studies on the effects of fluctuating temperatures on nitrification. However, several studies have shown that the nitrification rate under fluctuating low temperatures is less than those under a corresponding low mean temperature. Like

drying and re-wetting, freezing and thawing stimulates mineralisation of native soil organic matter and consequently there is a flush of nitrification (Haynes, 1986).

## 5. MOVEMENT OF NITROGEN IN SOILS

Organic N usually constitutes over 90% of total N in surface soil and its mobility in soils is generally low. Ammonium is unlikely to be leached from soils because (1)  $\text{NH}_4^+$ -ions are held in the soil by the processes of cation exchange and fixation within clay lattices, (2) organic matter can adsorb considerable amounts of ammonia ( $\text{NH}_3$ ), and (3)  $\text{NH}_4^+$  can be readily immobilised by the microbial biomass or alternatively nitrified to  $\text{NO}_3^-$ -N (Haynes, 1986).

In contrast to  $\text{NH}_4^+$ , there is little tendency for the  $\text{NO}_3^-$  ion to be absorbed by soil colloids, which possess a net negative charge. Nitrate is thus susceptible to diffusion and mass transport with soil water.

Leaching losses of  $\text{NO}_3^-$  to groundwater occur principally when soil  $\text{NO}_3^-$  levels are high and downward water movement is large like during a rainstorm which occur regularly during the summer months in South Africa. Nitrate leaching is least likely to take place during the winter (in the summer rainfall regions) when evapotranspiration usually exceeds precipitation. The magnitude of such losses depends on factors such as rainfall, evaporation, soil type and plant cover.

Most terrestrial ecosystems show a reasonably closed N cycle and only lose significant amounts of N through leaching, runoff, and erosion following severe disturbance such as burning, harvesting, irrigating, heavy rain, or fertilising. In contrast, losses can be large in agricultural ecosystems, which are often continually disturbed. Leaching is often the most important channel of N loss from cultivated field soils other than that accounted for in crop uptake.

The economic significance of such losses of N is self-evident especially in areas where the loss becomes a limiting factor for plant growth. These losses can also have environmental consequences since they can increase the productivity of microorganisms in surface waters, particularly lakes and estuaries. Eutrophication brings about many undesirable changes, including proliferation of algae, a decrease in water clarity, and a depletion of dissolved oxygen in bottom water. A high level of nitrate in drinking water has also been considered as a potential health hazard (Brock *et al.*, 1994).

Because of its solubility in the soil solution, nitrate readily moves downward out of the zone of root penetration. Nitrate and ammonium will also be removed to satisfy the nutrient demand of the plant cover.

## 6. GASEOUS LOSSES OF NITROGEN

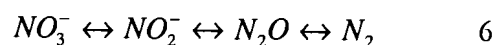
In the next 20 years, human population is expected to exceed 7.5 billion people. To feed these people food production must keep pace, generally without expanding production land area. In some areas of the world, China for example, increased economic wealth is leading to a change in diet from one that is essentially cereal-based to inclusion of more animal-based food products. The coupling of increased population with a move of a large sector of the world population to diets that require more energy and N input, will lead to continued increased anthropogenic input into the global N cycle. This scenario suggests that emissions of  $\text{NH}_3$ ,  $\text{NO}_x$  and  $\text{N}_2\text{O}$  from agricultural systems will continue to increase and impact global terrestrial and aquatic systems, even those far removed from agricultural production, to an ever growing extent, unless N resources can be used more efficiently or food consumption trends change (Mosier, 1999).

The greatest biological leak in the otherwise closed cycle is through denitrification; whereby nitrogen is removed entirely from the realm of ready accessibility because the end product of denitrification,  $\text{N}_2$ , is unavailable to most macro- and microorganisms.

Nitrous oxide (N<sub>2</sub>O) is an important greenhouse gas and it also participates in reactions, which destroy stratospheric ozone (Groffman, 1991, Atlas & Bartha, 1993). N<sub>2</sub>O is produced in soil by several processes, biotic and abiotic. Denitrification and nitrification are considered to be the most important biotic ones (Martikainen & De Boer, 1993, Hutchinson *et al.*, 1993). Abiotic production of N<sub>2</sub>O, and particularly NO, occurs primarily through a set of reactions collectively known as chemodenitrification (Hutchinson *et al.*, 1993).

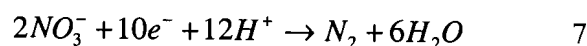
### 6.1 Denitrification

Denitrification is the microbial reduction of nitrate to produce nitrous oxide, N<sub>2</sub>O, and dinitrogen, N<sub>2</sub> by microorganisms that normally use O<sub>2</sub> for respiration.



The most common bacterial species performing denitrification is *Thiobacillus denitrificans* and *Pseudomonas denitrificans* (Prescott *et al.*, 1993). In *Klebsiella pneumoniae* N<sub>2</sub>O evolution has been reported to be associated with the use of NO<sub>2</sub><sup>-</sup> as sole terminal electron acceptor during anaerobic respiration (Firestone, 1982). Although the process has been demonstrated to occur in the presence of O<sub>2</sub>, vigorous denitrification activity is generally associated with anaerobic conditions in soil (Groffman, 1991).

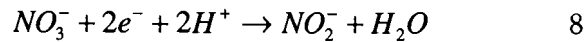
Some bacteria have electron transport chains that can operate with inorganic electron acceptors other than O<sub>2</sub>. Each nitrate will accept five electrons and the product is non-toxic.



### 6.2 Nitrification

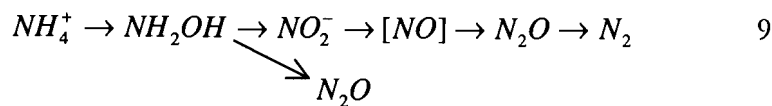
Nitrification is an important source of N<sub>2</sub>O in the environment (Goreau *et al.*, 1980). This process is of concern in atmospheric chemistry because it leads to the production of N<sub>2</sub>O by at least two mechanisms. First, intermediates between NH<sub>4</sub><sup>+</sup> and NO<sub>2</sub><sup>-</sup>, or NO<sub>2</sub><sup>-</sup> itself, can

chemically decompose to  $N_2O$ , especially under acid conditions. Nitrifying organisms can use nitrate as the electron acceptor at the end of their electron transport chain and still produce ATP (anaerobic respiration).  $N_2O$  is produced during the reduction of  $NO_2^-$  under anaerobic or microaerophilic conditions (Groffman, 1991, Verstraete, 1981). Nitrate may be reduced to nitrite by nitrate reductase, which replaces cytochrome oxidase.



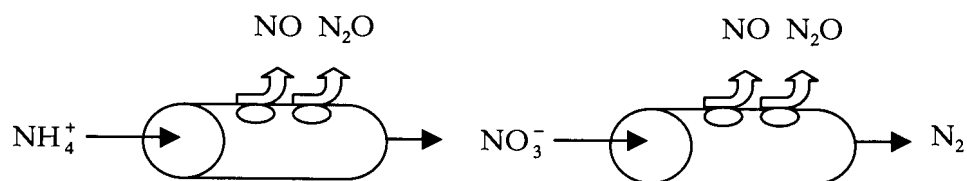
For microorganisms, the reduction of nitrate to nitrite is not a particularly effective way of making ATP because a large amount of nitrate is required for growth (a nitrate molecule will only accept two electrons). The nitrite formed is also quite toxic (Prescott *et al.*, 1993).

$NO_2^-$  can act as an intermediate in the production of  $N_2O$  through *dissimilatory nitriifier denitrification*.



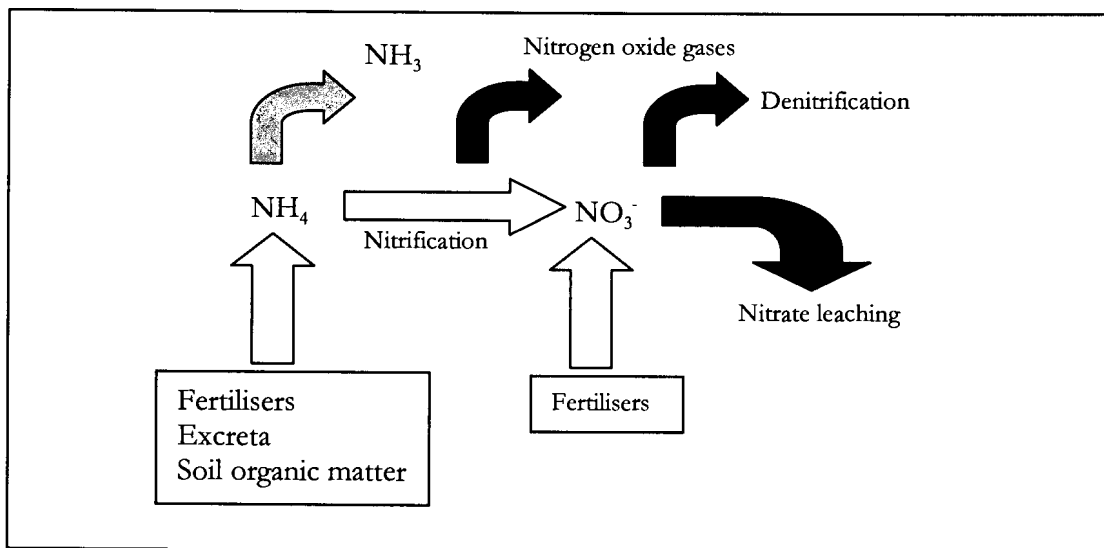
(Koops *et al.*, 1997)

Nitrification is often considered to be the dominant source of  $N_2O$  in “aerobic” soils (Groffman, 1991). It is illustrated in Figure 4, through the “hole-in-the-pipe” model from Firestone & Davidson (1989):



**Figure 4.** A conceptual model of the two levels of regulation of N trace gas production via nitrification and denitrification: (a) flux of N through the process “pipes” and (b) holes in the pipes through which trace N-gases “leak”





**Figure 5.** Nitrogen emissions from grassland soils (Jarvis, 1997)

$\text{NH}_3$  volatilisation is an important emission process (fig. 5) but will not be discussed here. Central to the control of emissions is the generation and removal of  $\text{NO}_3^-$ . Nitrate can be supplied directly through fertiliser addition or through nitrification of  $\text{NH}_4^+$  derived from fertilisers, dung, urine or mineralisation of soil organic matter. Nitrification is in itself important, not only as a supplier of  $\text{NO}_3^-$  but also through the release of  $\text{N}_2\text{O}$  and its transmission into the atmosphere. Not only is it likely that nitrification and denitrification can occur concurrently in adjacent microsites in soils under certain conditions, there is also the possibility that they are closely linked or coupled in some way. Many of the intermediates in each process are common to both, so that, the nitric oxide ( $\text{NO}$ ) released during nitrification could be consumed during denitrification perhaps in preference to  $\text{NO}_3^-$  (Jarvis, 1997).

### 6.3 Chemodenitrification

The term describes chemical reactions of  $\text{NO}_2^-$  ions within soils that result in the emission of a variety of nitrogenous gases (e.g.,  $\text{N}_2$ ,  $\text{NO}$ ,  $\text{NO}_2$  and sometimes  $\text{N}_2\text{O}$ ). Such gases are of nonbiological origin since they are also evolved from sterilised soil treated with  $\text{NO}_2^-$  (Haynes, 1986).

#### **6.4. Factors controlling $N_2O$ emissions**

##### 6.4.1 Soil aeration and moisture

The amount and rate of denitrification is proportional to soil moisture content. Under dryer soil conditions,  $N_2O$  is more likely to evolve than  $N_2$ . Denitrification of added nitrate is appreciable at high water levels and in localities having improper drainage. The effect of water is attributed to its role in governing the diffusion of  $O_2$  to sites of microbiological activity (Alexander, 1977, Haynes, 1986). With increasing soil moisture and decreasing  $O_2$  concentration the rate of denitrification generally increases.

In well-aerated soils, the anaerobic centres of soil aggregates provide suitable microsites for denitrification (see fig. 3, section 3.1.3) (Ross, 1989, Haynes, 1986). Under such conditions, emissions of  $N_2O$  are likely to originate, at least partially, through nitrification (Haynes, 1986).

Denitrification proceeds only when the  $O_2$  supply is insufficient to satisfy the microbial demand; at the same time,  $O_2$  is necessary for the formation of nitrite and nitrate, which are essential for denitrification (Alexander, 1977). The  $N_2O$  flux takes place just after the soil has become partially anoxic (Rappoldt & Corrè, 1997). Anaerobic microsites exist at microscopic sites in well-drained soils whenever the biological  $O_2$  demand exceeds the supply (see section 3.1.3).

In the absence of  $O_2$ ,  $NO_3^-$  or oxides derived from it serve as terminal electron acceptors for respiratory electron transport during the oxidation of the organic substrate and a more reduced N oxide or  $N_2$  is produced (Haynes, 1986).

Denitrification may also be inhibited by  $O_2$ . It may be the result of one or more effects on the responsible microbial populations. Because the active species are aerobes, the suppression cannot be attributable to some detrimental influence of  $O_2$  on their growth. In some instances, the suppression of nitrate reduction may merely be the preferential use of

oxygen as the electron acceptor. On the other hand, the inhibition may sometimes result from a detrimental effect on the enzymes bringing about nitrate and nitrite reduction, or  $O_2$  may even repress formation of the necessary denitrifying enzymes so that the catalysts required for the conversion never appear in the cells (Alexander, 1977).

#### 6.4.2 Temperature

Laboratory incubation studies have indicated that the optimum temperature for denitrification lies between 60 and 70° C. Other studies have shown that the optimum temperature is at 25° C but will not take place at 70° C (Alexander, 1977). Minimum temperatures for denitrification appear to be around 3 – 10° C (Scholefield & Hawkins, 1997, Haynes, 1986). The transformation proceeds slowly at 2° C, but increasing temperature enhances the rate of biological loss (Alexander, 1977). The  $N_2O:N_2$  was found by Nommik (1956, as quoted by Scholefield & Hawkins, 1997) to be large at low temperature and to decrease progressively over the temperature range. The optimum temperature for nitrification is often 25 to 30° C and it has been shown that  $N_2O$  production via nitrification increased as the temperature was raised from 10 to 30° C (Goodroad & Keeney, 1984).

There have been several observations of marked diurnal variability in the rate of  $N_2O$  emission. Maximum rates generally occurred in the afternoon and minimum rates during the night. Such results reflect the temperature dependence of both denitrification and nitrification. Denitrification appears to follow a seasonal trend with losses of  $N_2O$  plus  $N_2$  being markedly higher in summer than in winter (Haynes, 1986).

#### 6.4.3 pH

There is evidence that the kinetics of denitrification is affected by pH (Ellis *et al.*, 1997). Denitrifying bacteria are unable to live in acid conditions. Many of the bacteria that bring about denitrification are sensitive to high hydrogen ion concentrations, and hence various acid soils contain a sparse denitrifying population (Alexander, 1977). A decrease in soil pH generally decreases the proportion of  $N_2O$  and  $N_2$  produced (Jarvis, 1997). It has been found that below a pH of 4.8 there is a reduction in  $(N_2O + N_2)$ -N evolution, with maximum

denitrification at pH 8.0 (Groffman, 1991, Haynes, 1986). At pH-levels below 5.5, toxic levels of soil Al and Mn could limit microbial activity.

The optimum pH for nitrification appears to be pH 6 to 7. It has been found that nitrification can take place even at a pH of  $< 4$  but the  $N_2O$  production is still very little:  $< 0.001 \text{ mg N kg}^{-1} \text{ h}^{-1}$ . Acid soil pH favours nitrous oxide production via nitrification in some N fertilised forest-soils, preferentially selecting a dominant population of autotrophic nitrifiers (Ellis *et al.*, 1997). A limed soil (pH 6.7) showed a greater rate of nitrification of added  $NH_4^+$  and concomitant  $N_2O$  emission than from an unlimed (pH 4.7) control (Goodroad & Keeney, 1984).

#### 6.4.4 Source of carbon

In well-aerated soils autotrophic ammonium-oxidation can be the main source of  $N_2O$  (Blackmer *et al.*, 1980) but most denitrifying organisms are heterotrophic (Groffman, 1991, Haynes, 1986). The availability of organic matter is an important factor moderating both the rate and total extent of denitrification. Microbial degradation of organic matter supplies a readily available energy source for the denitrifiers and reduces the oxygen ( $O_2$ ) potential of the soil, which may create the necessary anaerobic conditions required for denitrification (Fowler *et al.*, 1997). The rate of denitrification is far slower in soils low in carbon than in land that is rich in organic matter (Alexander, 1977).

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## Chapter 2

### Extended abstract

**Research experiment 1** - The influence of substrate on nitrogen transformations

**Research experiment 2** - Mineralisation and nitrification in soil in the region of Pretoria (South Africa)

**Research experiment 3** - Nitrous oxide production and emission from South African soils

**General conclusions**



# CHAPTER 2

## RESEARCH EXPERIMENTS

### TABEL OF CONTENTS

#### Extended abstract

#### Research experiment 1 - The influence of substrate on nitrogen transformations in soil

|   |           |
|---|-----------|
| <b>INTRODUCTION</b> .....   | <b>2</b>  |
| <b>MATERIALS &amp; METHOD</b> .....   | <b>3</b>  |
| <i>SAMPLING</i> .....   | 3         |
| <i>PRE-INCUBATION</i> .....   | 3         |
| <i>INCUBATION</i> .....   | 4         |
| <i>MEASUREMENTS</i> .....   | 4         |
| <i>STATISTICAL ANALYSIS</i> .....   | 5         |
| <b>RESULTS &amp; DISCUSSION</b> .....   | <b>6</b>  |
| <i>N MINERALISATION/IMMOBILISATION AND NITRIFICATION</i> .....                  | 6         |
| <i>Influence of organic material incorporation</i> .....                        | 6         |
| <i>Figure 3. Influence of maize straw and fertiliser on nitrification</i> ..... | 7         |
| <i>Influence of added NH<sub>4</sub>NO<sub>3</sub> fertiliser</i> .....         | 8         |
| <i>INFLUENCE OF MOISTURE</i> .....  | 9         |
| <b>CONCLUSIONS</b> .....  | <b>10</b> |
| <b>REFERENCES</b> .....   | <b>10</b> |

#### Research experiment 2 - Mineralisation and nitrification in soil in the region of Pretoria (South Africa)

|                                     |           |
|-------------------------------------|-----------|
| <b>INTRODUCTION</b> .....           | <b>12</b> |
| <b>MATERIALS &amp; METHOD</b> ..... | <b>13</b> |
| <i>LOCALITY</i> .....               | 13        |
| <i>CLIMATIC CONDITIONS</i> .....    | 13        |
| <i>EXPERIMENTAL LAYOUT</i> .....    | 14        |
| <i>SOIL SAMPLING</i> .....          | 14        |
| <i>SOIL ANALYSIS</i> .....          | 15        |



|  |           |
|--|-----------|
| <b>RESULTS &amp; DISCUSSION .....</b>                                  | <b>14</b> |
| <b>NITROGEN MINERALISATION, IMMOBILISATION AND NITRIFICATION .....</b> | <b>14</b> |
| <i>Season 1997/1998 .....</i>  | <i>14</i> |
| <b>NITRATE LEACHING .....</b>  | <b>21</b> |
| <i>Season 1997/1998 .....</i>  | <i>21</i> |
| <i>Season 1998/1999 .....</i>  | <i>22</i> |
| <b>CONCLUSIONS .....</b>   | <b>24</b> |
| <b>REFERENCES.....</b>   | <b>25</b> |

## Research experiment 3 - Nitrous oxide production and emission from South African soils

|   |           |
|---|-----------|
| <b>INTRODUCTION.....</b>  | <b>27</b> |
| <b>MATERIALS &amp; METHOD.....</b>  | <b>28</b> |
| <b>SOIL SAMPLING.....</b>   | <b>28</b> |
| <b>PRE-INCUBATION.....</b>  | <b>29</b> |
| <b>TREATMENTS .....</b>   | <b>29</b> |
| <b>INCUBATION .....</b>   | <b>30</b> |
| <b>INHIBITORY HEADSPACE TREATMENTS .....</b>  | <b>30</b> |
| <b>REACTIONS .....</b>  | <b>31</b> |
| <b>MEASUREMENTS.....</b>  | <b>31</b> |
| <b>RESULTS &amp; DISCUSSION .....</b>   | <b>33</b> |
| <b>STATISTICAL ANALYSIS.....</b>  | <b>33</b> |
| <b>NET N<sub>2</sub>O EMISSION.....</b>   | <b>33</b> |
| <i>Stellenbosch soil.....</i>   | <i>34</i> |
| <i>Bethlehem soil.....</i>  | <i>35</i> |
| <i>Pretoria soil.....</i>   | <i>36</i> |
| <b>DIFFERENT N<sub>2</sub>O SOURCES.....</b>  | <b>36</b> |
| <b>Inhibitory headspace treatments.....</b>   | <b>37</b> |
| Effect of 10 kPa C <sub>2</sub> H <sub>2</sub> .....                                    | 37        |
| Effect of 10 Pa C <sub>2</sub> H <sub>2</sub> .....                                     | 38        |
| Effect of 100 kPa O <sub>2</sub> .....  | 38        |
| Combined effect of 100 kPa O <sub>2</sub> and 10 Pa C <sub>2</sub> H <sub>2</sub> ..... | 39        |
| Effect of organic material .....  | 39        |
| <i>Addition of NH<sub>4</sub>NO<sub>3</sub> fertiliser.....</i>                         | <i>40</i> |
| <b>CONCLUSIONS .....</b>  | <b>41</b> |
| <b>REFERENCES.....</b>  | <b>42</b> |

General conclusions



## EXTENDED ABSTRACT

The experimental data presented in the following three sections was obtained from experiments done as part of the bilateral scientific co-operation programme between Flanders and South Africa. The main activity under this initiative was to present a series of scientific contributions in which the characteristics and properties of intensive agriculture in Flanders and extensive agriculture in South Africa was demonstrated and discussed. The contributions highlighted the obvious differences and contrasts between both the agricultural systems. The Flemish soils are very rich in plant nutrients, organic matter and biological activities. The soils of South Africa in general, have poor nutrient levels, very low organic matter content and have low mineralisation activity. The immobilisation potential can be important when straw or plant residues are incorporated into the soil.

The institutes involved in this Flanders-South Africa bilateral project were:

- the Katholieke Universiteit Leuven (K.U. Leuven)
- the Rijks Universiteit Gent (R.U.G)
- the University of Stellenbosch
- the Small Grain Institute, Bethlehem
- the University of Pretoria.

Represented here are the experiments done by the University of Pretoria as part of the project.

Experiment 1 was an incubation study whereby the mineralisation potential of soil from the Hatfield experimental farm, Hatfield was measured under different conditions. The soil was incubated for 60 days at 20°C with the addition of maize straw and soya straw (at 4 ton ha<sup>-1</sup>) and NH<sub>4</sub>NO<sub>3</sub> fertiliser (at 20 kg). The soil samples were analysed according to the Kjeldahl method for NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N. The jars with distilled fluid were titrated with 0.01 N HCl.

Although the influence of moisture on mineralisation was not specifically measured in the study, it could be seen to greatly enhance microbial activity at the start of the incubation.

The addition of water after the pre-incubation greatly enhanced mineralisation. Values for both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in the first 7 days, measured around  $4 \text{ mg kg}^{-1}$ , with a maximum around day three. From day 7 the levels increased slightly (average  $2 \text{ mg kg}^{-1}$  for  $\text{NH}_4^+$  around day 15 and  $4 \text{ mg kg}^{-1}$  for  $\text{NO}_3^-$  around day 30) but a steady decline was measured up to the end of the incubation time for both treatments. The nitrogen was either lost through denitrifying activity or immobilised by microorganisms.

Experiment 2 was done as a field trial where the mineralisation and nitrification rate of the soil was measured as well as the potential for  $\text{NO}_3^-$  leaching. The trial was conducted over the growing season, thus October to May (8 months) over two years (1997 –1998 and 1998 –1999). Apart from a reference plot, one plot was amended with wheat straw (C:N 120) at a rate of  $4 \text{ ton ha}^{-1}$ , incorporated in the 0-30 cm soil layer. Soil samples were collected every two weeks for analysis of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . For collection of mineral N data under field conditions the soil core method was used. PVC pipes of 100 mm diameter were placed 30 cm into the soil. The PVC pipes were perforated with holes of 6 mm to allow easy transport of soil water. Two weeks before sampling four pipes were covered to avoid leaching and water penetration. The soil samples were also analysed according to the Kjeldahl method for  $\text{NH}_4^+$  and  $\text{NO}_3^-$ .

The rainfall pattern varied much over the two seasons, influencing mineralisation and nitrification in the soil. Compared to the rates measured in the incubation study, the rates measured in the field were much lower, due to different environmental conditions as well as the substrate added to the soil.  $\text{NH}_4^+$  rates were measured at around  $0.5 \text{ mg kg}^{-1}$  per two-week period. The nitrate levels were higher in the field than in the incubation study. Although most was immobilised through the season, at stages when the rainfall was high,  $\text{NO}_3^-$  levels of up to 10 and sometimes  $40 \text{ mg kg}^{-1}$  was measured.

Nitrate levels in the soil, up to 90 cm, picked up through both seasons. On average  $80 \text{ mg kg}^{-1} \text{ NO}_3^-$  was measured during March and April. In general the potential for  $\text{NO}_3^-$  leaching

is low. The high clay content (20.6 %) together with the limited rainfall restricts  $\text{NO}_3^-$  leaching. This soil is only subjected to possible  $\text{NO}_3^-$  leaching if heavy rains occur during periods of the year when no plants are grown and when sufficient residual N is present in the soil profile, which could be mineralised and/or nitrified.

The seasons responded very different concerning mineralisation and nitrification. It was not clear whether climatic variations caused the difference in N transformations during both seasons. Nor was it clear whether there was an effect of straw additions during the first season on N transformations in the second season. Probably both reasons play a role, since mineral N transformations in both straw and the control plot were altered.

In the third experiment, the contribution of nitrification and denitrification processes to gaseous N losses ( $\text{N}_2\text{O}$  and  $\text{N}_2$ ) was investigated with the acetylene ( $\text{C}_2\text{H}_2$ ) inhibition technique. In countries practising intensive agriculture, like in Europe,  $\text{N}_2\text{O}$  emission from soil is implicated in stratospheric ozone destruction. In South Africa the emphasis is rather on N loss for production than the potential for pollution. Three South African soils (Stellenbosch, Bethlehem and Pretoria) were used. The treatments included two moisture levels and combinations of C (straw) and N ( $\text{NH}_4\text{NO}_3$ ) amendments. Gaseous N losses were measured 1, 4 and 10 days after the C and N addition.  $\text{N}_2\text{O}$  emissions varied in a range of 0 to almost 3000 ng  $\text{N}_2\text{O-N kg}^{-1} \text{ h}^{-1}$ . The main source of  $\text{N}_2\text{O}$  was denitrification from the nitrifiers. The Stellenbosch soil has the lowest mineralisation and subsequent N loss potential of all three soils. The 100 kPa  $\text{O}_2$  treatment enhanced  $\text{N}_2\text{O}$  production as was found elsewhere. Carbon treatments gave the highest response to  $\text{N}_2\text{O}$  emission. Therefore, it is suggested that carbon is limiting both denitrification and heterotrophic nitrification in these soils.

The mineral N content of the soil is presently not used in N recommendations to either maize or wheat. Very little is known about the potential N losses due to leaching, volatilisation and the production of  $\text{N}_2\text{O}$ . In these experiments it showed large variation due to differences in climatic and soil conditions in different regions of South Africa.



## ON NITROGEN TRANSFORMATIONS IN SOIL

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### INTRODUCTION

Nitrogen transformations, biological and abiological, are very important processes in the nitrogen (N), sulphur (S) and carbon (C) cycles in soil. Many studies have been conducted over the world to determine e.g. the N transformations in the soil under different conditions. Not many of the results can be extrapolated to other places because of the different conditions at each site that govern the different processes.

Methods to determine the potential mineralisation indices of soil include laboratory and field studies; incubations with different water and organic matter contents under different environmental conditions, controlled and in the field; and <sup>15</sup>N labelled fertiliser techniques (Jarvis *et al.*, 1996).

The experiment was part of a larger study, co-ordinated with the University of Ghent, Belgium, to assess the mineralisation potential of some soils in South Africa. Field studies were done at three locations (Stellenbosch, Bethlehem and Pretoria) to obtain real figures of N transformations under extensive agricultural conditions, locally. Further studies on another aspect, N<sub>2</sub>O emissions, which has not been previously assessed under South African conditions, were conducted in Ghent in a laboratory. These results and that of the field studies are discussed elsewhere.

The aim of this experiment was to determine the extent of mineralisation of organic N and the transformations of inorganic nitrogen in the soil, specifically to NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> as influenced by organic material.

## MATERIALS & METHOD

### *Sampling*

Soil from the Hatfield experimental farm of the University of Pretoria, in Pretoria (26° 12' S 28° 10' E) was used. The main physical and chemical soil characteristics of the 0-30 cm region are listed in Table 1. Soil for analysis was taken from the top 30 cm of the profile. The soil was air dried, ground and sieved to pass through a 2 mm sieve. Care was taken to ensure that the soil is homogenous.

**Table 1.** Physical-chemical characteristics of the soils.

| Max<br>Soil depth | Textural<br>class  | C:N         |             |             | Total C<br>(g kg <sup>-1</sup> ) | Total N<br>(mg kg <sup>-1</sup> ) | Inorganic N   |   | pH<br>(H <sub>2</sub> O) | CaCO <sub>3</sub> |     |
|-------------------|--------------------|-------------|-------------|-------------|----------------------------------|-----------------------------------|---|---|--------------------------|-------------------|-----|
|                   |                    | Clay<br>(%) | Silt<br>(%) | Sand<br>(%) |                                  |                                   | NH <sub>4</sub> <sup>+</sup> -N<br>(mg kg <sup>-1</sup> ) | NO <sub>3</sub> <sup>-</sup> -N<br>(mg kg <sup>-1</sup> ) |                          |                   |     |
| 120 cm +          | Sandy<br>clay loam | 20.6        | 9.8         | 69.6        | 12.2                             | 6.1                               | 500   | 2.2   | 1.3                      | 5.6               | 0.0 |

### *Pre-incubation*

Glass jars (volume: 250 ml) were filled with 50 g soil. The soil in the jars was brought to ½ field capacity (FC). FC was determined as follows:

Three tall glass cylinders (± 40 cm tall, 3.5 cm wide) with open ends were filled with the chosen soil. A rubber stopper was inserted at one end and the soil compacted as close to natural conditions as possible. About 15 ml water was added to the soil column and another stopper inserted loosely at the top. It was left overnight for the water to distribute in the profile. The next day soil samples were taken from the middle of the column to ensure equal wetness. Care was taken to exclude the drier soil on top and the wetter soil at the bottom of the columns. The samples were weighed while wet and then dried at 105°C to extract all the moisture from the soil. The dry soil was then weighed again. The values obtained were used in this calculation to determine field capacity.

$$\%w = w/M_s \times 100$$

$$w = \text{wet mass} - \text{dry mass}$$

$$M_s = \text{mass solid particles (dry mass)}$$



Soil was pre-incubated for 7 days at 20°C. The jars were opened every second day to aerate the soil. Soil moisture content was kept as close to that intended by adding distilled H<sub>2</sub>O to compensate for losses due to evaporation.

The pre-incubation was done to stabilise the environment in the jars. Microbial communities were given time to increase their numbers and adjust to the environment.

### *Incubation*

After the seven-day pre-incubation period the soil was brought to  $\frac{3}{4}$  FC (15% moisture). Treatments were applied to the soil, consisting of different organic materials (maize straw, C:N 25 or soya straw, C:N 30) in combination with or without inorganic N fertiliser (NH<sub>4</sub>NO<sub>3</sub>) according to table 2.

0.4 g of the different organic material was added to the 50 g soil in the jars, representing a rate of 4 ton ha<sup>-1</sup> based on a soil density 1500 kg ha<sup>-1</sup> and a profile depth of 30 cm. Maize straw was added to half of the jars and soy straw to the other half. Half of the soil was treated with NH<sub>4</sub>NO<sub>3</sub> fertiliser. The fertiliser was added as 0.22 g NH<sub>4</sub>NO<sub>3</sub> to 50 g soil at a rate of 20 kg NH<sub>4</sub>NO<sub>3</sub> ha<sup>-1</sup>.

The jars were incubated at  $\frac{3}{4}$  FC at 20°C for 0, 1, 3, 7, 15, 30, 45 and 60 days. Four replicates were taken on the specific days to determine the NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N content of the soil samples.

### *Measurements*

On each day of measurement (0, 1, 3, 7, 15, 30, 45 and 60) the NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> of the soil samples were determined. To each jar 100 ml 1 N KCl was added and shaken for 1 hour and filtrated. The filtrate was analysed according to the Kjeldahl-method for NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> (Stanford & Smith, 1972). The jars with distilled fluid were titrated with 0.01 N HCl.



**Table 2.** Treatment combinations

| Combinations | Composition    |  |                     |
|--------------|----------------|--|---------------------|
|              | Organic matter | NH <sub>4</sub> NO <sub>3</sub> fertiliser |                     |
| N0           | +              | No   | plus maize straw    |
|              | -              |  | without maize straw |
|              | +              |  | plus soy straw      |
|              | -              |  | without soy straw   |
| N1           | +              | Yes  | plus maize straw    |
|              | -              |  | without maize straw |
|              | +              |  | plus soy straw      |
|              | -              |  | without soy straw   |

### *Statistical analysis*

Analysis of variance (ANOVA) was done on two factors, treatment (Table 2) and 8 time slots (day 0, 1, 3, 7, 15, 30, 45 and 60). Both the main effects and the day.treatment interaction were tested (Table 3 (a) & (b)) with the Fisher protected LSD test (Snedecor & Cochran, 1967).

**Table 3.** ANOVA of the production of (a) NH<sub>4</sub><sup>+</sup> and (b) NO<sub>3</sub><sup>-</sup> over time with different treatments

| (a)                 |         | (b)                 |     |
|---------------------|---------|---------------------|-----|
| Source of variation | Df      | Source of variation | Df  |
| Day                 | 7       | Day                 | 7   |
| Treatment           | 7       | Treatment           | 7   |
| Day.Treatment       | 49      | Day.Treatment       | 49  |
| Residual (error)    | 127 (1) | Residual (error)    | 128 |
| Total               | 190 (1) | Total               | 191 |

## RESULTS & DISCUSSION

The complete data sets of the incubation study are given in Appendix I. In this experimental report the data is shown graphically after the averages over the replicates have been calculated.

### *N mineralisation/immobilisation and nitrification*

#### Influence of organic material incorporation

The soil has a relatively low nitrogen and carbon content (Table 1). When carbonaceous substrates are incorporated into the soil, there is an immediate and marked drop in the  $O_2$  concentration and an increase in the  $CO_2$  content of the soil air. At the same time the oxidation-reduction potential ( $E_b$ ) is shifted to a more reduced condition. Microorganisms cause the change in  $E_b$  through the consumption of  $O_2$  and the liberation of reduced products, such as  $CO_2$  (Alexander, 1977). The  $NH_4^+$  is thus immobilised.

The soil treated with organic material showed immobilisation of N compared to the control soil from day 7 to 30. As the C:N ratios were lowered, a portion of the immobilised N was released through net mineralisation (fig. 1 & 2). Mineralisation and immobilisation occur simultaneously in the soil. The  $NH_4^+$  was exhausted to the end of the incubation in all the treatments.

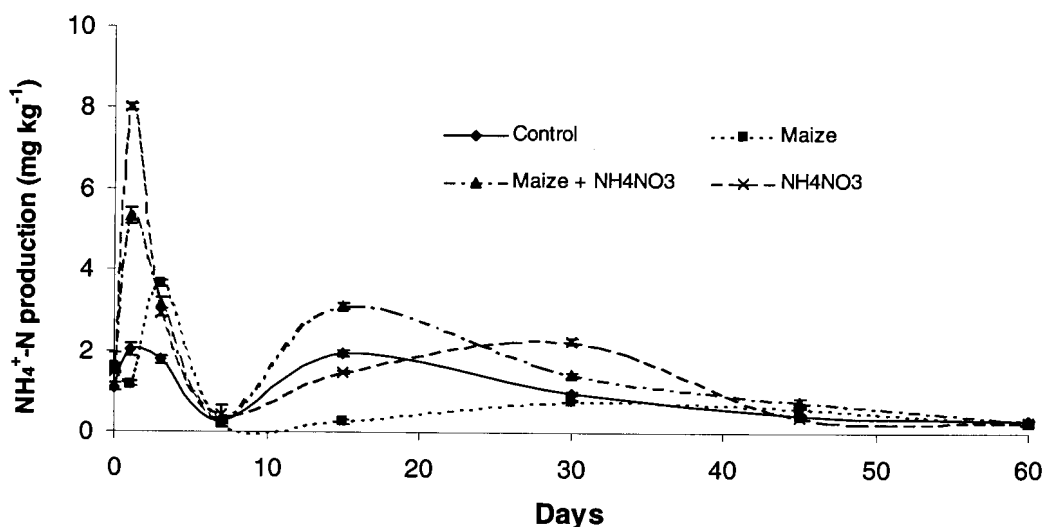


Figure 1. Influence of maize straw and fertiliser on mineralisation

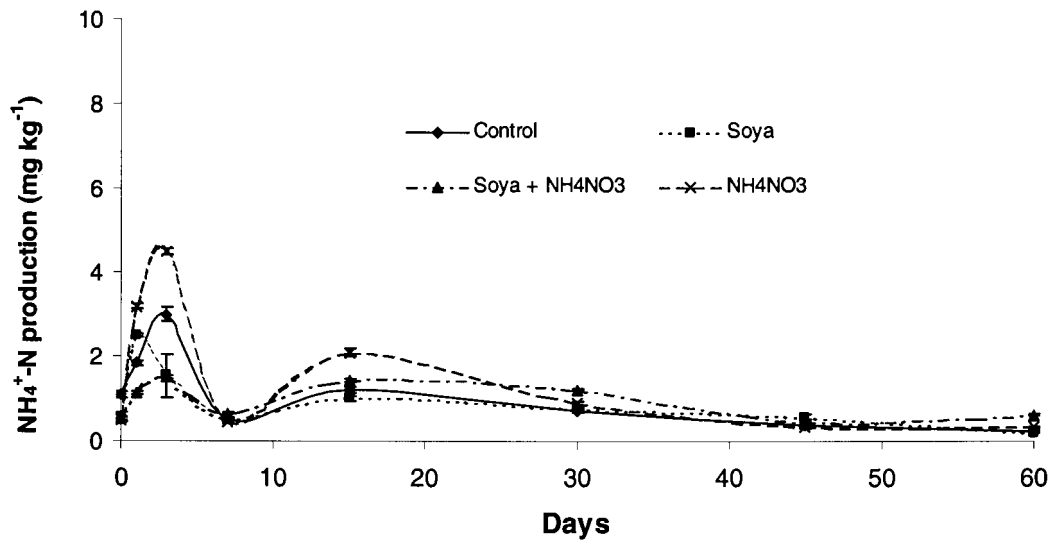


Figure 2. Influence of soya straw and fertiliser on mineralisation

$\text{NO}_3^-$  was consumed by microorganisms during immobilisation of the added organic material for the same three weeks (day 7 to 30). When conditions changed to net mineralisation, a part of the  $\text{NO}_3^-$  reappeared in the measured  $\text{NO}_3^-$  pool.

After three or four days, readily decomposable substrates were becoming depleted. Biomass C declined slowly during the rest of the incubation (assumed). At the end of the incubation study there was less than  $10 \text{ mg kg}^{-1} \text{ NH}_4^+$ -N left in the soil for the maize and soya treated soil.

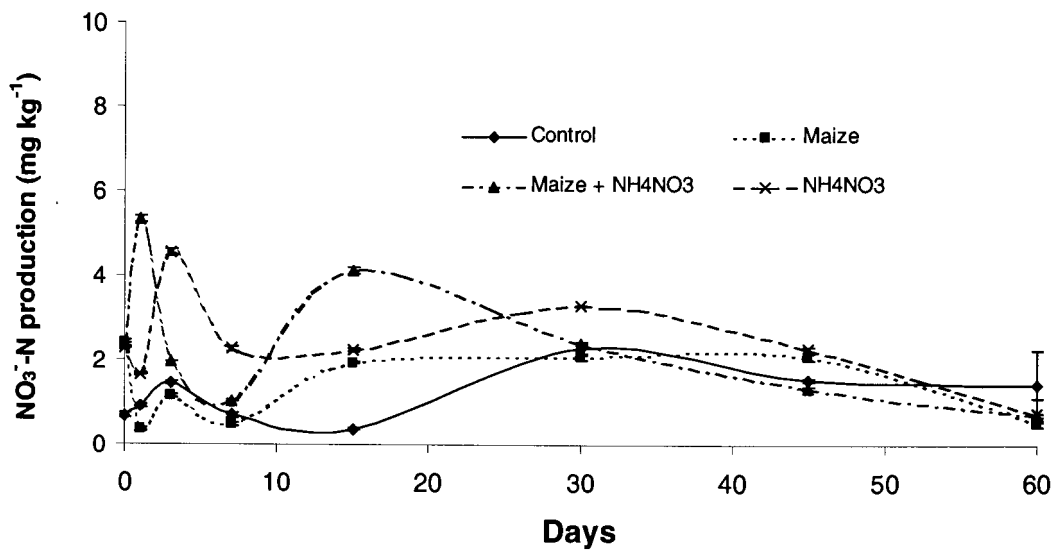
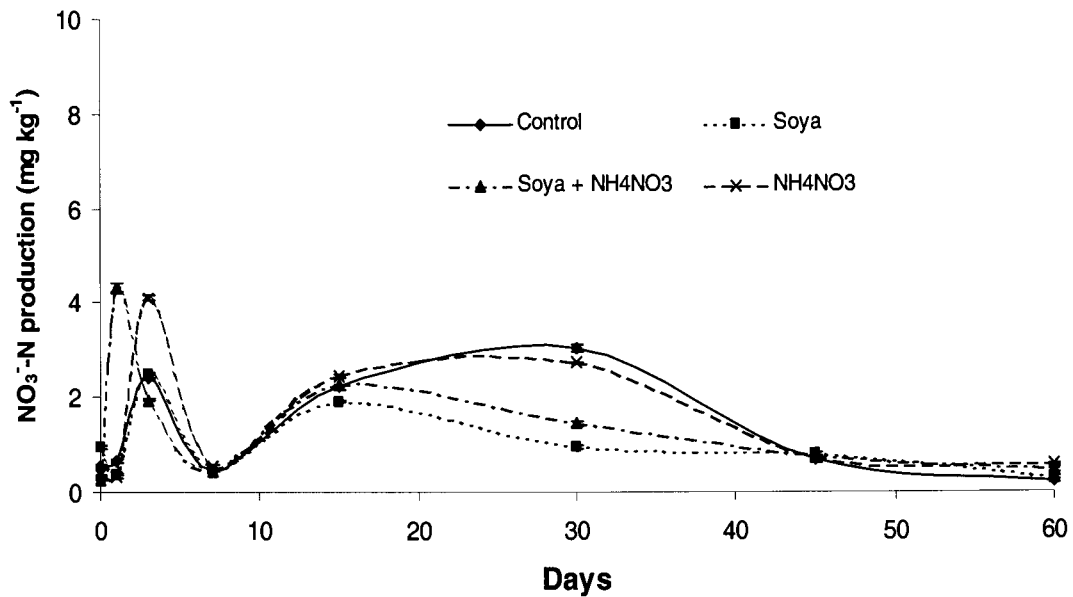


Figure 3. Influence of maize straw and fertiliser on nitrification



**Figure 4.** Influence of soya straw and fertiliser on nitrification

#### Influence of added NH<sub>4</sub>NO<sub>3</sub> fertiliser

The highest net mineralisation was measured in the soil treatments containing fertiliser N. Fertiliser N led to an increase in mineralisation of the native organic matter compared to the control and straw treatments.

Applications of inorganic fertiliser N have been reported to stimulate, depress or have no effect on the mineralisation of native soil organic N (Broadbent, 1970). In the treatments where the fertiliser was applied with the organic matter, high levels of NH<sub>4</sub><sup>+</sup> were measured from day 7 to day 30. The addition of NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> to straw or other nitrogen-deficient substrates greatly enhances decomposition (Alexander, 1977). Fertiliser addition gives rise to the preparing/priming effect, increasing mineralisation of native organic material.

Nitrification was also enhanced in the fertiliser treated soil. The control soils measured high NO<sub>3</sub><sup>-</sup> levels compared to the other treatments. The C:N in the soil was lower than that of the treated soil, which led to increased mineralisation and subsequent nitrification.



Nitrate is very sensitive to environmental factors, which could influence production of  $\text{NO}_3^-$ . When nitrogen is oxidised one gets a change in surface charge of particles. A part is charged positively (as in  $\text{NH}_4^+$ ) and other is charged negatively as in  $\text{NO}_3^-$ . The positively charged particles can be adsorbed onto the negatively charged clay particle where it is held but nitrate, with its negative charge will be pushed off the surface and could be subjected to loss through leaching or denitrification.

### *Influence of moisture*

Although moisture was not studied as one of the factors influencing mineralisation and nitrification, it definitely played a large role in these processes. Soil microbes are sensitive to changing moisture conditions as it influences the processes they perform. The soil moisture content was increased from  $\frac{1}{2}$  FC to  $\frac{3}{4}$  FC after the pre-incubation. The increase in moisture led to an increase in mineralisation of C and N during the subsequent incubation of the maize and soya treated soil. There is a distinct N flush at the start of each incubation (fig. 1, 2, 3 & 4). It reached a maximum on day 3. Within two days the readily decomposable substrates were depleted and mineralisation and nitrification decreased up to day 7.

This phenomenon is supported by other workers (Van Gestel *et al.*, 1993, Haynes, 1986) who found that when the moisture content of the soil is increased after pre-incubation, more amounts of C and N are mineralised during the subsequent mineralisation, when compared with mineralisation from soils that are kept at the same moisture level. It was also found that after remoistening of soil, microbial cells killed by drying, and other sources of soil organic matter which had become available during drying, were rapidly metabolised, leading to increases in biomass C.

Moisture also influences nitrification in that it influences the solubility of  $\text{O}_2$ , needed for nitrification. The increase in moisture enhanced nitrification because of adequate aeration.



## CONCLUSIONS

The study conducted here showed significant results concerning the different treatments over time. It also showed a good correlation with moisture.

After 60 days of incubation, the soil was exhausted of decomposable materials,  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N. The microorganisms could not sustain themselves any longer. It is thought that the incubation time is too long and that it can be stopped after 45 days rather than 60. It is difficult to extrapolate the data to field conditions because of the amplified mineralisation and nitrification due to the small, enclosed environment.

Moisture stimulates microbial growth as can be seen from the nitrogen flush at the beginning of the incubation. The enhanced microbial activity stimulates the production of more  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N.

Organic material immobilised most of the nitrogen in those treatments. Due to the age of the material all the easily decomposable material has been mineralised already. The tougher parts of the material were introduced to the soil and immobilisation of the nitrogen therein took place, with a very slow release to mineralise.

The addition of fertiliser N showed a good priming/preparing effect to mineralisation of the native organic material of the soil. Although it is advantageous for plant growth, care should be taken that it is not lost to the groundwater or atmosphere.

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## MINERALISATION AND NITRIFICATION IN SOIL IN THE REGION OF PRETORIA (SOUTH AFRICA)

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### INTRODUCTION

The incorporation of organic material in soil brings about many changes to the soil environment. The basic constituents of plant material can be classified into several identifiable fractions such as cellulose; hemicellulose; lignin; a water-soluble fraction composed of simple sugars, amino acids, and aliphatic acids; a protein fraction; and an ether- and alcohol-soluble fraction composed of fats, oils, waxes and resins (Coyne, 1999).

Microbes attack the organic matter, which serves as a carbon and energy source for them. The easily decomposable fractions like the sugars and amino acids are consumed first. The lignin and cellulose compounds are not easily decomposed and it will take months before it is totally decomposed (Coyne, 1999).

Nitrogen (N) is the most common constituent of plants after carbon (C) and oxygen (O). Depending on the C:N of the organic plant material being decomposed by heterotrophic organisms, the nitrogen will be mineralised or immobilised. Many factors influence N transformation processes such as mineralisation, nitrification and denitrification. These include climate (e.g. soil temperature and moisture), pH, organic matter and microbial activity. Increasing soil temperature and moisture up to optimum levels, speeds up the mineralisation of organic material by creating more suitable conditions for microbial activity. Microbes function best at a pH of 5-6. Mineralisation of organic N occurs over the whole pH range but the rate decreases progressively below a pH of 6 (Curtin *et al.*, 1998).

The study area lies in a region where extensive agriculture is practised. Crop residues are sometimes incorporated into the soil but the relatively warm winters in the Middleveld and Highveld regions gives way for decomposition, though at a slower rate. Crop residues are sometimes taken of the field and used as animal feed.

The main emphasis in this study was to determine the potential mineralisation, nitrification and leaching of nitrogen from the soil with and without the addition of wheat straw under field conditions.

## MATERIALS & METHOD

### *Locality*

A field experiment was conducted at the Hatfield experimental farm of the University of Pretoria (26°12' S 28°10' E) during the seasons 1997/1998 and 1998/1999 on a deep red sand-clay-loam soil. Soil characteristics are given in Table 1.

**Table 1.** Physical-chemical characteristics of the soil

| Max soil depth | Textural Class  | C:N      |          |          | Total C (g kg <sup>-1</sup> ) | Total N (mg kg <sup>-1</sup> ) | Inorganic N (mg kg <sup>-1</sup> ) |                                 | pH (H <sub>2</sub> O) | CaCO <sub>3</sub> |     |
|----------------|-----------------|----------|----------|----------|-------------------------------|--------------------------------|------------------------------------|---------------------------------|-----------------------|-------------------|-----|
|                |                 | Clay (%) | Silt (%) | Sand (%) |                               |                                | NH <sub>4</sub> <sup>+</sup> -N    | NO <sub>3</sub> <sup>-</sup> -N |                       |                   |     |
| 120 cm +       | Sandy Clay Loam | 20.6     | 9.8      | 69.6     | 12.2                          | 6.1                            | 500                                | 2.2                             | 1.3                   | 5.6               | 0.0 |

### *Climatic conditions*

The experimental farm lies in the summer rainfall region (table 2). The growing season in the region is from October to May. A wide range of crops is grown in the area due to a very suitable climate. The soil is also quite deep, which makes it a very suitable soil for agriculture. The only restricting factor is rainfall, which is unreliable (table 3).

**Table 2.** Climatic data<sup>†</sup> for the Hatfield experimental farm of the University of Pretoria

|               | Jan   | Feb  | March | April | May  | June | July | Aug  | Sept | Oct  | Nov   | Dec   |
|---------------|-------|------|-------|-------|------|------|------|------|------|------|-------|-------|
| Rainfall (mm) | 131.7 | 85.7 | 83.7  | 53.9  | 13.4 | 7.3  | 2.5  | 4.9  | 19.1 | 20.5 | 105.9 | 122.1 |
| Min temp      | 16.1  | 15.9 | 14.5  | 11.1  | 7.0  | 3.7  | 3.7  | 6.3  | 10.5 | 12.9 | 14.2  | 15.3  |
| Max temp      | 28.1  | 27.9 | 26.6  | 24.2  | 21.8 | 19.1 | 19.4 | 22.0 | 25.6 | 26.6 | 26.7  | 27.5  |
| Avg. temp*    | 22.1  | 21.9 | 20.6  | 17.7  | 14.4 | 11.4 | 11.6 | 14.2 | 18.1 | 19.8 | 20.5  | 21.4  |

<sup>†</sup>Forty year average

\*Mean daily temperature (°C)

**Table 3.** Annual seasonal weather data for 1997/1998 and 1998/1999

| Rainfall (mm)    | Oct  | Nov   | Dec   | Jan   | Feb   | March | April | May  | Sum   |
|------------------|------|-------|-------|-------|-------|-------|-------|------|-------|
| 1997/1998        | 24.5 | 14.9  | 85.2  | 133.0 | 118.4 | 48.7  | 1.5   | 0.0  | 426.2 |
| 1998/1999        | 51.9 | 104.2 | 146.2 | 41.1  | 28.8  | 71.8  | 84.9  | 30.2 | 559.1 |
| Temperature (°C) |      |       |       |       |       |       |       |      | Avg.  |
| 1997/1998        | 19.0 | 20.7  | 21.9  | 22.0  | 22.6  | 22.2  | 19.7  | 13.7 | 20.2  |
| 1998/1999        | 18.7 | 20.7  | 20.4  | 22.0  | 22.8  | 22.0  | 19.1  | 15.1 | 20.2  |

### *Experimental layout*

The experiment comprised two treatments; a control treatment where no straw was added and a straw treatment where the equivalent of 4000 kg of wheat straw ha<sup>-1</sup> was added each season. Wheat straw (C:N 120) was evenly spread on the soil and disked to a depth of 150 mm into the soil of the plot. Both plots were ploughed to a depth of 200 mm at the beginning of October. At the start of the season soil sampling was done on both plots (see table 1). PVC cylinders (300 mm long, 100 mm in diameter) were installed on both plots to a depth of 300 mm.

### *Soil sampling*

Soil sampling started in the first week of October. On every sampling day, at 14 day intervals, 8 tubes, of which four were sealed off 14 days prior and four left uncovered, were sampled from each plot up to 300 mm. The non-covered cylinders served as the blank treatment for the following sampling of the covered cylinders. One would then be able to see the influence of moisture and nutrient movement on the production and



movement of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N in the topsoil. Soil cores taken from the cylinders were divided into two sub-samples (0 – 150 mm and 150 – 300 mm soil profile), sealed in plastic bags and immediately transported to the laboratory. From an unsealed cylinder, samples were taken in 150 mm intervals up to 900 mm. That was done to access the amounts of N leached through the profile.

### *Soil analysis*

After drying at 40°C overnight, the soil samples were passed through a 2.0 mm sieve and analysed to determine the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  content of the soil according to the Kjeldahl method of analysis (Stanford & Smith, 1972).

## **RESULTS & DISCUSSION**

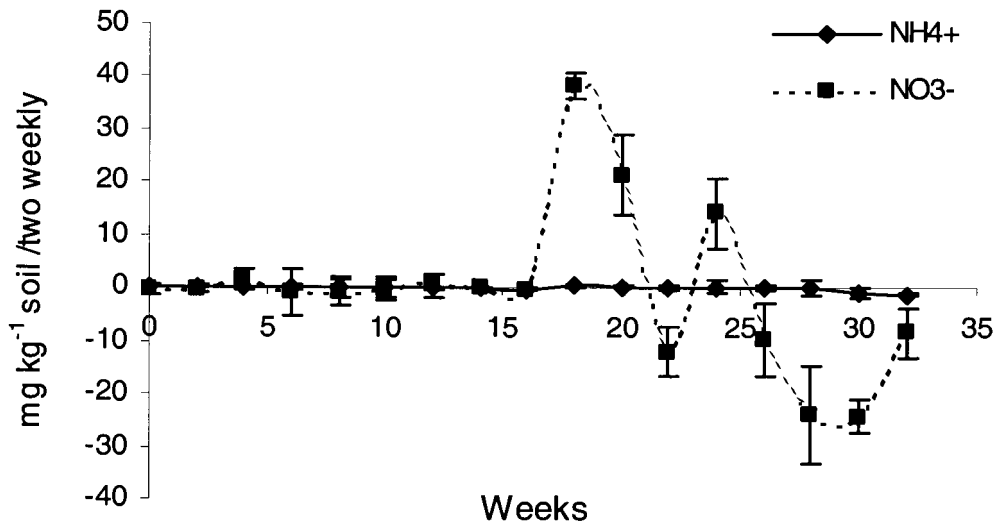
The complete data sets are represented in Appendix II. Net mineralisation and immobilisation rates as well as cumulative rates of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N values are represented in figures in the text.

### *Nitrogen mineralisation, immobilisation and nitrification*

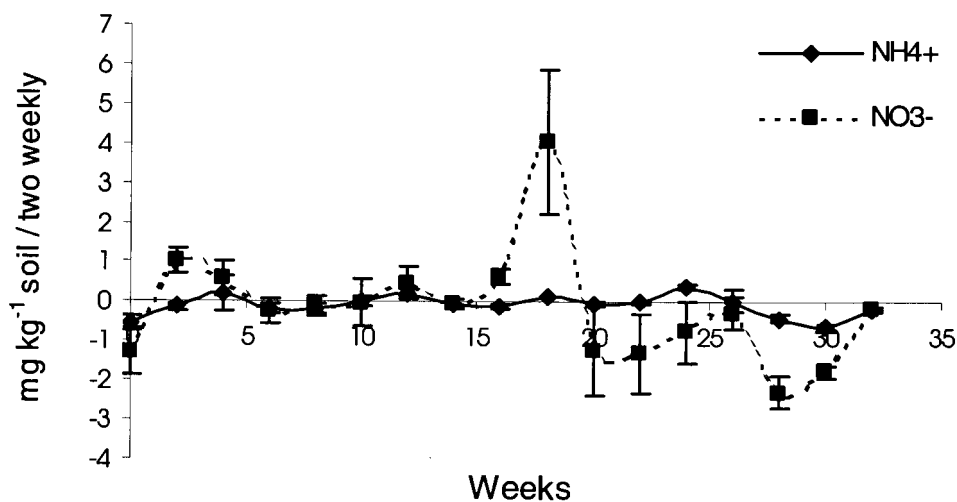
Season 1997/1998

The decay of organic residues in soil is accompanied by conversion of C and N into microbial tissue. In the process part of the C is liberated as  $\text{CO}_2$  (Stevenson, 1982). The nitrogen would remain in organic combination (immobilised) as long as the C:N is wide (Alexander, 1977). Since the wheat straw added to the soil of the experimental site had a wide C:N (120), it was slowly decayed because of the various constituents of the material. The easily decomposable components (e.g. carbohydrates and starches) were mineralised first and the tougher material (e.g. cellulose and lignin) later.

Figures 1 and 2 show the production and immobilisation rates of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N for the straw and control plots of the first season (1997 – 1998), respectively. From October until January (week 0 – 15)  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N production or immobilisation rates were very low. The first two months received very little rain (table 3) and it was generally very warm. Microbial activity was limited by the lack of moisture and high temperatures (Leiròs *et al.*, 1999).



**Figure 1.**  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N production (positive gradient) and immobilisation (negative gradient) rates in the top 30 cm of the straw plot, during the 1997-1998 season (week 0 is October 1, 1997).



**Figure 2.**  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N production (positive gradient) and immobilisation (negative gradient) rates in the top 30 cm of the control plot, during the 1997-1998 season (week 0 is October 1, 1997).



The major factor that influenced the mineralisation and nitrification during the season was the rainfall. Despite the heavy rains during December, January and February (week 8 – 20), the difference in  $\text{NH}_4^+$  measured compared to the other months was not very large (fig. 1 & 2). The control soil (fig. 2) showed more fluctuation in the  $\text{NH}_4^+$  production. Less immobilisation took place in the soil from the control plot. Figures 1 & 2 show that with the increased rainfall in January-February (week 12 – 20), net  $\text{NO}_3^-$ -N production rose sharply from week 16. The  $\text{NH}_4^+$ -N levels, however, remained low. The increase in moisture led to an increase in microbial activity and the  $\text{NH}_4^+$ -N was converted to  $\text{NO}_3^-$ -N as soon as it was formed (Prescott *et al.*, 1993). From March (week 20) the rainfall decreased again and no rain fell in the last month of the growing season (May, week 28). Six months after the incorporation of the organic material, the substrate was mostly decomposed. Immobilisation of the  $\text{NO}_3^-$ -N was observed until the end of the season. <sup>1</sup>Cheshire *et al.* (1999) found in studies using <sup>15</sup>N (Jansson, 1958) that microorganisms taking part in straw decomposition in the presence of soil preferred  $\text{NO}_3^-$  to  $\text{NH}_4^+$ . In the absence of straw,  $\text{NH}_4^+$  was preferred to  $\text{NO}_3^-$  by heterotrophic organisms colonizing the straw. The difference was explained in terms of the competing reactions in soil for exchangeable  $\text{NH}_4^+$  such as  $\text{NH}_4^+$  fixation, which is characteristic of 2:1 clay minerals such as illite and vermiculite.

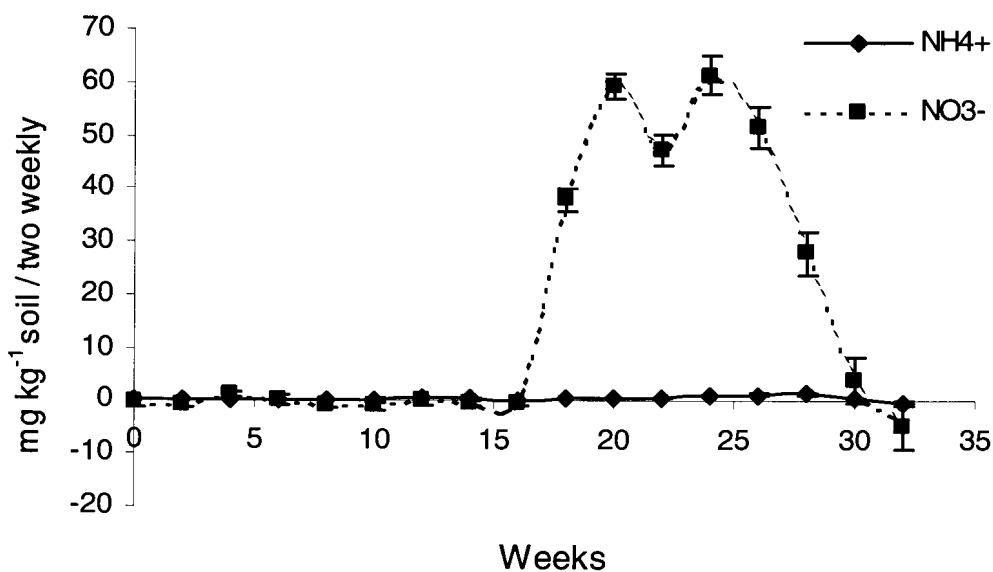
Figure 3 and 4 show that  $\text{NO}_3^-$  accumulated from week 16 (beginning of February) almost until the end of the study. In the straw plot (fig. 3)  $\text{NO}_3^-$  levels dropped again around week 30 (middle May) and earlier in the control plot, almost two weeks (beginning of May). Accumulation of  $\text{NO}_3^-$  in the control soil was smaller, though. If the rainy period would have come earlier or with additional irrigation, the  $\text{NO}_3^-$  accumulation peak in the soil could have shifted earlier in the season. It would be more beneficial for plant growth, having nitrate available sooner for plant uptake.

#### Season 1998/1999

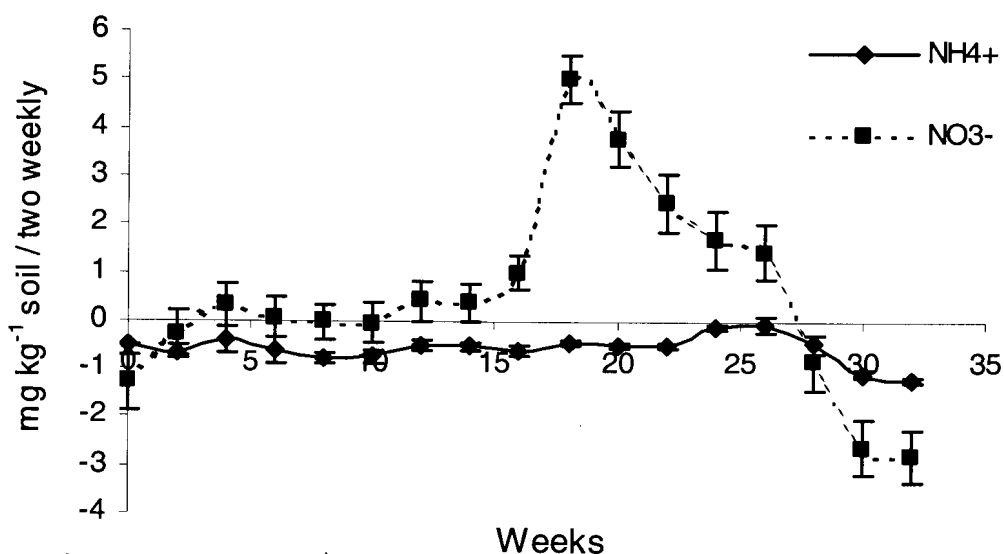
The rainfall pattern during this season was completely different from the 1997 – 1998 season the previous year (table 3). Most of the rain fell during November and December



(week 4 – 12) as well as some substantial rain at the end of the season (week 24 –32). The total amount of rainfall was approximately 130 mm higher. The variations of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  content of the straw and control plots were much smaller than the previous season (fig. 5 & 6).

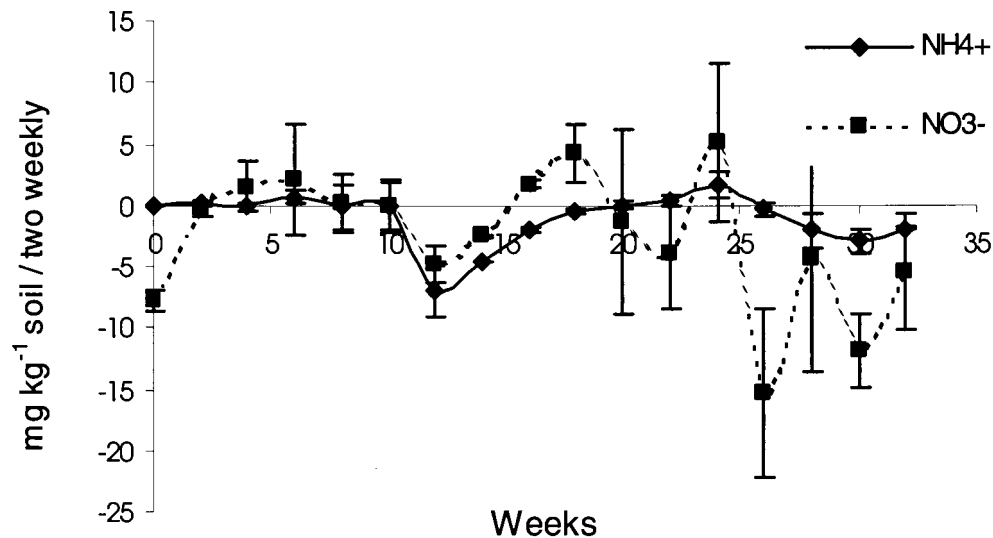


**Figure 3.** Cumulative  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N production (positive gradient) and immobilisation (negative gradient) rates in the top 30 cm of the straw plot, during the 1997-1998 season (week 0 is October 1, 1997).



**Figure 4.** Cumulative  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N production (positive gradient) and immobilisation (negative gradient) rates in the top 30 cm of the control plot, during the 1997-1998 season (week 0 is October 1, 1997).



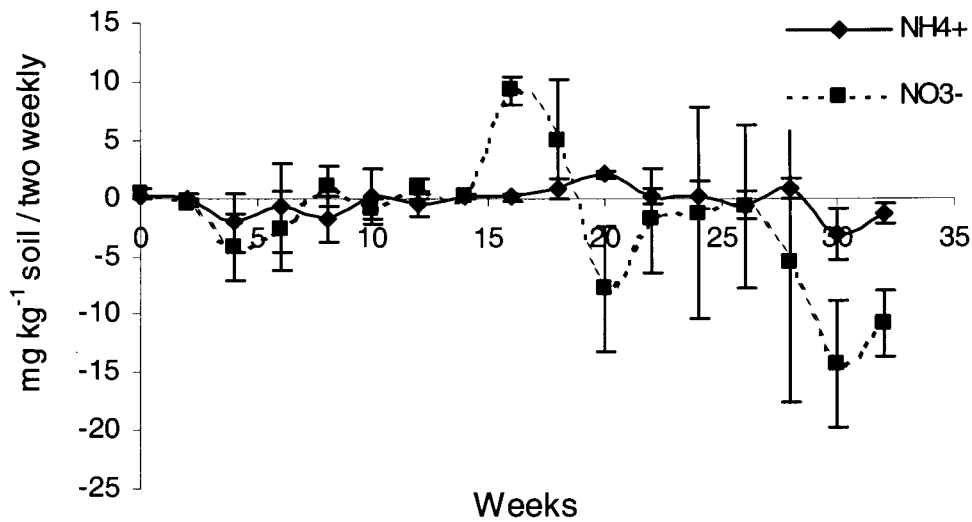


**Figure 5.**  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N production (positive gradient) and immobilisation (negative gradient) rates in the top 30 cm of the straw plot, during the 1998-1999 season (week 0 is October 1, 1998).

During the first month (week 0 – 4) of the second season (1998 – 1999) very little  $\text{NH}_4^+$ -N was measured from the straw treated soil (fig. 5). Initial immobilisation of nitrogen took place because there was not enough N available in the substrate to convert all the C into biomass (Coyne, 1999). From week 4 the  $\text{NH}_4^+$  was released to the soil and could be measured. Both in the straw and control plots,  $\text{NO}_3^-$  formation were almost absent during this season. At week 14/15 (middle to end January) there was a slight increase in  $\text{NO}_3^-$  production at both plots. It was probably a lag effect due to the high rainfall of the previous few weeks. After four weeks the production declined. Net  $\text{NO}_3^-$  immobilisation and/or depletion persisted until the end of the season. It will be discussed later but high levels of  $\text{NO}_3^-$  were measured in the subsoil in that period (fig. 9, 10, 11 & 12).

During November and December (week 4 – 12) (1998 – 1999) with the heavy rains, net mineralisation took place (fig. 5). The soil water content was very high and thus immobilisation could not take place. Oxygen is needed for immobilisation to take place because the microbes use  $\text{O}_2$  with the uptake and assimilation of organic matter, with the liberation of  $\text{CO}_2$  (amount of C is progressively reduced, thus the C:N narrows) (Haynes, 1986). High moisture levels reduced microbial activities indirectly by hindering the movement of air and thus reducing  $\text{O}_2$  supply (Alexander, 1977). That agrees with results

found by <sup>2</sup>Cheshire *et al.* (1999) where net immobilisation could not take place because of high soil moisture content.

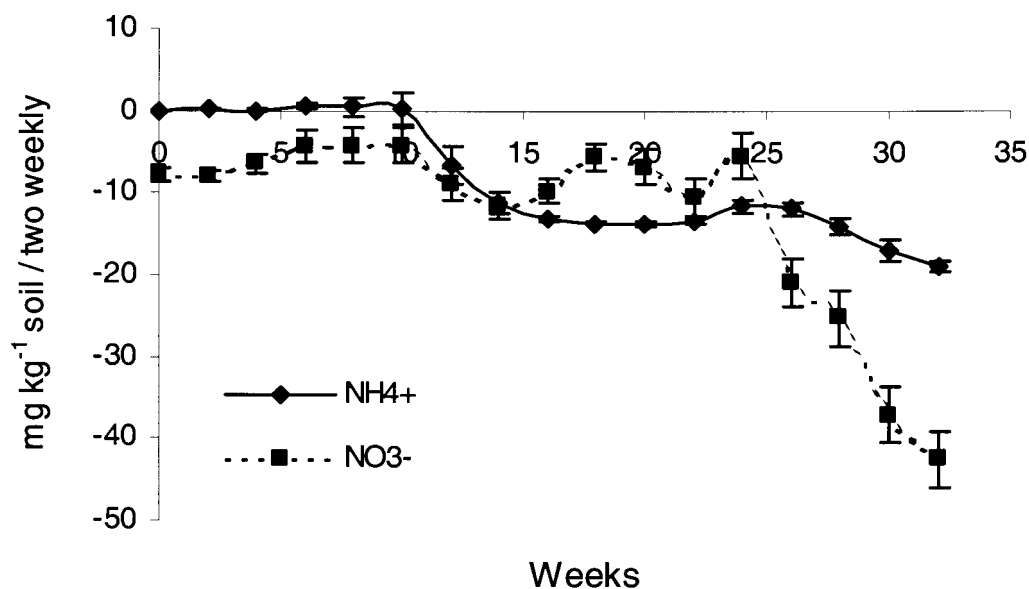


**Figure 6.** NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N production (positive gradient) and immobilisation (negative gradient) rates in the top 30 cm of the control plot, during the 1998-1999 season (week 0 is October 1, 1998).

Mineralisation and immobilisation takes place simultaneously in the soil. From week 10 (middle December) there was net immobilisation of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> in the straw treated plot (fig. 7), which could contribute to improving the economy of the nitrogen use in the soil. It would be wise to quantify in a field situation when considering effective fertiliser application on the grounds of rollover of N from one season to the next.

The effect of temperature on microbial growth was not measured during the season. Temperatures differ between the topsoil and soil 5 to 10 cm below. It could have a marked influence on the mineralisation and nitrification in the soil.

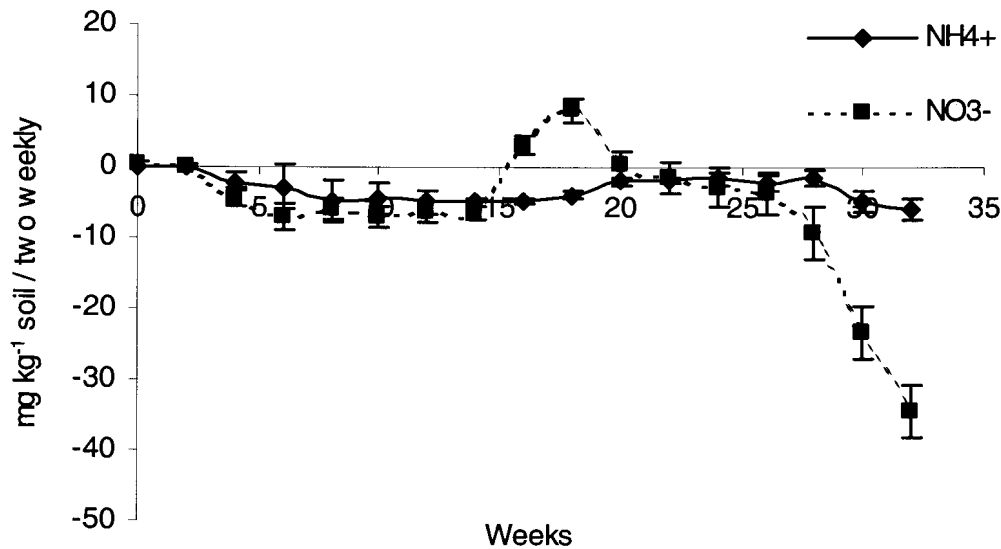
Immobilisation is normally found to occur from the NH<sub>4</sub><sup>+</sup> pool but there was not enough NH<sub>4</sub><sup>+</sup> in this case (because of very low mineralisation). NO<sub>3</sub><sup>-</sup> was thus assimilated by the soil microorganisms. To do this, they first reduce the NO<sub>3</sub><sup>-</sup> to NO<sub>2</sub><sup>-</sup> using an assimilatory NO<sub>3</sub><sup>-</sup> reductase enzyme. They then reduce the NO<sub>2</sub><sup>-</sup> to NH<sub>4</sub><sup>+</sup> via an enzyme called assimilatory NO<sub>2</sub><sup>-</sup> reductase (Coyne, 1999). The control soil showed net mineralisation.



**Figure 7.** Cumulative  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N production (positive gradient) and immobilisation (negative gradient) rates in the top 30 cm of the straw plot, during the 1998-1999 season (week 0 is October 1, 1998).

Moisture plays an important role in mineralisation and nitrification alike. Moisture must be adequate for decomposition to proceed. There are many other factors to be considered here, like the microbial populations and the degradation of the straw material under these circumstances. Microorganisms are quite sensitive to moisture in the soil and a change in the water status influences their activity.

The production rates in both seasons declined noticeably from the fifth month to the end of the season. Fresh nitrogen sources were depleted. Much of the nitrogen that was mineralised was further reduced to  $\text{NO}_3^-$ . Due to the lack of adequate substrate microbial activity decreased and the  $\text{NO}_3^-$  probably assimilated by the microorganisms for growth.



**Figure 8.** Cumulative  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N production (positive gradient) and immobilisation (negative gradient) rates in the top 30 cm of the control plot, during the 1998-1999 season (week 0 is October 1, 1998).

### *Nitrate leaching*

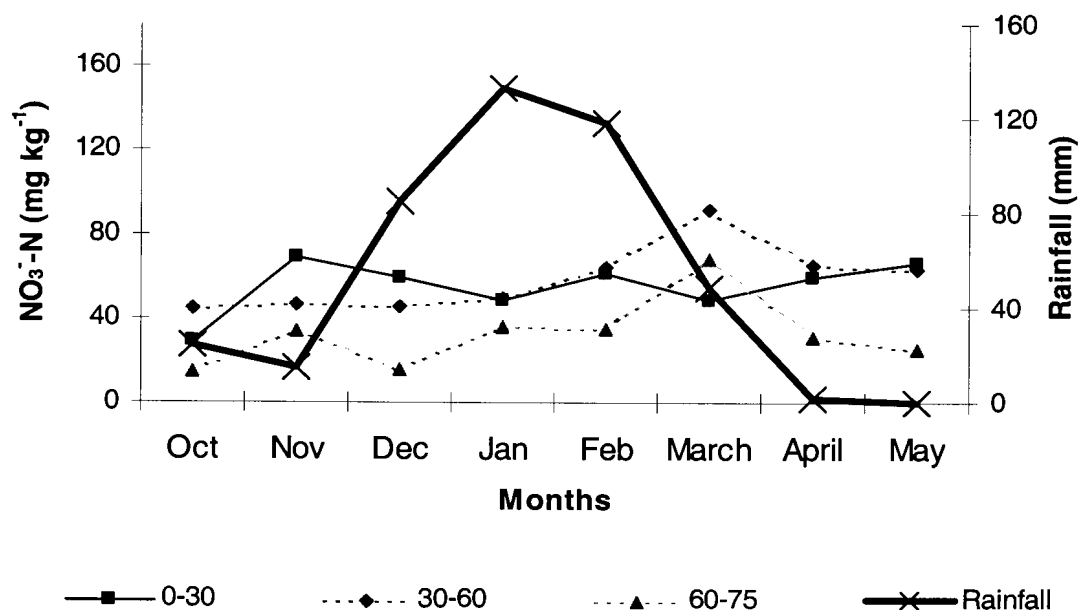
Season 1997/1998

The control plot (fig. 9) did not show much variation in  $\text{NO}_3^-$ -N compared to the straw treated soil. The subsoil continually measured less  $\text{NO}_3^-$ -N than the subsoil throughout the season. Little mineralisable substrates reside in the subsoil, thus mineralisation and nitrification activity would be less. From middle April  $\text{NO}_3^-$ -N levels in the subsoil declined. During the heavy rainfall period denitrification could have taken place, especially in the subsoil. Oxygen is limited in the subsoil, especially when the pore space is filled with water. That is ideal conditions for nitrate to be reduced to  $\text{N}_2\text{O}$  or  $\text{NO}$ . A slight lag can be seen in the topsoil where more  $\text{NO}_3^-$ -N was measured after the rain lessened.

In the straw treated plot the nitrate measured in the topsoil (0 – 30 cm) stayed almost constant throughout the season (fig. 10). After the few months of heavy rain (December through February) much more nitrate was measured in the subsoil (30 – 60 cm) without loss from the topsoil. It could have been that when the subsoil was wet enough, there



was a substantial increase in microbial activity responsible for nitrification. The nitrogen could also have been released from immobilisation during the drier period near the end of the season. On average the highest  $\text{NO}_3^-$ -N levels were measured in the 60 - 90 cm region.



**Figure 9.** Nitrate content in the control soil profile during 1997 – 1998

Season 1998/1999

The nitrogen content throughout the profile coincided very well with the rainfall pattern during the season (fig. 11 & 12). After heavy rain a flush of nitrate was measured almost in each soil layer tested.

Although increased nitrate levels were measured down the profile as the season progressed, the most  $\text{NO}_3^-$ -N was still measured in the topsoil (fig. 11 & 12). With the wet conditions also late in the season, rapid nitrification took place in the topsoil. The source was the mineralised  $\text{NH}_4^+$ -N accumulated during the season. The subsoil, from 60-90 cm contained a substantial amount of concretions and stones. Soil microbial processes would have been very low under those circumstances (Parkin & Meisinger, 1989). The soil at that depth was also more or less always moist anaerobic and nitrogen could have been lost as  $\text{N}_2\text{O}$  and  $\text{NO}$ .

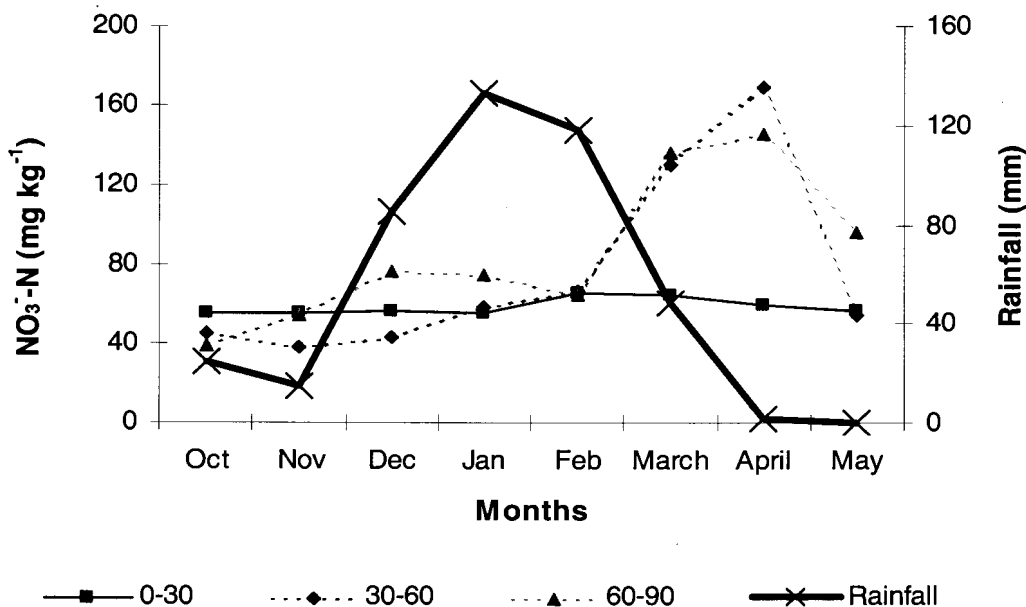


Figure 10. Nitrate content in the straw treated soil profile during 1997 – 1998

There was no significant difference between the straw treated plot and the control plot although the control plot had a little more  $\text{NO}_3^-$ -N measured (fig. 11). Some of the nitrogen in the straw treated plot could have been fixated/immobilised as have been found by other authors (Cheshire *et al.*, 1999, Jarvis *et al.*, 1996).

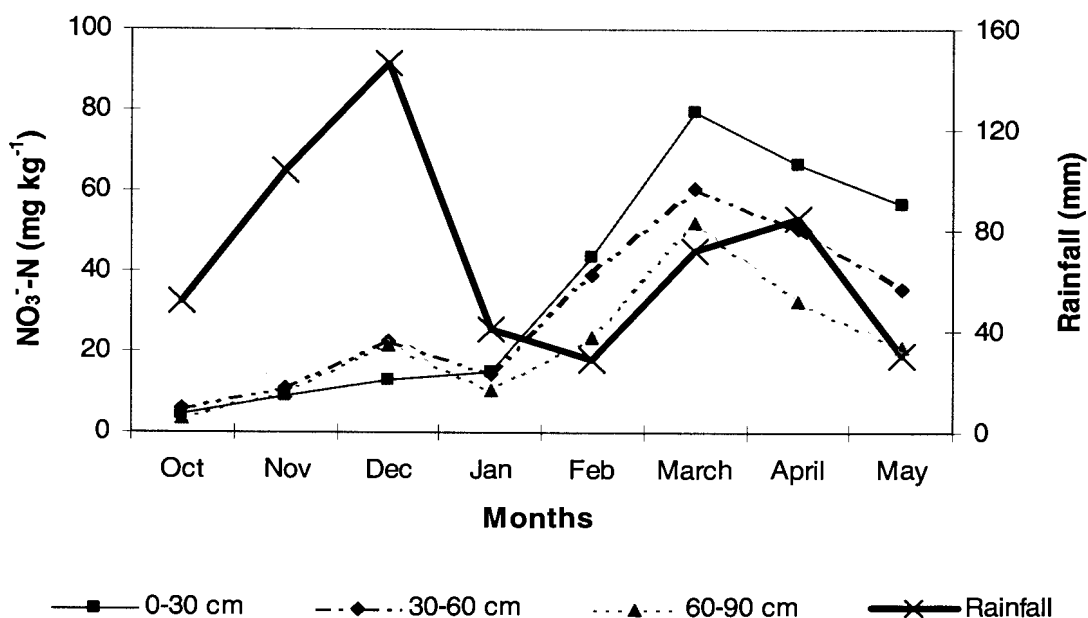
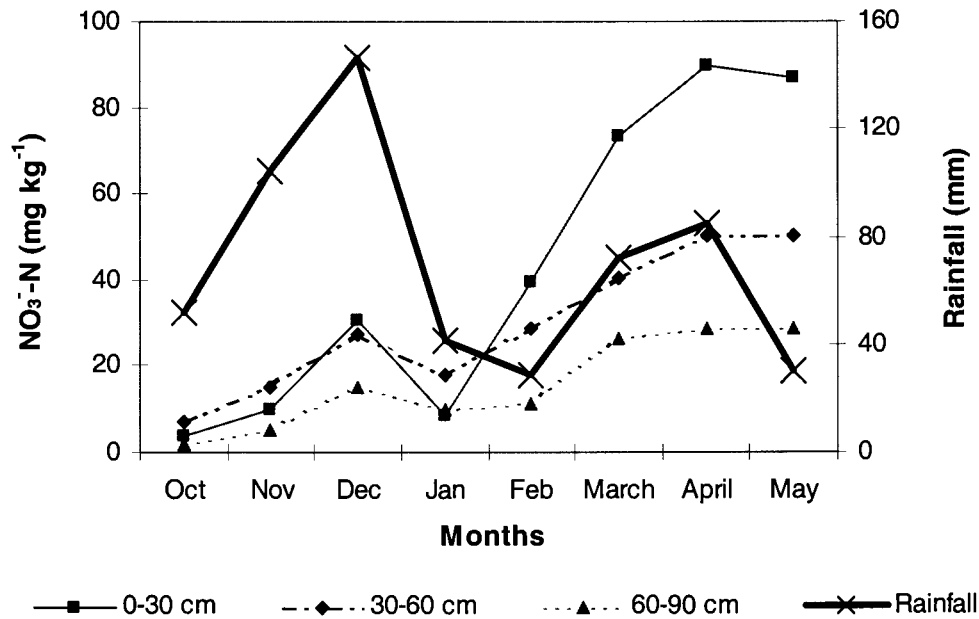


Figure 11. Nitrate content in the control soil profile during 1998 – 1999



**Figure 12.** Nitrate content in the straw treated soil profile during 1998 – 1999

In general more nitrate was measured in the last three months because of subsequent nitrification of the mineralised nitrogen. Denitrification could also have taken place in the subsoil where oxygen is limited. Subsequently lower  $\text{NO}_3^-$ -N was measured (fig. 11 & 12).

## CONCLUSIONS

Rainfall had the largest influence on the mineralisation and nitrification processes over both seasons. Microbial activity was adequately stimulated, due to sufficient moisture and aeration, to ensure mineralisation and immobilisation of nitrogen (ammonium and nitrate).

Denitrification could have taken place at stages in microsites in the soil when excess moisture was present. Denitrification products were not measured though. It is proposed because immobilisation and nitrification require  $\text{O}_2$  to occur.

Definitive periods of net immobilisation were recorded at the straw treated plot due to the incorporation of organic material with a high C:N. Immobilisation was also noted in the control soil. From April, during both seasons, due to the deficiency of mineralisable





substrate and little rainfall, microbes still present in the soil assimilated all the nitrogen they could, to ensure survival. A cover crop could be planted during the winter to ensure that the nitrogen is not lost when the spring rain arrives, bringing moisture, necessary for microbial metabolism.

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## NITROUS OXIDE PRODUCTION AND EMISSION FROM SOUTH AFRICAN SOILS

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### INTRODUCTION

N<sub>2</sub>O is of great concern since it is implicated in stratospheric ozone destruction and global warming. The increase in N<sub>2</sub>O gas concentration in Europe has been estimated at 50% as compared to pre-industrial times (Vermoesen *et al.*, 1996). It has been estimated that nitrous oxide (N<sub>2</sub>O) emissions from agricultural soils account for 41% of total N<sub>2</sub>O emissions (Freibauer & Montanarella, 1999). This is largely due to the relatively high availability of inorganic nitrogen compounds and organic carbon. Nitrogen availability is high due to two sources of nitrogen: application of N-fertiliser and the mineralisation of organic nitrogen, harvest residues and soil organic matter (Vermoesen *et al.*, 1996).

Extensive work on N<sub>2</sub>O emissions has been done in Europe and the U.S.A. to assess the contribution to the destruction of stratospheric ozone (Firestone & Davidson, 1989, Goossens & Van Cleemput, 1999). It is of great concern in Europe and North America since the use of nitrogen fertiliser per ha is much more compared to South Africa. In South Africa, little if any studies have been done to assess the pollution of nitrogen from N<sub>2</sub>O production. In Western Europe the current nitrogen use leads to considerable N losses. The N (as N<sub>2</sub>O) lost to the atmosphere will ultimately cause a reduction of O<sub>3</sub> in the stratosphere and the ability to absorb ultraviolet rays (Stevenson, 1982).

Of more concern under South African conditions is the N lost under extensive agricultural conditions and the lack of knowledge on N-recommendations since mineral-N content of the soil is presently not taken into account (Vlassak & Agenbag, 1999).



Most  $N_2O$  originates from soil processes, as an intermediate product of microbial nitrification and denitrification. During denitrification,  $N_2O$  arises as an intermediate from dissimilatory  $NO_3^-$  and/or  $NO_2^-$  reduction to  $N_2$  under anaerobic conditions.  $N_2O$  production by nitrifying bacteria may arise either from dissimilatory  $NO_2^-$  reduction when  $O_2$  supply is limited, i.e. nitrifier denitrification, or during  $NH_4^+$  oxidation to  $NO_2^-$ , i.e. non-denitrifying nitrification (Webster & Hopkins, 1996).

The aim of this study was to distinguish between the different  $N_2O$  sources in soils from different locations when exposed to varying environmental conditions. A laboratory incubation study was conducted similar to that of Webster & Hopkins (1996) where different inhibitors (acetylene and oxygen) were added to three South African soils under different conditions. The inhibiting treatments on nitrification and denitrification made it possible to distinguish between different  $N_2O$  sources as they occurred under different levels of organic material and inorganic nitrogen.

## **MATERIALS & METHOD**

The method used was adapted from the method used by Webster & Hopkins (1996) in their study. In this study there is the added factor of inorganic and organic nitrogen supply through organic material and fertiliser and two moisture regimes.

### ***Soil sampling***

Representative soil samples from certain locations were taken from the top 30 cm of arable land and were used in the incubation study. Some chemical and physical characteristics are presented in table 1. Soils were collected from Stellenbosch, Bethlehem and Pretoria. Although it was an incubation study under fixed conditions, a summary of the climatic conditions of the areas is given (Table 2). Stellenbosch ( $33^\circ 17'$  S,  $18^\circ 42'$  E) lies in a winter rainfall region, agricultural production taking place from May to October. Bethlehem ( $28^\circ 7'$  S,  $28^\circ 10'$  E) lies in a summer rainfall region with winter crops like wheat being produced from April to August. Pretoria ( $25^\circ 43'$  S,  $28^\circ 15'$  E) also lies in a summer rainfall region and crops are produced from October to May.

**Table 1.** Physical-chemical characteristics of the three different soils

|  | Stellenbosch | Bethlehem  | Pretoria        |
|--|--------------|------------|-----------------|
| Textural class   | Sandy loam   | Sandy loam | Sandy clay loam |
| Clay (%)   | 8.5          | 12.5       | 20.6            |
| Silt (%)   | 22.3         | 17.1       | 9.8             |
| Sand (%)   | 69.3         | 70.4       | 69.6            |
| C:N  | 11.6         | 10.0       | 12.2            |
| Total C (g kg <sup>-1</sup> )                          | 7.3          | 6.0        | 6.1             |
| Total N (mg kg <sup>-1</sup> )                         | 630          | 600        | 500             |
| Inorganic N  |              |            |                 |
| NH <sub>4</sub> <sup>+</sup> -N (mg kg <sup>-1</sup> ) | 1.7          | 2.1        | 2.2             |
| NO <sub>3</sub> <sup>-</sup> -N (mg kg <sup>-1</sup> ) | 55.3         | 0.7        | 1.3             |
| pH (H <sub>2</sub> O)                                  | 6.0          | 5.7        | 5.6             |
| CaCO <sub>3</sub>                                      | 0.0          | 0.0        | 0.0             |

### *Pre-incubation*

Glass jars with a volume of 250 ml were filled with 30 g soil (soil density taken as 1.2 kg m<sup>3</sup>). The soil was initially wetted to 50% of field capacity. The bottles were then pre-incubated at different temperatures (Stellenbosch 15°C, Bethlehem 15°C and Pretoria 20°C) for 7 days. This was done to simulate the natural environmental conditions under which production normally takes place (Table 2) and to stabilise the environment in the bottles before the commencement of the incubation.

### *Treatments*

After the pre-incubation four treatments were applied, which were replicated three times:

- (1) Control (Co)
- (2) 40 kg N (NH<sub>4</sub>NO<sub>3</sub>) ha<sup>-1</sup> (N)
- (3) 0.5% wheat straw (C:N 113) (C) and
- (4) 40 kg N (NH<sub>4</sub>NO<sub>3</sub>) ha<sup>-1</sup> plus 0.5% wheat straw (C&N).



To assess whether microbial growth and metabolism under these conditions are influenced by different moisture regimes, incubation was done at two moisture levels. Half the jars were wetted to  $\frac{1}{4}$  FC for Pretoria and Stellenbosch soil and  $\frac{1}{2}$  FC for Bethlehem soil and the other half to  $\frac{3}{4}$  FC.

**Table 2.** Climatic data for the three different locations

|              | †Temperature (°C) |        | Rainfall (mm) |        | Aridity index |        |
|--------------|-------------------|--------|---------------|--------|---------------|--------|
|              | April             | August | April         | August | April         | August |
| Stellenbosch | 19.3              | 13.0   | 30.5          | 61.7   | 5.6           | 1.4    |
|              | January           | May    | January       | May    | Annual        |        |
| Bethlehem    | 20.5              | 9.9    | 122.8         | 25.2   | 0.36          |        |
| Pretoria     | 17.7              | 14.2   | 53.9          | 4.9    | 0.28          |        |

† Mean daily temperature using 40 year averages

### *Incubation*

The jars with soil were then incubated for ten days. The Pretoria soil was kept at 20°C and the Bethlehem and Stellenbosch soils at 15°C.

### *Inhibitory headspace treatments*

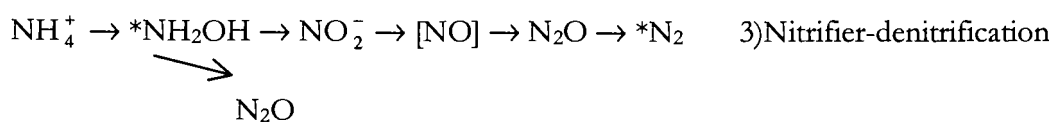
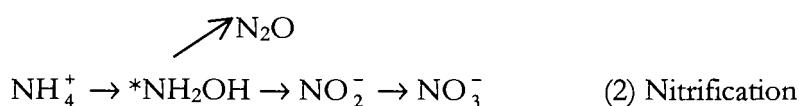
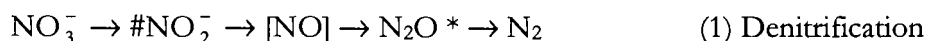
To distinguish between different contributors of N<sub>2</sub>O production, five inhibitory headspace (area of space above soil in bottle) treatments were applied to the soil according to the principle that certain gasses and concentrations of it are supposed to inhibit specific microbial processes (Table 3). The gas was injected 24 hours before measurement and the bottles closed with rubber stoppers and returned to the incubators.

Acetylene severely inhibits nitrification at concentrations far below those that affect N<sub>2</sub>O reductase in soil while oxygen in sufficient quantities inhibits respiratory denitrification (Robertson & Tjiede, 1987).



## Reactions

Acetylene and oxygen inhibit certain products from forming during nitrification and denitrification.



\*Blockage by acetylene

# Blockage by oxygen

(Koops, Van Beusichem & Oenema, 1997).

## Measurements

N<sub>2</sub>O production (as ng kg<sup>-1</sup> h<sup>-1</sup>) was determined after 1, 4 and 10 days. The bottles were closed for 24 hours before measurement. N<sub>2</sub>O was determined using a Chrompack 437A gas chromatograph with a 4.87 m, 0.32 cm stainless steel Chromosorb 102 column (80/120 mesh) and a <sup>63</sup>Ni ECD detector under the following conditions: injector temperature 90°C, oven temperature 90°C and detector temperature 300°C. The flow rate of the carrier gas N<sub>2</sub> was 25 - 40 ml min<sup>-1</sup>. The chromatograms were calibrated against N<sub>2</sub>O standard gas (50.3 ppmv in He). All chromatograms were analysed using “Winner of Windows” software (Spectra-Physics Analytical Systems, Fremont, USA).



**Table 3.** Inhibitory headspace-filling principle

| Treatment  | Function   |
|--|--|
| A = control (air)  | Net N <sub>2</sub> O emission  |
| B = 10 kPa C <sub>2</sub> H <sub>2</sub>                         | Inhibit reduction of N <sub>2</sub> O to N <sub>2</sub><br>Thus preventing N <sub>2</sub> O consumption by denitrifiers                  |
| C = 10 Pa C <sub>2</sub> H <sub>2</sub>                          | Inhibit oxidation of NH <sub>4</sub> <sup>+</sup> by nitrifiers<br>Thus preventing N <sub>2</sub> O production by autotrophic nitrifiers |
| D = 100 kPa O <sub>2</sub>                                       | Restricts the number of anaerobic microsites<br>Thus preventing N <sub>2</sub> O production and consumption by denitrifiers              |
| E = 10 Pa C <sub>2</sub> H <sub>2</sub> + 100 kPa O <sub>2</sub> | Prevents the activity of nitrifiers and denitrifiers   |

Analysis of variance (ANOVA) (Snedecor & Cochran, 1967) was done on three factors namely

TREATMENT –

- (1) Control (Co)
- (2) 40 kg N (NH<sub>4</sub>NO<sub>3</sub>) ha<sup>-1</sup> (N)
- (3) 0.5% wheat straw (C) and
- (4) 40 kg N (NH<sub>4</sub>NO<sub>3</sub>) ha<sup>-1</sup> plus 0.5% wheat straw (C&N)

DAYS (of incubation) –

- (1) day 1
- (2) day 4 and
- (3) day 10

and

GAS (inhibitory headspace treatments) – see table 3.



## RESULTS & DISCUSSION

### *Statistical analysis*

The main effects of DAY, GAS and the DAY.GAS interaction accounted for 91.1% on the Stellenbosch soil, 82.9% on the Bethlehem soil and 73.4% on the Pretoria soil. This interaction is thus the most important effect to be discussed. The effect of moisture ( $\frac{1}{4}$  and  $\frac{3}{4}$  FC) was less than 0.5% of the total variation in N<sub>2</sub>O on all the soil types and thus excluded from further statistical analysis.

**Table 4.** ANOVA for the Stellenbosch, Bethlehem and Pretoria soil

|                     | Stellenbosch |      | Bethlehem |      | Pretoria |      |
|---------------------|--------------|------|-----------|------|----------|------|
| F-probability       | <0.001       |      | <0.001    |      | <0.001   |      |
| Source of variation | Df           | ss%  | Df        | ss%  | Df       | ss%  |
| Day                 | 2            | 15.6 | 2         | 21.9 | 2        | 8.0  |
| Gas                 | 4            | 52.1 | 4         | 27.5 | 4        | 51.2 |
| Treatment           | 3            | 0.3  | 3         | 0.3  | 3        | 0.7  |
| Day.Gas             | 8            | 23.4 | 8         | 33.5 | 8        | 13.7 |
| Day.Treatment       | 6            | 1.1  | 6         | 0.4  | 6        | 0.7  |
| Gas.Treatment       | 12           | 1.6  | 12        | 2.8  | 12       | 0.8  |
| Day.Gas.Treatment   | 24           | 3.2  | 24        | 5.8  | 24       | 1.8  |
| Residual (error)    | 293 (7)      |      | 294 (6)   |      | 300      |      |
| Total               | 352 (7)      |      | 353 (6)   |      | 359      |      |

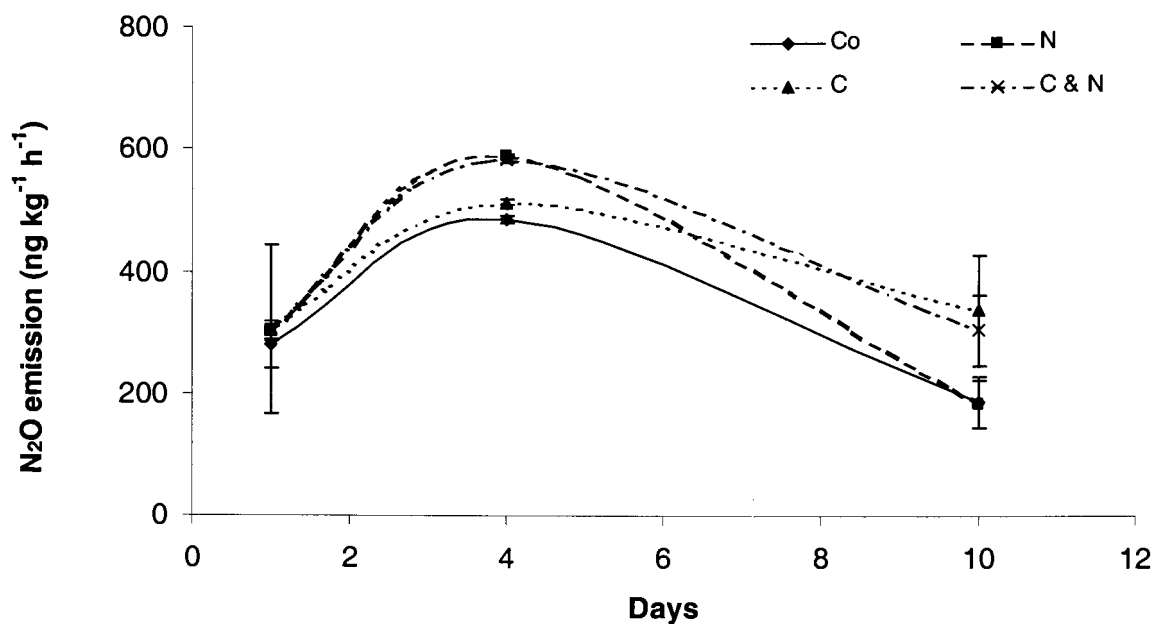
### *Net N<sub>2</sub>O emission*

The net emission of N<sub>2</sub>O from soil depends on the rate of N<sub>2</sub>O formation, the rate of diffusion out of the soil and the consumption of N<sub>2</sub>O during denitrification (Bandibas *et al.*, 1994). N<sub>2</sub>O was taken as the N<sub>2</sub>O produced from the soil where no inhibitory gas treatments were applied.

The complete set of N<sub>2</sub>O emission values from the Stellenbosch, Bethlehem and Pretoria soil are given in Appendix III, page XIII.

The highest N<sub>2</sub>O emission originated from the Bethlehem soil for both moisture regimes. The Stellenbosch soil showed the lowest emission rate at both moisture regimes. There was no significant difference between the two moisture regimes (1/4 and 3/4 FC) for all three soils. The different moisture levels did not influence nitrification and denitrification much in this instance.

Stellenbosch soil



**Figure 1.** N<sub>2</sub>O emission from the Stellenbosch soil

The initial NO<sub>3</sub><sup>-</sup>-N content of the Stellenbosch soil was markedly higher (55.3 mg kg<sup>-1</sup>) compared to that of the Bethlehem (0.7 mg kg<sup>-1</sup>) and Pretoria (1.3 mg kg<sup>-1</sup>) soils. High concentrations of NO<sub>3</sub><sup>-</sup> almost completely inhibit the reduction of N<sub>2</sub>O to N<sub>2</sub> by denitrifying microorganisms (Bremner, 1997). This is reflected in the data where one can see the accumulation of N<sub>2</sub>O (fig. 1). The production of N<sub>2</sub>O, as intermediate product from the reaction, reached a maximum on day four of the incubation. It is possible that the denitrifying bacteria were dominant in the soil. When the NO<sub>3</sub><sup>-</sup>-N was exhausted, N<sub>2</sub>O could be reduced to N<sub>2</sub>, as in figure 1.

The soil has a total C content of  $7.3 \text{ mg kg}^{-1}$  (Table 1). The microbes used the native organic carbon compounds as electron donors for energy and for synthesis of cellular constituents (Aulakh, 1992). There was not much difference between the individual treatments as such, although as expected the control (Co) showed the lowest and the (C & N) treatment the highest emission.

Most denitrifying organisms like *Pseudomonas* and *Bacillus* are heterotrophic (see Glossary) and are strongly dependent on C availability (Coyne, 1999). The Stellenbosch soil had the highest total C content ( $7.3 \text{ mg kg}^{-1}$ ) measured in the soil compared to the other two locations. Insufficient C means that electrons are unavailable to further reduce  $\text{N}_2\text{O}$ . Carbon supply is determined by  $\text{H}_2\text{O}$  (solubility), plants, physical disruption, competition, and excretion by other organisms in soil (Coyne, 1999).  $\text{N}_2\text{O}$  was further reduced in the soil, because much less  $\text{N}_2\text{O}$  was measured compared to the Bethlehem and Pretoria soil (fig. 1, 2 & 3).

#### Bethlehem soil

$\text{N}_2\text{O}$  emission rates measured on the first day were the highest, thereafter the rates declined and from day four until the last day of measurement (day 10) the rates were almost constant.

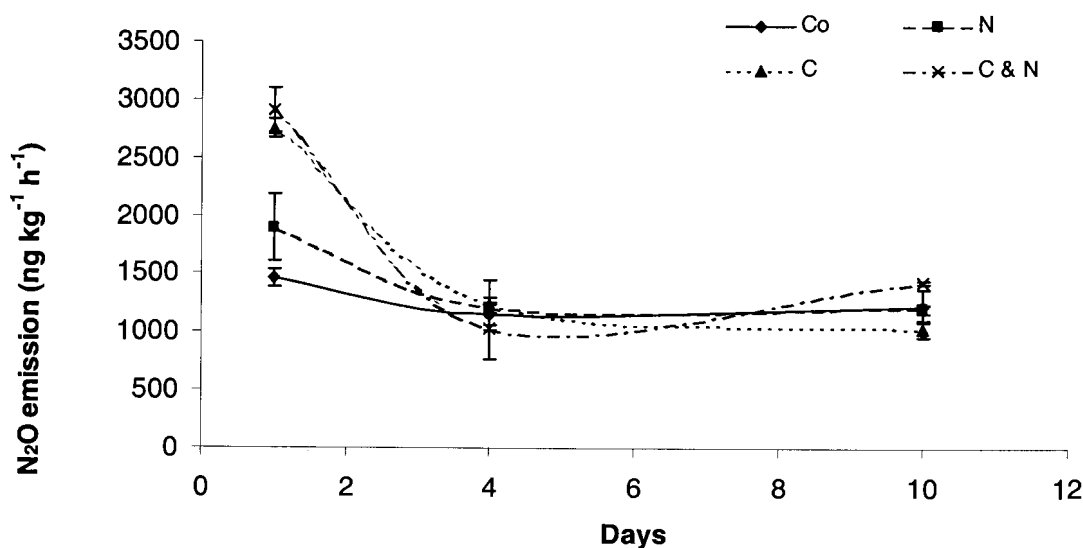
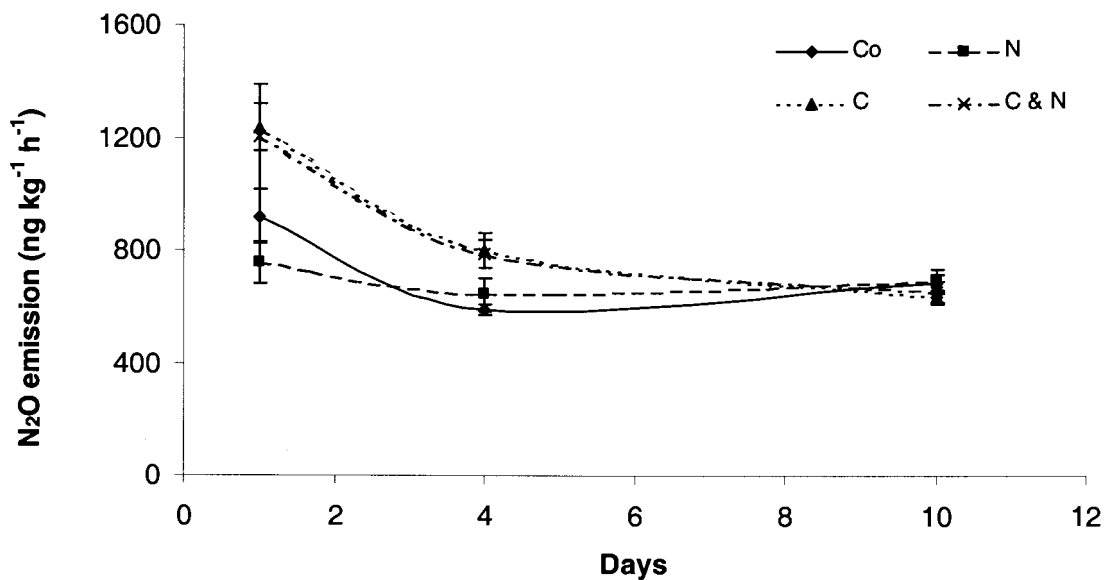


Figure 2.  $\text{N}_2\text{O}$  emission from the Bethlehem soil

On day one there were still significant differences (at 5%) between the different treatments. The (C & N) and C soil started out with the highest emission and the control the lowest. The addition of carbon stimulates the reduction of  $N_2O$  to  $N_2$ . It is noticeable from the steep decline in measured  $N_2O$  from those treatments. From day 4 to the end of the incubation, however, there were no distinct differences between the treatments, concerning  $N_2O$  emission.  $N_2O$  was constantly reduced to  $N_2$ . Nitrifying and denitrifying organisms both contributed to the high emission rate of  $N_2O$  from the soil.

Pretoria soil



**Figure 3.**  $N_2O$  emission from the Pretoria soil

The control soil showed quite a high emission rate compared to the other treatments, probably due to the reduction of  $N_2O$  to  $N_2$  during denitrification in the other treatments. The rates declined steadily from the start of the incubation, showing that the  $N_2O$  was constantly being reduced to  $N_2$ .

### *Different $N_2O$ sources*

The inhibitory gas treatments had the largest influence on the production and emission of  $N_2O$  from the different soils compared to the treatments with fertiliser and organic



material. A distinction can be made between different sources of gaseous nitrogen production in these soils according to the net rates of  $N_2O$  emissions from the different gas treatments (Table 3). These methods for partitioning the  $N_2O$  between different processes are, however, neither completely effective nor completely selective (Webster & Hopkins, 1996). Many negative values were calculated with the partitioning of the  $N_2O$ .  $N_2O$  consumption is one very strong possibility, explaining the phenomena. The  $N_2O$  could have been consumed by denitrifiers in the production of  $N_2$ . This is promoted by  $NO_3^-$  and the addition of organic C.

The pH values of the soils were very similar (6.0, 5.7 and 5.6 respectively for Stellenbosch, Bethlehem and Pretoria). Although the optimum pH values for nitrification and denitrification lie between 6 and 7, no marked decrease in production was noticed.

Inhibitory headspace treatments

#### Effect of 10 kPa $C_2H_2$

10 kPa  $C_2H_2$  inhibits the reduction of  $N_2O$  to  $N_2$ , thus the consumption of  $N_2O$  by denitrifiers.  $N_2O$  derived from denitrification differs from  $N_2O$  produced during nitrifier denitrification since denitrification is performed by denitrifying organisms. In this case  $NO_3^-$  and/or  $NO_2^-$  is reduced, under anaerobic conditions, to  $N_2$  with  $N_2O$  as intermediate (Webster & Hopkins, 1996).

From the Stellenbosch soil, at  $\frac{1}{4}$  FC, all the treatments except the control measured less  $N_2O$  compared to the soil receiving no acetylene (A). Thus, the  $N_2O$  came from another source, namely nitrification. At  $\frac{3}{4}$  FC, three of the four treatments (Co, N and C&N) measured higher values than the control soil (A).  $N_2O$  was not reduced to  $N_2$ , the source of it being the denitrifier organisms. This can be expected at a higher moisture level, where more anaerobic sites would be found. The amount of  $N_2O$  reduced to  $N_2$  in the Bethlehem soil was small compared to the amount of  $N_2O$  produced. The amounts were less than those of the control soil (A). The rates measured in the Pretoria soil were lower



than those of the control soil at both moisture regimes. 10 kPa  $C_2H_2$  is not only supposed to inhibit  $N_2O$  reduction, but nitrifier activity as well. This was the case here since the rates were less than those measured in the control soil (Co).

#### Effect of 10 Pa $C_2H_2$

10 Pa  $C_2H_2$  prevents  $N_2O$  production by autotrophic nitrifiers by inhibiting the oxidation of  $NH_4^+$  by nitrifiers.

At  $\frac{1}{4}$  FC 10 Pa  $C_2H_2$  in the Stellenbosch soil was effective in inhibiting nitrification in three of the four treatments compared to the control soil (A). At  $\frac{3}{4}$  FC the effect was not noticed. At anoxic microsites, denitrification activity was probably more pronounced and probably exceeded nitrification in  $N_2O$  production. Nitrification was inhibited successfully in all treatments of the Bethlehem soil at both moisture regimes. A large amount of  $N_2O$  was thus produced from denitrification. 10 Pa  $C_2H_2$  inhibits  $NH_4^+$  oxidation. This correlates well with the data from the Pretoria soil because compared to the control, the soil treated with 10 Pa  $C_2H_2$  measured lower rates at both  $\frac{1}{4}$  and  $\frac{3}{4}$  FC.

#### Effect of 100 kPa $O_2$

In all the soils, especially the Bethlehem soil, the addition of  $O_2$  stimulated the production and emission of  $N_2O$ . In a normal soil atmosphere denitrifiers consume  $N_2O$ . Under the  $O_2$  atmosphere denitrifiers were inhibited so that  $N_2O$  produced by other sources accumulated rather than being further reduced to  $N_2$  by denitrifiers. This corresponds with Webster & Hopkins (1996) who found similar results. The large amounts measured on the Bethlehem, and to a lesser extent Pretoria, soil were probably because there were far less  $NO_3^-$  (0.7 mg  $kg^{-1}$  and 1.3 mg  $kg^{-1}$  respectively) in those soils than in the Stellenbosch soil (55.3 mg  $kg^{-1}$ ). Low concentrations of  $NO_3^-$  delay reduction of  $N_2O$  to  $N_2$  by denitrifying organisms.





### Combined effect of 100 kPa O<sub>2</sub> and 10 Pa C<sub>2</sub>H<sub>2</sub>

The N<sub>2</sub>O production values measured from the Stellenbosch soil were also very high, in fact higher than the O<sub>2</sub> treatment alone. The O<sub>2</sub> stimulated the production of N<sub>2</sub>O by nitrifying organisms through nitrifier denitrification. The contribution of N<sub>2</sub>O from denitrifiers after stimulation from 10 Pa C<sub>2</sub>H<sub>2</sub> could not be discerned. Non-denitrifying bacteria probably contributed much of the N<sub>2</sub>O. For the Bethlehem soil the rates measured were also very high. Nitrifying organisms were probably the sole contributors everything to the production of N<sub>2</sub>O over the whole 10-day incubation period. N<sub>2</sub>O production rates from the Pretoria soil treated with this combination were also very high. Most of the N<sub>2</sub>O probably came from the nitrifier microorganisms.

### Effect of organic material

The presence of organic C promotes N<sub>2</sub>O consumption (Webster & Hopkins, 1996). N<sub>2</sub>O consumption from the Stellenbosch soil was noted at both 1/4 and 3/4 FC with low rates measured compared to the control soil (a). If the available C concentration in the environment is high relative to the concentration of NO<sub>3</sub><sup>-</sup>, then NO<sub>3</sub><sup>-</sup> is used as an electron dump and NH<sub>4</sub><sup>+</sup> formation (oxidation to NO<sub>2</sub><sup>-</sup>) is favoured. At 3/4 FC, the soil treated with 10 Pa C<sub>2</sub>H<sub>2</sub> (inhibiting nitrifier activity), higher N<sub>2</sub>O values were measured in the control soil. O<sub>2</sub> levels were probably too low for any nitrifier activity.

At 1/2 FC, N<sub>2</sub>O consumption took place in the Bethlehem soils where denitrifying activity was supposed to be inhibited. Denitrification was either not completely inhibited or the N<sub>2</sub>O came from another source. Since O<sub>2</sub> enhanced N<sub>2</sub>O production, it probably also exceeded N<sub>2</sub>O consumption in two of the three treatments where the soil treated with organic material measured less than the control soil. Most of the N<sub>2</sub>O came from denitrifying nitrifier activity at both 1/2 and 3/4 FC.

At 3/4 FC, all the Bethlehem soil treated with organic material measured less N<sub>2</sub>O than the control soil. Denitrification in soils under anaerobic conditions is controlled largely by the supply of water soluble or readily decomposable organic matter. Organic material



can be a limiting factor when it comes to its availability for utilisation by denitrifiers for reduction to nitrate (Bremner, 1997). There was net  $N_2O$  production, since the microbes did not use the  $N_2O$  as an energy source. At  $\frac{1}{4}$  FC, the control soil (a), soil treated with 10 Pa  $C_2H_2$  and the soil treated with 100 kPa  $O_2$  from the Pretoria soils showed signs of  $N_2O$  consumption. The soil treated with 10 kPa  $C_2H_2$  showed a higher value than the control soil (Co) since 10 kPa  $C_2H_2$  inhibits the reduction of  $N_2O$  to  $N_2$ . At  $\frac{3}{4}$  FC all the soil treated with organic material (C) measured higher rates than those of the control soil (A). This indicates that organic material did increase the consumption of  $N_2O$  at that moisture level.  $N_2O$  was produced from denitrifying nitrifiers and to a lesser extent, denitrification.

#### Addition of $NH_4NO_3$ fertiliser

Most of the  $N_2O$  produced from the fertiliser treated Stellenbosch soil came from non-denitrifying nitrifiers at both  $\frac{1}{4}$  and  $\frac{3}{4}$  FC - thus, the addition of  $NH_4^+$  rather than  $NO_3^-$ . Chemoautotrophic nitrifiers that perform this action include *Nitrosomonas europaea*. Bremner (1997) found that most of the  $N_2O$  evolved from aerobic soils treated with ammonium or ammonium-yielding fertilisers such as urea is produced during oxidation of ammonium to nitrate by nitrifying microorganisms. The organisms use  $NO_2^-$  as an electron acceptor, especially when  $O_2$  is limiting.  $CO_2$  and adequate  $O_2$  must be available for nitrification to take place.

The factor limiting nitrification in most soils is the availability of  $NH_4^+$ . The relatively high concentration of  $NO_3^-$  improved the ability to reduce  $N_2O$  to  $N_2$ , which explains why the values are overall a little lower than in the control soil (Co). High concentrations of  $NO_3^-$ , on the other hand, almost completely inhibit the process (Bremner, 1997). Most  $N_2O$  produced from the Bethlehem soil came from the denitrifying nitrifiers. At  $\frac{1}{2}$  FC only the 10 kPa  $C_2H_2$  treated soil and the 100 kPa  $O_2$  plus 10 Pa  $C_2H_2$  treated soil showed net production of  $N_2O$ . Overall the control soil (Co) had higher rates than the fertilised soil (N).



Non-denitrifying nitrifiers were responsible for most of the  $N_2O$  produced from the Pretoria soil. It was not clear if the addition of fertiliser had such a distinct effect on the reduction of  $N_2O$  to  $N_2$ . There were no clear differences in  $N_2O$  production rate values between the control (Co) and fertiliser (N) treated soils. Values varied only about 2% from each other at both moisture regimes.

## CONCLUSIONS

The differential gas treatments showed good results in inhibiting certain processes that contribute to  $N_2O$  production. It should be kept in mind that the efficiency of 10 Pa  $C_2H_2$  and 100 kPa  $O_2$  in suppressing nitrification and denitrification, respectively, will have been affected by the rates at which the gas diffused into the soil samples and dissolved in water (Webster & Hopkins, 1996). The enhancement of  $N_2O$  production from the 100 kPa  $O_2$  treated soil is consistent with results found elsewhere (Robertson & Tiedje, 1987).

In this study nitrifying bacteria made the highest contribution to the production of  $N_2O$  from all the soils. It came mostly from denitrifying nitrifiers. Webster & Hopkins (1996) have found that under drier soil conditions  $N_2O$  produced from denitrifiers and nitrifiers were almost equal (32% and 31% respectively). Under wet conditions, though, most of the  $N_2O$  produced came from the denitrifiers. Robertson & Tiedje (1987) found on a certain soil that most of the  $N_2O$  produced in this site is from sources other than nitrification and denitrification.

The soil in this study was not autoclaved. It could therefore not be determined if other  $N_2O$  sources contributed to the production other than nitrifiers and denitrifiers. Fungi are considered an important source of  $N_2O$  according to some authors (Robertson & Tiedje, 1987, Bleakley & Tiedje, 1982). Since they prefer acidic conditions for growth, contributions would probably have been unlikely.

The highest N<sub>2</sub>O production came from the Bethlehem soil and the lowest from the Stellenbosch soil. That indicates that the soil from Stellenbosch has the lowest mineralisation and subsequent N loss potential of all three soils.

Statistically there were no distinct differences in the production of N<sub>2</sub>O between the different water regimes. The lower or higher moisture content of the soil did, thus not impair microbial growth in this study.

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## GENERAL CONCLUSIONS

From the experiments performed to present characteristics and properties of soil under extensive cultivation in South Africa, conclusions could be made, which is presented in the following paragraphs.

The incubation study showed significant results concerning the different treatments over time. It also showed a good correlation with moisture. After 60 days of incubation, the soil was exhausted of decomposable materials,  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N. The microorganisms could not sustain themselves any longer. It is thought that the incubation time is too long and that it can be stopped after 45 days rather than 60. It is difficult to extrapolate the data to field conditions because of the amplified mineralisation and nitrification due to the small, enclosed environment.

Moisture stimulates microbial growth as can be seen from the nitrogen flush at the beginning of the incubation. The enhanced microbial activity stimulates the production of more  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N.

Organic material immobilised most of the nitrogen in those treatments. Due to the age of the material all the easily decomposable material has been mineralised already. The tougher parts of the material were introduced to the soil and immobilisation of the nitrogen therein took place, with a very slow release to mineralise.

The addition of fertiliser N showed a good priming/preparing effect to mineralisation of the native organic material of the soil. Although it is advantageous for plant growth, care should be taken that it is not lost to the groundwater or atmosphere.

In the field trial during the seasons 1997/1998 and 1998/1999, rainfall had the largest influence on the mineralisation and nitrification processes over both seasons. Microbial activity was adequately stimulated, due to sufficient moisture and aeration, to ensure mineralisation and immobilisation of nitrogen (ammonium and nitrate).

Denitrification could have taken place at stages in microsites in the soil when excess moisture was present. Denitrification products were not measured though. It is proposed because immobilisation and nitrification require  $O_2$  to occur.

Definitive periods of net immobilisation were recorded at the straw treated plots in both seasons due to the incorporation of organic material with a high C:N. Immobilisation was also noted in the control soil. From April, during both seasons, due to the deficiency of mineralisable substrate and little rainfall, microbes still present in the soil assimilated all the nitrogen they could, to ensure survival. A cover crop could be planted during the winter to ensure that the nitrogen is not lost when the spring rain arrives, bringing moisture, necessary for microbial metabolism.

In the study to discern between different  $N_2O$  sources in South African soils, the differential gas treatments showed good results in inhibiting certain processes that contribute to  $N_2O$  production. It should be kept in mind that the efficiency of 10 Pa  $C_2H_2$  and 100 kPa  $O_2$  in suppressing nitrification and denitrification, respectively, will have been affected by the rates at which the gas diffused into the soil samples and dissolved in water (Webster & Hopkins, 1996). The enhancement of  $N_2O$  production from the 100 kPa  $O_2$  treated soil is consistent with results found elsewhere (Robertson & Tiedje, 1987).

In this study nitrifying bacteria made the highest contribution to the production of  $N_2O$  from all the soils. It came mostly from denitrifying nitrifiers. Webster & Hopkins (1996) have found that under drier soil conditions  $N_2O$  produced from denitrifiers and nitrifiers were almost equal (32% and 31% respectively). Under wet conditions, though, most of the  $N_2O$  produced came from the denitrifiers. Robertson & Tiedje (1987) found on a certain soil that most of the  $N_2O$  produced in this site is from sources other than nitrification and denitrification.



The soil in this study was not autoclaved. It could therefore not be determined if other  $N_2O$  sources contributed to the production other than nitrifiers and denitrifiers. Fungi are considered an important source of  $N_2O$  according to some authors (Robertson & Tiedje, 1987, Bleakley & Tiedje, 1982). Since they prefer acidic conditions for growth, contributions would probably have been unlikely.

The highest  $N_2O$  production came from the Bethlehem soil and the lowest from the Stellenbosch soil. That indicates that the soil from Stellenbosch has the lowest mineralisation and subsequent N loss potential of all three soils.



## Chapter 3

### Abbreviations

### Glossary of Soil and Soil Microbiological terms

### Appendices

## CHAPTER 3

### TABLE OF CONTENTS

|  |          |
|--|----------|
| ABBREVIATIONS OF TERMS AND CHEMICAL FORMULAS USED IN THE THESIS .....                                | II       |
| GLOSSARY OF SOIL MICROBIOLOGY TERMS .....  | III      |
| <b>APPENDICES</b> .....  | <b>V</b> |
| APPENDIX I: INFLUENCE OF SUBSTRATE ON NITROGEN TRANSFORMATIONS.....                                  | VI       |
| APPENDIX II: MINERALISATION AND NITRIFICATION IN SOIL IN THE REGION OF PRETORIA (SOUTH AFRICA) ..... | VIII     |
| APPENDIX III: NITROUS OXIDE PRODUCTION AND EMISSION FROM SOUTH AFRICAN SOILS.....                    | XVIII    |



## ABBREVIATIONS OF TERMS AND CHEMICAL FORMULAS USED IN THE THESIS

**ATP** – Adenosine 5'-triphosphate

The triphosphate of the nucleoside adenosine, which is a high energy molecule and serves as the cell's major form of energy currency.

**C** – Carbon

**CEC** – Cation exchange capacity

The sum of exchangeable bases plus total soil acidity at a specific pH, values, usually 7.0 or 8.0. When acidity is expressed as salt extractable acidity, the cation exchange capacity is called the effective cation exchange capacity (ECEC) because this is considered to be the CEC of the exchanger at the native pH value. It is usually expressed in centimoles of charge per kilogram of exchanger ( $\text{cmol}_c\text{kg}^{-1}$ ) or millimoles of charge per kilogram of exchanger.

**CH<sub>4</sub>** – Methane

**C:N** - Carbon-organic nitrogen ratio

The ratio of the mass of organic carbon to the mass of organic nitrogen in soil, organic material, plants, or microbial cells.

**CO<sub>2</sub>** – Carbon dioxide

**FC** – Field capacity, in situ (field water capacity)

The content of water, on a mass or volume basis, remaining in a soil 2 or 3 days after having been wetted with water and after free drainage is negligible.

**MIT** – Mineralisation-immobilisation turnover

**N** – Nitrogen

**N<sub>2</sub>** – Dinitrogen (molecular nitrogen)

**NO** – Nitric oxide

**NH<sub>4</sub><sup>+</sup>** - Ammonium

**NH<sub>3</sub>** - Ammoniac

**NO<sub>2</sub><sup>-</sup>** - Nitrite

**NO<sub>3</sub><sup>-</sup>** - Nitrate

**N<sub>2</sub>O** – Dinitrogen oxide

**O<sub>2</sub>** – Oxygen gas



**pH** – The pH of a solution is the negative algorithm to the base ten of the hydrogen ion activity in the solution:  $\text{pH} = -\log_{10}a_{\text{H}}$

Soil pH is the degree of acidity of a soil as determined by means of a glass or other suitable electrode or indicator at a specified soil to suspension medium ratio of specified soil water content, expressed in terms of the pH scale.

Suspension media commonly used are distilled water, 1 M KCl and 0.1 M CaCl<sub>2</sub>.

**SMB** – Soil microbial biomass

**Urea** – CO(NH<sub>2</sub>)<sub>2</sub>



## GLOSSARY OF SOIL MICROBIOLOGY TERMS

**Aerobic** - (i) having molecular oxygen as a part of the environment. (ii) Growing only in the presence of molecular oxygen, such as aerobic organisms. (iii) Occurring only in the presence of molecular oxygen (said of chemical or biochemical processes such as aerobic decomposition).

**Anaerobic** - (i) The absence of molecular oxygen. (ii) Growing in the absence of molecular oxygen (such as anaerobic bacteria). (iii) Occurring in the absence of molecular oxygen (as a biochemical process).

**Anaerobic respiration** - The metabolic process whereby electrons are transferred from a reduced compound (usually organic) to an inorganic acceptor molecule other than oxygen. The most common acceptors are carbonate, sulphate, and nitrate.

**Autotroph** (autotrophic) - An organism capable of utilising CO<sub>2</sub> or carbonates as a sole source of carbon and obtaining energy for carbon reduction and biosynthetic processes from radiant energy (photoautotroph or photolithotroph) or oxidation of inorganic substances (chemoautotroph or chemolithotroph).

**Chemodenitrification** – Nonbiological (abiotic) processes leading to the production of gaseous forms of nitrogen (molecular nitrogen or an oxide of nitrogen, N<sub>2</sub>O and particularly NO).

**Chemolithotroph** (chemolithotrophic) - An organism capable of using CO<sub>2</sub> or carbonates as the sole source of carbon for cell biosynthesis, and deriving energy from the oxidation of reduced inorganic or organic compounds. Used synonymously with "chemolithoautotroph" and "chemotroph."  
[**Chemoautotroph** (chemoautotrophic)]

**Dissimilation** (dissimilatory nitrifier denitrification) - The release from cells of inorganic or organic substances formed by metabolism.

**Eutrophication** (eutrophic) – having concentrations of nutrients optimal, or nearly so, for plant, animal, or microbial growth. It is said of nutrient or soil solutions and bodies of water.

The enrichment of an aquatic environment with nutrients. Can also be groundwater.

**Heterotroph** (heterotrophic) – An organism able to derive carbon and energy for growth and cell synthesis by utilising organic compounds. Used synonymously with chemoorganotroph.



**Microaerophile** (microaerophilic) - An organism that requires a low concentration of oxygen for growth. Sometimes used to indicate an organism that will carry out its metabolic activities under aerobic conditions but that will grow much better under anaerobic conditions.

**Microbial biomass** - (i) The total mass of living microorganisms in a given volume or mass of soil. (ii) The total weight of all microorganisms in a particular environment.

**Photolithotroph** (photolithotrophic) - an organism that uses light as a source of energy and CO<sub>2</sub> or carbonates as the source of carbon for cell biosynthesis. See also **autotroph**.

[**Photoautotroph** (photoautotrophic)]

**Protoplasm** (protoplasmic) – the complete cellular contents, cytoplasmic membrane, cytoplasm, and nucleus; usually considered to be the living portion of the cell, thus excluding those layers peripheral to the cytoplasmic membrane.

**Respiration** (respiring) – an energy-yielding process in which an electron donor is oxidised using an inorganic electron acceptor. The acceptor may be either oxygen (aerobic respiration) or another inorganic acceptor (anaerobic respiration).

**Substrate** - (i) That which is laid or spread under an underlying layer, such as the subsoil. (ii) The substance, base, or nutrient on which an organism grows. (iii) Compounds or substances that are acted upon by enzymes or catalysts and changed to other compounds in the chemical reaction





## **APPENDICES**

**APPENDIX I:** INFLUENCE OF MOISTURE AND SUBSTRATE ON  
NITROGEN TRANSFORMATIONS

**APPENDIX II:** MINERALISATION AND NITRIFICATION IN SOIL IN THE  
REGION OF PRETORIA (SOUTH AFRICA)

**APPENDIX III:** NITROUS OXIDE PRODUCTION AND EMISSION FROM  
SOUTH AFRICAN SOILS

**APPENDIX I: INFLUENCE OF SUBSTRATE ON NITROGEN TRANSFORMATIONS**

Maize treatment

 $\text{NH}_4^+\text{-N}$  ( $\text{mg kg}^{-1}$ )

Blank

0.12

| Days | Control | Maize | Maize + $\text{NH}_4\text{NO}_3$ | $\text{NH}_4\text{NO}_3$ |
|------|---------|-------|----------------------------------|--------------------------|
| 0    | 1.11    | 1.75  | 1.64                             | 1.45                     |
|      | 1.00    | 1.82  | 1.65                             | 1.48                     |
|      | 1.18    | 1.17  | 1.66                             | 1.45                     |
| 1    | 1.90    | 1.16  | 5.49                             | 8.00                     |
|      | 2.01    | 1.14  | 5.12                             | 8.12                     |
|      | 2.21    | 1.26  | 5.41                             | 7.95                     |
| 3    | 1.68    | 3.58  | 3.05                             | 2.96                     |
|      | 1.75    | 3.62  | 3.12                             | 2.81                     |
|      | 1.89    | 3.74  | 3.31                             | 3.00                     |
| 7    | 0.25    | 0.10  | 0.29                             | 0.70                     |
|      | 0.24    | 0.20  | 0.31                             | 0.31                     |
|      | 0.31    | 0.25  | 0.29                             | 0.27                     |
| 15   | 2.00    | 0.21  | 3.05                             | 1.47                     |
|      | 1.85    | 0.26  | 3.12                             | 1.45                     |
|      | 1.96    | 0.29  | 3.19                             | 1.50                     |
| 30   | 0.95    | 0.79  | 1.37                             | 2.16                     |
|      | 0.93    | 0.77  | 1.42                             | 2.28                     |
|      | 1.00    | 0.82  | 1.49                             | 2.34                     |
| 45   | 0.46    | 0.66  | 0.70                             | 0.35                     |
|      | 0.39    | 0.61  | 0.76                             | 0.39                     |
|      | 0.43    | 0.65  | 0.85                             | 0.43                     |
| 60   | 0.29    | 0.20  | 0.30                             | 0.26                     |
|      | 0.32    | 0.26  | 0.29                             | 0.28                     |
|      | 0.31    | 0.29  | 0.36                             | 0.33                     |

Maize treatment

 $\text{NO}_3^-\text{-N}$  ( $\text{mg kg}^{-1}$ )

Blank

0.12

| Days | Control | Maize | Maize + $\text{NH}_4\text{NO}_3$ | $\text{NH}_4\text{NO}_3$ |
|------|---------|-------|----------------------------------|--------------------------|
| 0    | 0.66    | 2.44  | 2.35                             | 2.33                     |
|      | 0.63    | 2.31  | 2.34                             | 2.29                     |
|      | 0.75    | 2.46  | 2.40                             | 2.26                     |
| 1    | 0.90    | 0.37  | 5.37                             | 1.68                     |
|      | 0.95    | 0.33  | 5.41                             | 1.64                     |
|      | 0.87    | 0.39  | 5.25                             | 1.64                     |
| 3    | 1.42    | 1.16  | 1.95                             | 4.58                     |
|      | 1.48    | 1.13  | 1.96                             | 4.61                     |
|      | 1.48    | 1.12  | 1.96                             | 4.46                     |
| 7    | 0.69    | 0.47  | 1.03                             | 2.21                     |
|      | 0.74    | 0.53  | 1.00                             | 2.34                     |
|      | 0.63    | 0.42  | 1.06                             | 2.25                     |
| 15   | 0.38    | 1.95  | 4.05                             | 2.21                     |
|      | 0.37    | 1.95  | 4.13                             | 2.25                     |
|      | 0.34    | 1.90  | 4.19                             | 2.29                     |
| 30   | 2.18    | 2.00  | 2.42                             | 3.31                     |
|      | 2.20    | 2.13  | 2.36                             | 3.31                     |
|      | 2.14    | 2.15  | 2.37                             | 3.30                     |
| 45   | 1.49    | 2.10  | 1.30                             | 2.27                     |
|      | 1.59    | 2.16  | 1.34                             | 2.31                     |
|      | 1.50    | 2.00  | 1.35                             | 2.19                     |
| 60   | 0.90    | 0.57  | 0.73                             | 0.36                     |
|      | 0.93    | 0.56  | 0.70                             | 0.94                     |
|      | 2.38    | 0.56  | 0.68                             | 0.97                     |

## Soy treatment

 $\text{NH}_4^+$ -N ( $\text{mg kg}^{-1}$ )

Blank 0.12

| Days | Control | Soy  | Soy + $\text{NH}_4\text{NO}_3$ | $\text{NH}_4\text{NO}_3$ |
|------|---------|------|--------------------------------|--------------------------|
| 0    | 1.03    | 0.66 | 0.47                           | 0.47                     |
|      | 1.12    | 0.59 | 0.51                           | 0.49                     |
|      | 1.15    | 0.50 | 0.56                           | 0.46                     |
| 1    | 1.88    | 2.45 | 1.16                           | 3.26                     |
|      | 1.84    | 2.53 | 1.09                           | 3.18                     |
|      | 1.79    | 2.51 | 1.12                           | 3.12                     |
| 3    | 2.79    | 1.21 | 1.47                           | 4.58                     |
|      | 3.06    | 1.27 | 1.50                           | 4.46                     |
|      | 3.12    | 2.13 | 1.53                           | 4.43                     |
| 7    | 0.53    | 0.51 | 0.63                           | 0.43                     |
|      | 0.54    | 0.54 | 0.67                           | 0.46                     |
|      | 0.56    | 0.53 | 0.64                           | 0.50                     |
| 15   | 1.21    | 0.95 | 1.47                           | 2.00                     |
|      | 1.12    | 1.02 | 1.45                           | 2.16                     |
|      | 1.27    | 1.06 | 1.40                           | 2.12                     |
| 30   | 0.68    | 0.76 | 1.23                           | 0.95                     |
|      | 0.70    | 0.79 | 1.20                           | 0.89                     |
|      | 0.75    | 0.75 | 1.23                           | 0.85                     |
| 45   | 0.32    | 0.56 | 0.40                           | 0.29                     |
|      | 0.38    | 0.56 | 0.45                           | 0.33                     |
|      | 0.41    | 0.63 | 0.50                           | 0.39                     |
| 60   | 0.20    | 0.18 | 0.61                           | 0.34                     |
|      | 0.25    | 0.22 | 0.63                           | 0.30                     |
|      | 0.22    | 0.22 | 0.59                           | 0.34                     |

## Soy treatment

 $\text{NO}_3^-$ -N ( $\text{mg kg}^{-1}$ )

Blank 0.12

| Days | Control | Soy  | Soy + $\text{NH}_4\text{NO}_3$ | $\text{NH}_4\text{NO}_3$ |
|------|---------|------|--------------------------------|--------------------------|
| 0    | 0.52    | 0.96 | 0.26                           | 0.32                     |
|      | 0.54    | 0.98 | 0.24                           | 0.37                     |
|      | 0.57    | 0.92 | 0.29                           | 0.37                     |
| 1    | 0.63    | 0.37 | 4.32                           | 0.37                     |
|      | 0.65    | 0.35 | 4.22                           | 0.32                     |
|      | 0.70    | 0.39 | 4.41                           | 0.32                     |
| 3    | 2.47    | 2.47 | 1.95                           | 4.05                     |
|      | 2.43    | 2.41 | 1.96                           | 4.12                     |
|      | 2.36    | 2.52 | 1.93                           | 4.14                     |
| 7    | 0.47    | 0.47 | 0.48                           | 0.50                     |
|      | 0.43    | 0.42 | 0.42                           | 0.51                     |
|      | 0.50    | 0.52 | 0.46                           | 0.58                     |
| 15   | 2.21    | 1.94 | 0.21                           | 2.47                     |
|      | 2.27    | 1.92 | 2.22                           | 2.41                     |
|      | 2.16    | 1.88 | 2.34                           | 2.49                     |
| 30   | 3.00    | 0.99 | 1.42                           | 2.74                     |
|      | 3.10    | 0.96 | 1.49                           | 2.74                     |
|      | 2.95    | 0.91 | 1.42                           | 2.69                     |
| 45   | 0.70    | 0.79 | 0.78                           | 0.70                     |
|      | 0.71    | 0.76 | 0.76                           | 0.74                     |
|      | 0.62    | 0.83 | 0.74                           | 0.70                     |
| 60   | 0.21    | 0.27 | 0.46                           | 0.58                     |
|      | 0.24    | 0.28 | 0.42                           | 0.56                     |
|      | 0.20    | 0.33 | 0.51                           | 0.60                     |

**APPENDIX II: MINERALISATION AND NITRIFICATION IN SOIL IN THE REGION OF PRETORIA (SOUTH AFRICA)**

Season: 1997/1998

Treatment: Wheat straw

NH<sub>4</sub><sup>+</sup>-N (mg kg<sup>-1</sup>)

| Days | Covered    | Non-covered | *Rate  | Days | Covered | Non-covered | Rate   | Days                            | Covered | Non-covered | Rate   |
|------|------------|-------------|--------|------|---------|-------------|--------|---------------------------------|---------|-------------|--------|
| 0    | [REDACTED] | 0.19        | 0.129  | 16   | 0.26    | 0.39        | -0.347 | 30                              | 0.46    | 0.67        | -0.471 |
|      |            | 0.19        | 0.129  |      | 0.32    | 0.39        | -0.353 |                                 | 0.62    | 0.73        | -0.541 |
|      |            | 0.19        | 0.129  |      | 0.39    | 0.32        | -0.294 |                                 | 0.48    | 0.77        | -0.582 |
|      |            | 0.19        | 0.193  |      | 0.39    | 0.32        | -0.288 |                                 | 0.62    | 0.71        | -0.516 |
| 2    | 3.22       | 0.13        | 0.064  | 18   | 0.04    | 0.03        | 0.392  | 32                              | 0.20    | 0.57        | -0.571 |
|      | 3.22       | 0.13        | 0.064  |      | 0.03    | 0.03        | 0.403  |                                 | 0.19    | 0.49        | -0.487 |
|      | 3.22       | 0.19        | 0.322  |      | 0.03    | 0.03        | 0.409  |                                 | 0.19    | 0.50        | -0.498 |
|      | 3.86       | 0.39        | 0.129  |      | 0.03    | 0.02        | 0.381  |                                 | 0.19    | 0.46        | -0.465 |
| 4    | 2.57       | 0.06        | 0.000  | 20   | 0.42    | 0.03        | 0.342  | *Rate was measured fort-nightly |         |             |        |
|      | 2.57       | 0.19        | 0.064  |      | 0.43    | 0.03        | 0.342  |                                 |         |             |        |
|      | 5.15       | 0.06        | 0.257  |      | 0.44    | 0.03        | 0.291  |                                 |         |             |        |
|      | 3.22       | 0.39        | -0.193 |      | 0.40    | 0.04        | 0.308  |                                 |         |             |        |
| 6    | 0.64       | 6.31        | -0.129 | 22   | 0.37    | 0.45        | -0.084 |                                 |         |             |        |
|      | 2.57       | 6.95        | 0.129  |      | 0.38    | 0.41        | -0.017 |                                 |         |             |        |
|      | 3.22       | 5.66        | -0.129 |      | 0.32    | 0.40        | 0.067  |                                 |         |             |        |
|      | 1.93       | 5.66        | -0.064 |      | 0.35    | 0.39        | -0.028 |                                 |         |             |        |
| 8    | 1.29       | 3.09        | 0.000  | 24   | 0.36    | 0.31        | 0.297  |                                 |         |             |        |
|      | 2.57       | 5.66        | -0.064 |      | 0.40    | 0.30        | 0.230  |                                 |         |             |        |
|      | 1.93       | 5.66        | 0.000  |      | 0.47    | 0.35        | 0.314  |                                 |         |             |        |
|      | 1.93       | 8.88        | -0.129 |      | 0.36    | 0.34        | 0.241  |                                 |         |             |        |
| 10   | 1.29       | 5.02        | 0.193  | 26   | 0.61    | 0.37        | 0.269  |                                 |         |             |        |
|      | 0.64       | 4.38        | 0.193  |      | 0.53    | 0.38        | 0.336  |                                 |         |             |        |
|      | 1.93       | 3.73        | 0.257  |      | 0.66    | 0.54        | 0.129  |                                 |         |             |        |
|      | 1.29       | 7.60        | 0.193  |      | 0.58    | 0.61        | 0.196  |                                 |         |             |        |
| 12   | 4.51       | 11.46       | 0.193  | 28   | 0.64    | 0.45        | 0.006  |                                 |         |             |        |
|      | 3.22       | 13.39       | 0.129  |      | 0.72    | 0.52        | 0.106  |                                 |         |             |        |
|      | 4.51       | 8.88        | 0.193  |      | 0.67    | 0.46        | 0.017  |                                 |         |             |        |
|      | 5.15       | 8.24        | 0.193  |      | 0.81    | 0.54        | 0.078  |                                 |         |             |        |
| 14   | 4.51       | 7.92        | -0.193 |      |         |             |        |                                 |         |             |        |
|      | 3.86       | 7.79        | -0.064 |      |         |             |        |                                 |         |             |        |
|      | 4.51       | 7.98        | -0.193 |      |         |             |        |                                 |         |             |        |
|      | 4.51       | 7.79        | -0.064 |      |         |             |        |                                 |         |             |        |

Season: 1997/1998

Treatment: Control

NH<sub>4</sub><sup>+</sup>-N (mg kg<sup>-1</sup>)

| Days | Covered    | Non-covered | Rate  | Days                            | Covered | Non-covered | Rate  | Days                            | Covered | Non-covered | Rate  |
|------|------------|-------------|-------|---------------------------------|---------|-------------|-------|---------------------------------|---------|-------------|-------|
| 0    | [REDACTED] | 0.45        | -0.64 | 16                              | 0.32    | 0.45        | 0.00  | 30                              | 0.22    | 0.58        | -0.44 |
|      |            | 0.45        | -0.64 |                                 | 0.39    | 0.51        | -0.19 |                                 | 0.20    | 0.76        | -0.61 |
|      |            | 0.45        | -0.45 |                                 | 0.32    | 0.45        | -0.06 |                                 | 0.17    | 0.55        | -0.40 |
|      |            | 0.45        | -0.32 |                                 | 0.45    | 0.45        | -0.13 |                                 | 0.15    | 0.57        | -0.42 |
| 2    | -0.19      | 0.51        | -0.19 | 18                              | 0.45    | 0.39        | 0.14  | 32                              | 0.15    | 0.15        | -0.15 |
|      | -0.19      | 0.51        | 0.00  |                                 | 0.32    | 0.32        | 0.17  |                                 | 0.15    | 0.12        | -0.12 |
|      | 0.00       | 0.51        | -0.06 |                                 | 0.39    | 0.39        | 0.15  |                                 | 0.15    | 0.13        | -0.13 |
|      | 0.13       | 0.51        | -0.26 |                                 | 0.32    | 0.32        | 0.18  |                                 | 0.15    | 0.15        | -0.15 |
| 4    | 0.32       | 0.26        | -0.39 | 20                              | 0.53    | 0.39        | -0.10 | *Rate was measured fort-nightly |         |             |       |
|      | 0.51       | 0.00        | 0.26  |                                 | 0.49    | 0.39        | -0.08 |                                 |         |             |       |
|      | 0.45       | -0.13       | 0.51  |                                 | 0.54    | 0.39        | -0.06 |                                 |         |             |       |
|      | 0.26       | -0.13       | 0.51  |                                 | 0.50    | 0.32        | 0.07  |                                 |         |             |       |
| 6    | -0.13      | 0.45        | -0.19 | 22                              | 0.29    | 0.51        | 0.00  | *Rate was measured fort-nightly |         |             |       |
|      | 0.26       | 0.58        | -0.26 |                                 | 0.30    | 0.49        | -0.01 |                                 |         |             |       |
|      | 0.39       | 0.32        | 0.00  |                                 | 0.33    | 0.50        | 0.04  |                                 |         |             |       |
|      | 0.39       | 0.64        | -0.26 |                                 | 0.39    | 0.53        | -0.05 |                                 |         |             |       |
| 8    | 0.26       | 0.39        | -0.19 | 24                              | 0.52    | 0.31        | 0.43  | *Rate was measured fort-nightly |         |             |       |
|      | 0.32       | 0.39        | -0.32 |                                 | 0.48    | 0.33        | 0.31  |                                 |         |             |       |
|      | 0.32       | 0.39        | -0.13 |                                 | 0.54    | 0.29        | 0.44  |                                 |         |             |       |
|      | 0.39       | 0.32        | -0.06 |                                 | 0.48    | 0.32        | 0.38  |                                 |         |             |       |
| 10   | 0.19       | 0.39        | 0.06  | 26                              | 0.74    | 0.37        | -0.38 | *Rate was measured fort-nightly |         |             |       |
|      | 0.06       | 0.32        | 0.00  |                                 | 0.64    | 0.43        | 0.14  |                                 |         |             |       |
|      | 0.26       | 0.39        | -0.13 |                                 | 0.73    | 0.50        | 0.14  |                                 |         |             |       |
|      | 0.26       | 0.32        | 0.19  |                                 | 0.69    | 0.48        | 0.27  |                                 |         |             |       |
| 12   | 0.45       | 0.26        | 0.19  | 28                              | -0.01   | 0.47        | -0.25 | *Rate was measured fort-nightly |         |             |       |
|      | 0.32       | 0.13        | 0.26  |                                 | 0.57    | 0.71        | -0.50 |                                 |         |             |       |
|      | 0.26       | 0.13        | 0.32  |                                 | 0.64    | 0.55        | -0.39 |                                 |         |             |       |
|      | 0.51       | 0.26        | 0.13  |                                 | 0.74    | 0.57        | -0.41 |                                 |         |             |       |
| 14   | 0.45       | 0.32        | 0.00  | *Rate was measured fort-nightly |         |             |       |                                 |         |             |       |
|      | 0.39       | 0.26        | 0.13  |                                 |         |             |       |                                 |         |             |       |
|      | 0.45       | 0.45        | -0.13 |                                 |         |             |       |                                 |         |             |       |
|      | 0.39       | 0.51        | -0.06 |                                 |         |             |       |                                 |         |             |       |

Season: 1997/1998

Treatment: Wheat straw

NO<sub>3</sub><sup>-</sup>-N (mg kg<sup>-1</sup>)

| Days | Covered | Non-covered | Rate  | Days                            | Covered | Non-covered | Rate   | Days                            | Covered | Non-covered | Rate   |
|------|---------|-------------|-------|---------------------------------|---------|-------------|--------|---------------------------------|---------|-------------|--------|
| 0    | ■       | 1.80        | -1.42 | 16                              | 1.40    | 1.90        | 0.34   | 30                              | 10.86   | 22.51       | -8.49  |
|      |         | 1.22        | 0.39  |                                 | 1.01    | 1.57        | -0.45  |                                 | 10.64   | 16.35       | -2.34  |
|      |         | 1.74        | -0.32 |                                 | 1.29    | 1.57        | -0.62  |                                 | 9.91    | 25.31       | -11.29 |
|      |         | 1.42        | -0.26 |                                 | 1.34    | 1.46        | -0.45  |                                 | 12.43   | 32.26       | -18.30 |
| 2    | 0.39    | 1.42        | -0.51 | 18                              | 2.24    | 1.79        | 39.76  | 32                              | 14.03   | 18.54       | -18.54 |
|      | 1.61    | 1.35        | 0.00  |                                 | 1.12    | 1.12        | 47.66  |                                 | 14.02   | 12.32       | -12.32 |
|      | 1.42    | 1.80        | -0.45 |                                 | 0.95    | 1.29        | 50.01  |                                 | 14.02   | 13.38       | -13.38 |
|      | 1.16    | 0.97        | 0.26  |                                 | 1.01    | 1.18        | 16.35  |                                 | 13.96   | 10.98       | -10.98 |
| 4    | 0.90    | -0.58       | 1.36  | 20                              | 41.55   | 1.12        | 20.16  | *Rate was measured fort-nightly |         |             |        |
|      | 1.35    | -0.64       | 1.32  |                                 | 48.78   | 1.06        | 15.51  |                                 |         |             |        |
|      | 1.35    | -0.51       | 1.75  |                                 | 51.30   | 1.46        | 23.24  |                                 |         |             |        |
|      | 1.22    | -0.71       | 2.11  |                                 | 17.53   | 1.23        | 26.43  |                                 |         |             |        |
| 6    | 0.78    | 1.06        | 0.67  | 22                              | 21.28   | 32.65       | -8.23  | *Rate was measured fort-nightly |         |             |        |
|      | 0.67    | 1.06        | 0.90  |                                 | 16.58   | 33.60       | -15.51 |                                 |         |             |        |
|      | 1.23    | 4.03        | -1.90 |                                 | 24.70   | 23.80       | -23.05 |                                 |         |             |        |
|      | 1.40    | 4.09        | -2.69 |                                 | 27.66   | 19.49       | -1.18  |                                 |         |             |        |
| 8    | 1.74    | 2.30        | -1.18 | 24                              | 24.42   | 31.53       | -4.09  | *Rate was measured fort-nightly |         |             |        |
|      | 1.96    | 2.13        | -1.34 |                                 | 18.09   | 25.14       | 20.33  |                                 |         |             |        |
|      | 2.13    | 1.62        | -0.22 |                                 | 0.75    | 16.46       | 11.98  |                                 |         |             |        |
|      | 1.40    | 1.62        | -0.56 |                                 | 18.31   | 21.06       | 28.22  |                                 |         |             |        |
| 10   | 1.12    | 1.34        | -0.11 | 26                              | 27.44   | 21.06       | -6.55  | *Rate was measured fort-nightly |         |             |        |
|      | 0.78    | 1.46        | -0.17 |                                 | 45.47   | 31.30       | -18.37 |                                 |         |             |        |
|      | 1.40    | 2.30        | -1.40 |                                 | 28.45   | 24.53       | -9.86  |                                 |         |             |        |
|      | 1.06    | 1.68        | -0.11 |                                 | 49.28   | 23.69       | -4.48  |                                 |         |             |        |
| 12   | 1.23    | 0.73        | 0.73  | 28                              | 14.50   | 43.34       | -32.48 | *Rate was measured fort-nightly |         |             |        |
|      | 1.29    | 1.18        | 1.06  |                                 | 12.94   | 35.62       | -24.98 |                                 |         |             |        |
|      | 0.90    | 1.23        | 0.84  |                                 | 14.67   | 32.59       | -22.68 |                                 |         |             |        |
|      | 1.57    | 1.06        | 1.18  |                                 | 19.21   | 27.10       | -14.67 |                                 |         |             |        |
| 14   | 1.46    | 1.29        | 0.11  | *Rate was measured fort-nightly |         |             |        |                                 |         |             |        |
|      | 2.24    | 1.23        | -0.22 |                                 |         |             |        |                                 |         |             |        |
|      | 2.07    | 1.46        | -0.17 |                                 |         |             |        |                                 |         |             |        |
|      | 2.24    | 1.40        | -0.06 |                                 |         |             |        |                                 |         |             |        |

Season: 1997/1998

Treatment: Control

NO<sub>3</sub><sup>-</sup>-N (mg kg<sup>-1</sup>)

| Days | Covered    | Non-covered | Rate  | Days                            | Covered | Non-covered | Rate  | Days                            | Covered | Non-covered | Rate  |
|------|------------|-------------|-------|---------------------------------|---------|-------------|-------|---------------------------------|---------|-------------|-------|
| 0    | [REDACTED] | 1.87        | -1.74 | 16                              | 1.35    | 1.54        | 0.39  | 30                              | 0.17    | 1.83        | -1.64 |
|      |            | 2.06        | -1.80 |                                 | 1.29    | 1.74        | 0.58  |                                 | 0.15    | 1.68        | -1.48 |
|      |            | 1.54        | -0.90 |                                 | 1.29    | 1.16        | 0.71  |                                 | 0.16    | 1.92        | -1.73 |
|      |            | 1.42        | -0.71 |                                 | 1.22    | 1.22        | 0.84  |                                 | 0.17    | 1.59        | -1.40 |
| 2    | 0.13       | 0.90        | 1.03  | 18                              | 1.93    | 1.16        | 1.51  | 32                              | 0.19    | 0.17        | -0.17 |
|      | 0.26       | 0.51        | 1.29  |                                 | 2.32    | 1.42        | 4.34  |                                 | 0.20    | 0.21        | -0.21 |
|      | 0.64       | 1.03        | 1.22  |                                 | 1.87    | 1.61        | 4.34  |                                 | 0.19    | 0.25        | -0.25 |
|      | 0.71       | 1.16        | 0.58  |                                 | 2.06    | 1.35        | 5.88  |                                 | 0.19    | 0.14        | -0.14 |
| 4    | 1.93       | 0.90        | 0.00  | 20                              | 2.67    | 3.22        | -1.16 | *Rate was measured fort-nightly |         |             |       |
|      | 1.80       | 0.51        | 0.84  |                                 | 5.75    | 5.15        | -2.90 |                                 |         |             |       |
|      | 2.25       | 1.03        | 0.97  |                                 | 5.95    | 2.57        | -0.47 |                                 |         |             |       |
|      | 1.74       | 0.97        | 0.45  |                                 | 7.23    | 2.38        | -0.40 |                                 |         |             |       |
| 6    | 0.90       | 1.54        | -0.06 | 22                              | 2.06    | 3.15        | -1.98 | *Rate was measured fort-nightly |         |             |       |
|      | 1.35       | 1.87        | -0.06 |                                 | 2.25    | 2.29        | -0.46 |                                 |         |             |       |
|      | 2.00       | 1.93        | -0.71 |                                 | 2.11    | 2.36        | -0.46 |                                 |         |             |       |
|      | 1.42       | 1.54        | -0.13 |                                 | 1.98    | 3.30        | -2.38 |                                 |         |             |       |
| 8    | 1.48       | 1.35        | 0.06  | 24                              | 1.16    | 2.85        | -1.37 | *Rate was measured fort-nightly |         |             |       |
|      | 1.80       | 1.61        | -0.45 |                                 | 1.83    | 2.99        | -1.08 |                                 |         |             |       |
|      | 1.22       | 1.09        | 0.06  |                                 | 1.89    | 1.75        | 0.36  |                                 |         |             |       |
|      | 1.42       | 1.74        | -0.06 |                                 | 0.91    | 2.54        | -0.96 |                                 |         |             |       |
| 10   | 1.42       | 1.29        | 0.64  | 26                              | 1.48    | 1.89        | -0.59 | *Rate was measured fort-nightly |         |             |       |
|      | 1.16       | 1.35        | -0.32 |                                 | 1.91    | 1.79        | -0.57 |                                 |         |             |       |
|      | 1.16       | 0.90        | 0.26  |                                 | 2.11    | 1.08        | 0.29  |                                 |         |             |       |
|      | 1.67       | 1.42        | -0.64 |                                 | 1.58    | 1.64        | -0.19 |                                 |         |             |       |
| 12   | 1.93       | 1.54        | 0.06  | 28                              | 1.30    | 2.69        | -2.53 | *Rate was measured fort-nightly |         |             |       |
|      | 1.03       | 1.42        | 0.39  |                                 | 1.22    | 2.88        | -2.73 |                                 |         |             |       |
|      | 1.16       | 1.29        | 0.39  |                                 | 1.36    | 2.23        | -2.07 |                                 |         |             |       |
|      | 0.77       | 1.29        | 1.03  |                                 | 1.44    | 2.00        | -1.83 |                                 |         |             |       |
| 14   | 1.61       | 1.54        | -0.19 | *Rate was measured fort-nightly |         |             |       |                                 |         |             |       |
|      | 1.80       | 1.35        | -0.06 |                                 |         |             |       |                                 |         |             |       |
|      | 1.67       | 1.29        | 0.00  |                                 |         |             |       |                                 |         |             |       |
|      | 2.32       | 1.16        | 0.06  |                                 |         |             |       |                                 |         |             |       |



## Nitrate profile

Season: 1997/1998

Treatment: Straw

NO<sub>3</sub><sup>-</sup>-N (mg kg<sup>-1</sup>)

| Weeks | Depth (cm) |          |          |          |          |          |          |
|-------|------------|----------|----------|----------|----------|----------|----------|
|       | 0-15 cm    | 15-30 cm | 30-45 cm | 45-60 cm | 60-75 cm | 75-90 cm | Rainfall |
| 2     | 14.00      | 14.00    | 14.00    | 14.00    | 11.20    | 11.20    | 18.9     |
| 4     | 7.84       | 19.04    | 9.50     | 7.84     | 3.36     | 5.04     | 5.6      |
| 6     | 12.32      | 13.44    | 10.08    | 8.96     | 10.08    | 6.72     | 64.3     |
| 8     | 12.32      | 16.80    | 7.84     | 10.64    | 12.88    | 14.00    | 76.6     |
| 10    | 17.92      | 16.24    | 11.36    | 10.64    | 19.60    | 20.72    | 35.2     |
| 12    | 10.08      | 11.76    | 10.64    | 10.64    | 11.20    | 10.08    | 50       |
| 14    | 15.68      | 12.88    | 15.68    | 13.44    | 19.60    | 11.76    | 91.6     |
| 16    | 14.00      | 12.88    | 16.24    | 13.44    | 14.56    | 14.00    | 41.4     |
| 18    | 11.76      | 12.32    | 14.00    | 10.08    | 11.20    | 15.68    | 10.9     |
| 20    | 19.04      | 22.40    | 26.88    | 15.68    | 12.32    | 12.32    | 107.5    |
| 22    | 13.72      | 18.88    | 37.24    | 36.90    | 36.01    | 26.82    | 15.4     |
| 24    | 13.61      | 18.03    | 27.83    | 28.22    | 23.46    | 23.18    | 33.3     |
| 26    | 14.39      | 22.62    | 25.20    | 24.75    | 24.14    | 16.24    | 1.5      |
| 28    | 7.39       | 14.84    | 53.92    | 65.24    | 42.34    | 33.71    | 0        |
| 30    | 13.70      | 20.01    | 17.86    | 13.83    | 34.27    | 18.64    | 0        |
| 32    | 11.31      | 10.98    | 10.19    | 12.32    | 11.46    | 13.10    | 0        |

## Nitrate profile

Season: 1997/1998

Treatment: Control

NO<sub>3</sub><sup>-</sup>-N (mg kg<sup>-1</sup>)

| Weeks | Depth   |          |          |          |          |          |  |
|-------|---------|----------|----------|----------|----------|----------|--|
|       | 0-15 cm | 15-30 cm | 30-45 cm | 45-60 cm | 60-75 cm | Rainfall |  |
| 2     | 9.5     | 7.3      | 19.6     | 12.3     | 7.8      | 18.9     |  |
| 4     | 7.28    | 5.04     | 5.60     | 7.84     | 5.04     | 5.6      |  |
| 6     | 24.01   | 28       | 14.56    | 19.04    | 21.84    | 64.3     |  |
| 8     | 7.28    | 10.08    | 7.28     | 5.6      | 8.4      | 76.6     |  |
| 10    | 13.44   | 19.6     | 20.72    | 8.96     | 6.72     | 35.2     |  |
| 12    | 14      | 12.88    | 10.08    | 6.16     | 7.28     | 50       |  |
| 14    | 7.84    | 6.72     | 6.72     | 10.08    | 14.56    | 91.6     |  |
| 16    | 19.6    | 15.2     | 18.48    | 14.56    | 17.36    | 41.4     |  |
| 18    | 12.32   | 14.56    | 16.24    | 15.68    | 14       | 10.9     |  |
| 20    | 22.4    | 12.32    | 11.2     | 21.28    | 17.36    | 107.5    |  |
| 22    | 14.78   | 16.24    | 28.84    | 22.01    | 27.44    | 15.4     |  |
| 24    | 10.08   | 7.39     | 22.56    | 18.87    | 33.6     | 33.3     |  |
| 26    | 13.77   | 13.94    | 13.83    | 10.42    | 13.22    | 1.5      |  |
| 28    | 12.88   | 19.48    | 23.19    | 18.02    | 14.67    | 0        |  |
| 30    | 10.25   | 26.88    | 13.34    | 17.3     | 11.76    | 0        |  |
| 32    | 15.12   | 14       | 14.56    | 17.92    | 10.64    | 0        |  |

Season: 1998/1999

Treatment: Wheat straw

NH<sub>4</sub><sup>+</sup>-N (mg kg<sup>-1</sup>)

| Days | Covered    | Non-covered | Rate  | Days                            | Covered | Non-covered | Rate  | Days                            | Covered | Non-covered | Rate  |
|------|------------|-------------|-------|---------------------------------|---------|-------------|-------|---------------------------------|---------|-------------|-------|
| 0    | [REDACTED] | 0.19        | 0.00  | 16                              | 3.41    | 4.89        | -2.00 | 30                              | 2.12    | 2.45        | -0.80 |
|      |            | 0.19        | 0.00  |                                 | 3.28    | 5.41        | -2.32 |                                 | 4.06    | 3.41        | -1.81 |
|      |            | 0.19        | 0.00  |                                 | 3.28    | 4.70        | -1.67 |                                 | 2.12    | 1.80        | -0.14 |
|      |            | 0.19        | 0.00  |                                 | 3.22    | 4.83        | -1.87 |                                 | 2.77    | 4.06        | -2.39 |
| 2    | 0.19       | 0.13        | 0.19  | 18                              | 2.90    | 3.48        | -0.39 | 32                              | 1.64    | 1.80        | -1.80 |
|      | 0.19       | 0.13        | 0.19  |                                 | 3.09    | 3.41        | -0.64 |                                 | 1.60    | 1.80        | -1.80 |
|      | 0.19       | 0.19        | 0.13  |                                 | 3.03    | 3.54        | -0.77 |                                 | 1.66    | 1.93        | -1.91 |
|      | 0.19       | 0.39        | 0.00  |                                 | 2.96    | 3.41        | -0.32 |                                 | 1.66    | 2.12        | -2.12 |
| 4    | 0.32       | 0.06        | 0.19  | 20                              | 3.09    | 2.77        | 0.32  | *Rate was measured fort-nightly |         |             |       |
|      | 0.32       | 0.19        | -0.06 |                                 | 2.77    | 3.09        | -0.32 |                                 |         |             |       |
|      | 0.32       | 0.06        | -0.26 |                                 | 2.77    | 2.77        | 0.32  |                                 |         |             |       |
|      | 0.39       | 0.39        | -0.51 |                                 | 3.09    | 3.09        | -0.32 |                                 |         |             |       |
| 6    | 0.26       | 6.31        | 0.64  | 22                              | 3.09    | 3.09        | 0.97  | *Rate was measured fort-nightly |         |             |       |
|      | 0.13       | 6.95        | 0.00  |                                 | 2.77    | 3.09        | 0.00  |                                 |         |             |       |
|      | -0.19      | 5.66        | 0.64  |                                 | 3.09    | 3.09        | 0.00  |                                 |         |             |       |
|      | -0.13      | 5.66        | 1.29  |                                 | 2.77    | 3.09        | 0.32  |                                 |         |             |       |
| 8    | 6.95       | 3.09        | 2.57  | 24                              | 4.06    | 3.41        | 3.22  | *Rate was measured fort-nightly |         |             |       |
|      | 6.95       | 5.66        | -0.64 |                                 | 3.09    | 3.41        | 0.64  |                                 |         |             |       |
|      | 6.31       | 5.66        | -0.64 |                                 | 3.09    | 3.41        | 0.97  |                                 |         |             |       |
|      | 6.95       | 8.88        | -1.93 |                                 | 3.41    | 3.73        | 1.93  |                                 |         |             |       |
| 10   | 5.66       | 5.02        | -1.29 | 26                              | 6.63    | 5.34        | -0.64 | *Rate was measured fort-nightly |         |             |       |
|      | 5.02       | 4.38        | 0.00  |                                 | 4.06    | 5.66        | -0.97 |                                 |         |             |       |
|      | 5.02       | 3.73        | 2.57  |                                 | 4.38    | 4.38        | 0.00  |                                 |         |             |       |
|      | 6.95       | 7.60        | -1.93 |                                 | 5.66    | 4.06        | 0.32  |                                 |         |             |       |
| 12   | 3.73       | 11.46       | -7.72 | 28                              | 4.70    | 5.02        | -2.90 | *Rate was measured fort-nightly |         |             |       |
|      | 4.38       | 13.39       | -9.66 |                                 | 4.70    | 4.06        | 0.00  |                                 |         |             |       |
|      | 6.31       | 8.88        | -4.51 |                                 | 4.38    | 5.02        | -2.90 |                                 |         |             |       |
|      | 5.66       | 8.24        | -5.79 |                                 | 4.38    | 5.34        | -2.57 |                                 |         |             |       |
| 14   | 3.73       | 7.92        | -4.51 | *Rate was measured fort-nightly |         |             |       |                                 |         |             |       |
|      | 3.73       | 7.79        | -4.51 |                                 |         |             |       |                                 |         |             |       |
|      | 4.38       | 7.98        | -4.70 |                                 |         |             |       |                                 |         |             |       |
|      | 2.45       | 7.79        | -4.57 |                                 |         |             |       |                                 |         |             |       |

Season: 1998/1999

Treatment: Control

NH<sub>4</sub><sup>+</sup>-N (mg kg<sup>-1</sup>)

| Days | Covered | Non-covered | Rate   | Days | Covered | Non-covered | Rate   | Days                            | Covered | Non-covered | Rate   |
|------|---------|-------------|--------|------|---------|-------------|--------|---------------------------------|---------|-------------|--------|
| 0    |         | 0.45        | 0.067  | 16   | 1.48    | 1.35        | 0.322  | 30                              | 5.99    | 6.31        | -0.407 |
|      |         | 0.45        | 0.067  |      | 1.54    | 1.54        | -0.064 |                                 | 5.02    | 1.80        | 4.053  |
|      |         | 0.45        | 0.067  |      | 1.35    | 1.35        | 0.257  |                                 | 4.70    | 2.12        | 3.335  |
|      |         | 0.45        | 0.067  |      | 1.61    | 1.67        | -0.257 |                                 | 7.27    | 2.45        | 3.967  |
| 2    | 0.51    | 0.26        | -0.451 | 18   | 1.67    | 1.54        | 2.189  | 32                              | 5.90    | 0.51        | -0.515 |
|      | 0.51    | 0.00        | -0.193 |      | 1.48    | 1.42        | 0.708  |                                 | 5.86    | 2.45        | -2.446 |
|      | 0.51    | -0.13       | 0.129  |      | 1.61    | 1.48        | 0.322  |                                 | 5.46    | 1.48        | -1.480 |
|      | 0.51    | -0.13       | 0.257  |      | 1.42    | 1.54        | 0.257  |                                 | 6.41    | 1.16        | -1.159 |
| 4    | -0.19   | 5.66        | -1.287 | 20   | 3.73    | 1.74        | 2.317  | *Rate was measured fort-nightly |         |             |        |
|      | -0.19   | 12.10       | -5.793 |      | 2.12    | 1.16        | 1.931  |                                 |         |             |        |
|      | 0.00    | 5.66        | -0.644 |      | 1.80    | 1.22        | 2.189  |                                 |         |             |        |
|      | 0.13    | 6.95        | -0.644 |      | 1.80    | 1.16        | 1.609  |                                 |         |             |        |
| 6    | 4.38    | 8.88        | -3.862 | 22   | 4.06    | 3.73        | -0.322 |                                 |         |             |        |
|      | 6.31    | 4.38        | 4.506  |      | 3.09    | 2.45        | 0.966  |                                 |         |             |        |
|      | 5.02    | 5.66        | -0.644 |      | 3.41    | 2.77        | 0.322  |                                 |         |             |        |
|      | 6.31    | 12.10       | -3.218 |      | 2.77    | 3.41        | -0.322 |                                 |         |             |        |
| 8    | 5.02    | 8.88        | -4.506 | 24   | 3.41    | 3.41        | 0.644  |                                 |         |             |        |
|      | 8.88    | 5.66        | -1.287 |      | 3.41    | 3.09        | 1.609  |                                 |         |             |        |
|      | 5.02    | 6.31        | -1.287 |      | 3.09    | 4.70        | -0.966 |                                 |         |             |        |
|      | 8.88    | 5.66        | 0.000  |      | 3.09    | 3.73        | -0.322 |                                 |         |             |        |
| 10   | 4.38    | 3.73        | 3.218  | 26   | 4.06    | 5.66        | -1.609 |                                 |         |             |        |
|      | 4.38    | 5.66        | -2.575 |      | 4.70    | 3.73        | 0.322  |                                 |         |             |        |
|      | 5.02    | 3.73        | 0.000  |      | 3.73    | 5.34        | -1.931 |                                 |         |             |        |
|      | 5.66    | 5.02        | 0.000  |      | 3.41    | 4.70        | 0.322  |                                 |         |             |        |
| 12   | 6.95    | 3.09        | -1.931 | 28   | 4.06    | 4.38        | 1.609  |                                 |         |             |        |
|      | 3.09    | 2.45        | -0.644 |      | 4.06    | 4.38        | 0.644  |                                 |         |             |        |
|      | 3.73    | 0.51        | 0.644  |      | 3.41    | 5.02        | -0.322 |                                 |         |             |        |
|      | 5.02    | 1.80        | 0.000  |      | 5.02    | 5.99        | 1.287  |                                 |         |             |        |
| 14   | 1.16    | 1.67        | -0.193 |      |         |             |        |                                 |         |             |        |
|      | 1.80    | 1.29        | 0.257  |      |         |             |        |                                 |         |             |        |
|      | 1.16    | 1.29        | 0.064  |      |         |             |        |                                 |         |             |        |
|      | 1.80    | 1.48        | 0.129  |      |         |             |        |                                 |         |             |        |

Season: 1998/1999

Treatment: Wheat straw

NO<sub>3</sub><sup>-</sup>-N (mg kg<sup>-1</sup>)

| Days | Covered | Non-covered | Rate  | Days                            | Covered | Non-covered | Rate   | Days                            | Covered | Non-covered | Rate   |
|------|---------|-------------|-------|---------------------------------|---------|-------------|--------|---------------------------------|---------|-------------|--------|
| 0    |         | 9.66        | -8.82 | 16                              | 5.15    | 11.715      | 1.61   | 30                              | 13.97   | 14.93       | 0.30   |
|      |         | 9.14        | -6.82 |                                 | 4.89    | 12.294      | 1.42   |                                 | 10.43   | 11.71       | 3.25   |
|      |         | 9.46        | -7.60 |                                 | 5.02    | 12.230      | 2.00   |                                 | 21.37   | 7.85        | 7.24   |
|      |         | 9.33        | -7.79 |                                 | 5.02    | 12.359      | 1.93   |                                 | 12.36   | 13.00       | 2.04   |
| 2    | 0.84    | 1.42        | -1.03 | 18                              | 13.32   | 16.543      | 2.25   | 32                              | 15.23   | 11.71       | -11.71 |
|      | 2.32    | 1.35        | 0.26  |                                 | 13.71   | 17.766      | 6.18   |                                 | 14.97   | 8.50        | -8.50  |
|      | 1.87    | 1.80        | -0.39 |                                 | 14.23   | 17.508      | 6.44   |                                 | 15.10   | 9.14        | -9.14  |
|      | 1.54    | 0.97        | 0.19  |                                 | 14.29   | 17.444      | 2.00   |                                 | 15.05   | 6.57        | -6.57  |
| 4    | 0.39    | -0.58       | 0.06  | 20                              | 18.80   | 18.795      | -4.83  | *Rate was measured fort-nightly |         |             |        |
|      | 1.61    | -0.64       | 0.13  |                                 | 23.94   | 31.025      | -9.66  |                                 |         |             |        |
|      | 1.42    | -0.51       | 4.51  |                                 | 23.94   | 20.083      | 7.72   |                                 |         |             |        |
|      | 1.16    | -0.71       | 1.48  |                                 | 19.44   | 19.439      | 1.61   |                                 |         |             |        |
| 6    | -0.51   | 8.50        | 0.00  | 22                              | 13.97   | 28.129      | -7.08  | *Rate was measured fort-nightly |         |             |        |
|      | -0.51   | 3.35        | 5.15  |                                 | 21.37   | 26.520      | -6.76  |                                 |         |             |        |
|      | 3.99    | 3.35        | 6.44  |                                 | 27.81   | 24.589      | -4.51  |                                 |         |             |        |
|      | 0.77    | 5.28        | -3.22 |                                 | 21.05   | 19.117      | 2.90   |                                 |         |             |        |
| 8    | 8.50    | 3.35        | 3.22  | 24                              | 21.05   | 18.795      | 5.47   | *Rate was measured fort-nightly |         |             |        |
|      | 8.50    | 5.92        | -1.29 |                                 | 19.76   | 14.933      | 14.16  |                                 |         |             |        |
|      | 9.78    | 3.99        | 0.64  |                                 | 20.08   | 22.979      | -0.64  |                                 |         |             |        |
|      | 2.06    | 5.28        | -1.93 |                                 | 22.01   | 26.520      | 1.61   |                                 |         |             |        |
| 10   | 6.57    | 2.06        | 2.57  | 26                              | 24.27   | 37.140      | -23.49 | *Rate was measured fort-nightly |         |             |        |
|      | 4.63    | 4.63        | -1.29 |                                 | 29.09   | 24.589      | -12.23 |                                 |         |             |        |
|      | 4.63    | 5.28        | 0.64  |                                 | 22.34   | 23.301      | -7.72  |                                 |         |             |        |
|      | 3.35    | 5.28        | -1.93 |                                 | 28.13   | 33.600      | -18.02 |                                 |         |             |        |
| 12   | 4.63    | 7.21        | -5.15 | 28                              | 13.65   | 19.117      | -5.15  | *Rate was measured fort-nightly |         |             |        |
|      | 3.35    | 3.99        | -2.57 |                                 | 12.36   | 22.657      | -12.23 |                                 |         |             |        |
|      | 5.92    | 6.57        | -5.79 |                                 | 15.58   | 12.359      | 9.01   |                                 |         |             |        |
|      | 3.35    | 6.57        | -5.79 |                                 | 15.58   | 21.370      | -9.01  |                                 |         |             |        |
| 14   | 2.06    | 7.53        | -2.38 | *Rate was measured fort-nightly |         |             |        |                                 |         |             |        |
|      | 1.42    | 7.27        | -2.38 |                                 |         |             |        |                                 |         |             |        |
|      | 0.77    | 7.60        | -2.57 |                                 |         |             |        |                                 |         |             |        |
|      | 0.77    | 7.53        | -2.51 |                                 |         |             |        |                                 |         |             |        |

Season: 1998/1999

Treatment: Control

NO<sub>3</sub><sup>-</sup>-N (mg kg<sup>-1</sup>)

| Days | Covered    | Non-covered | Rate  | Days                            | Covered | Non-covered | Rate   | Days                            | Covered | Non-covered | Rate   |
|------|------------|-------------|-------|---------------------------------|---------|-------------|--------|---------------------------------|---------|-------------|--------|
| 0    | [REDACTED] | 1.87        | 0.13  | 16                              | 7.79    | 8.05        | 10.75  | 30                              | 21.05   | 22.01       | -19.90 |
|      |            | 2.06        | 0.13  |                                 | 7.92    | 8.11        | 8.75   |                                 | 26.20   | 13.97       | -11.82 |
|      |            | 1.54        | 0.90  |                                 | 8.05    | 8.17        | 8.05   |                                 | 25.23   | 10.75       | -8.68  |
|      |            | 1.42        | 0.06  |                                 | 7.85    | 8.05        | 9.46   |                                 | 8.82    | 10.11       | -8.08  |
| 2    | 2.00       | 0.90        | -0.77 | 18                              | 18.80   | 18.80       | 3.22   | 32                              | 2.12    | 14.93       | -14.93 |
|      | 2.19       | 0.51        | -0.26 |                                 | 16.86   | 18.15       | 10.94  |                                 | 2.15    | 9.14        | -9.14  |
|      | 2.45       | 1.03        | -0.39 |                                 | 16.22   | 16.86       | 7.08   |                                 | 2.06    | 9.14        | -9.14  |
|      | 1.48       | 0.97        | -0.26 |                                 | 17.51   | 16.86       | -1.16  |                                 | 2.02    | 10.11       | -10.11 |
| 4    | 0.13       | 5.92        | -0.64 | 20                              | 22.01   | 23.75       | -1.74  | *Rate was measured fort-nightly |         |             |        |
|      | 0.26       | 6.57        | -3.22 |                                 | 29.09   | 32.06       | -6.82  |                                 |         |             |        |
|      | 0.64       | 12.36       | -6.44 |                                 | 23.94   | 22.66       | -7.72  |                                 |         |             |        |
|      | 0.71       | 11.71       | -6.44 |                                 | 15.71   | 34.95       | -14.87 |                                 |         |             |        |
| 6    | 5.28       | 9.78        | -7.72 | 22                              | 22.01   | 20.73       | -7.72  | *Rate was measured fort-nightly |         |             |        |
|      | 3.35       | 5.28        | -0.64 |                                 | 25.23   | 18.80       | 2.90   |                                 |         |             |        |
|      | 5.92       | 3.99        | -1.93 |                                 | 14.93   | 22.98       | 0.00   |                                 |         |             |        |
|      | 5.28       | 6.57        | -0.64 |                                 | 20.08   | 21.05       | -2.90  |                                 |         |             |        |
| 8    | 2.06       | 4.63        | -0.64 | 24                              | 13.00   | 27.81       | -7.40  | *Rate was measured fort-nightly |         |             |        |
|      | 4.63       | 5.92        | 0.00  |                                 | 21.69   | 30.38       | -10.30 |                                 |         |             |        |
|      | 2.06       | 4.63        | 3.22  |                                 | 22.98   | 22.34       | 8.69   |                                 |         |             |        |
|      | 5.92       | 5.92        | 1.29  |                                 | 18.15   | 23.94       | 3.86   |                                 |         |             |        |
| 10   | 3.99       | 3.35        | -1.93 | 26                              | 20.40   | 17.19       | 0.97   | *Rate was measured fort-nightly |         |             |        |
|      | 5.92       | 2.06        | 0.00  |                                 | 20.08   | 23.62       | -5.47  |                                 |         |             |        |
|      | 7.85       | 3.35        | -1.29 |                                 | 31.03   | 27.16       | -7.08  |                                 |         |             |        |
|      | 7.21       | 3.99        | -0.64 |                                 | 27.81   | 22.01       | 8.37   |                                 |         |             |        |
| 12   | 1.42       | 2.06        | 0.00  | 28                              | 18.15   | 22.98       | -1.93  | *Rate was measured fort-nightly |         |             |        |
|      | 2.06       | 3.99        | 0.00  |                                 | 18.15   | 23.94       | 2.25   |                                 |         |             |        |
|      | 2.06       | 2.70        | 1.93  |                                 | 20.08   | 24.27       | 0.97   |                                 |         |             |        |
|      | 3.35       | 2.70        | 1.29  |                                 | 30.38   | 32.31       | -23.49 |                                 |         |             |        |
| 14   | 2.06       | 7.79        | 0.00  | *Rate was measured fort-nightly |         |             |        |                                 |         |             |        |
|      | 3.99       | 7.98        | -0.06 |                                 |         |             |        |                                 |         |             |        |
|      | 4.63       | 7.72        | 0.32  |                                 |         |             |        |                                 |         |             |        |
|      | 3.99       | 7.79        | 0.06  |                                 |         |             |        |                                 |         |             |        |

## Nitrate profile

Season: 1998/1999

Treatment: Straw

NO<sub>3</sub><sup>-</sup>-N (mg kg<sup>-1</sup>)

| Weeks | Depth (cm) |          |          |          |          |          | Rainfall |
|-------|------------|----------|----------|----------|----------|----------|----------|
|       | 0-15 cm    | 15-30 cm | 30-45 cm | 45-60 cm | 60-75 cm | 75-90 cm |          |
| 2     | 0.81       | 1.53     | 1.39     | 1.81     | 1.63     | 1.31     | 18       |
| 4     | 0.67       | 1.29     | 1.12     | 1.57     | 1.40     | 1.18     | 33.9     |
| 6     | 0.78       | 2.02     | 1.01     | 0.78     | 0.34     | 0.56     | 15       |
| 8     | 1.79       | 4.59     | 3.47     | 5.71     | 3.47     | 11.31    | 89.2     |
| 10    | 2.91       | 3.47     | 3.47     | 4.59     | 5.71     | 12.04    | 57.8     |
| 12    | 3.47       | 2.91     | 10.19    | 4.03     | 6.83     | 10.19    | 88.4     |
| 14    | 2.35       | 7.39     | 3.47     | 5.15     | 6.77     | 4.59     | 18.7     |
| 16    | 1.79       | 3.47     | 1.79     | 4.03     | 4.03     | 1.79     | 22.4     |
| 18    | 7.19       | 7.21     | 3.50     | 7.31     | 6.14     | 7.09     | 21.2     |
| 20    | 14.87      | 14.04    | 14.24    | 14.00    | 9.61     | 15.01    | 7.6      |
| 22    | 18.59      | 19.15    | 18.59    | 14.39    | 12.99    | 25.31    | 18.5     |
| 24    | 21.67      | 20.27    | 13.83    | 13.83    | 20.27    | 24.75    | 53.3     |
| 26    | 19.71      | 12.99    | 9.07     | 9.63     | 12.43    | 13.27    | 1.4      |
| 28    | 16.91      | 16.91    | 21.11    | 10.47    | 12.15    | 14.39    | 83.5     |
| 30    | 14.39      | 14.95    | 9.35     | 8.23     | 8.23     | 8.51     | 1.5      |
| 32    | 14.21      | 12.78    | 9.01     | 8.74     | 8.02     | 8.79     | 28.7     |

## Nitrate profile

Season: 1998/1999

Treatment: Control

NO<sub>3</sub><sup>-</sup>-N (mg kg<sup>-1</sup>)

| Weeks | Depth (cm) |          |          |          |          |          | Rainfall |
|-------|------------|----------|----------|----------|----------|----------|----------|
|       | 0-15 cm    | 15-30 cm | 30-45 cm | 45-60 cm | 60-75 cm | 75-90 cm |          |
| 2     | 1.14       | 0.85     | 2.19     | 1.501    | 0.96     | 0.89     | 18       |
| 4     | 1.0        | 0.7      | 1.904    | 1.2      | 0.8      | 0.6      | 33.9     |
| 6     | 0.728      | 0.504    | 0.56     | 0.784    | 0.504    | 0.392    | 15       |
| 8     | 4.592      | 4.032    | 5.712    | 7.952    | 3.472    | 4.032    | 89.2     |
| 10    | 4.032      | 7.392    | 5.712    | 10.752   | 4.592    | 6.772    | 57.8     |
| 12    | 6.772      | 12.432   | 6.832    | 4.032    | 5.712    | 6.772    | 88.4     |
| 14    | 1.792      | 2.912    | 4.592    | 5.152    | 5.712    | 5.152    | 18.7     |
| 16    | 2.912      | 0.672    | 2.352    | 5.712    | 2.912    | 1.792    | 22.4     |
| 18    | 8.314      | 5.73     | 3.012    | 8.542    | 3.04     | 3.814    | 21.2     |
| 20    | 16.132     | 9.351    | 6.851    | 10.154   | 4.231    | 7.242    | 7.6      |
| 22    | 23.352     | 13.608   | 8.512    | 14.00    | 7.392    | 9.184    | 18.5     |
| 24    | 24.752     | 11.592   | 8.792    | 9.072    | 14.672   | 10.472   | 53.3     |
| 26    | 28.112     | 21.392   | 13.832   | 12.432   | 16.072   | 5.992    | 1.4      |
| 28    | 28.67      | 11.31    | 12.43    | 11.59    | 11.87    | 11.87    | 83.5     |
| 30    | 24.19      | 19.15    | 13.27    | 11.31    | 12.15    | 10.47    | 1.5      |
| 32    | 23.15      | 20.13    | 14.58    | 11.21    | 12.05    | 11.12    | 28.7     |

### APPENDIX III: NITROUS OXIDE PRODUCTION AND EMISSION FROM SOUTH AFRICAN SOILS

#### Key to symbols used:

##### Location of soil -

S = Stellenbosch

B = Bethlehem

P = Pretoria

D = 100 kPa O<sub>2</sub>

E = 100 kPa O<sub>2</sub> plus 10 Pa C<sub>2</sub>H<sub>2</sub>

##### Headspace treatment used -

A = control

B = 10 kPa C<sub>2</sub>H<sub>2</sub>

C = 10 Pa C<sub>2</sub>H<sub>2</sub>

##### Soil treatment used –

K = control (Co)

N = 40 kg N as NH<sub>4</sub>NO<sub>3</sub> (N)

O = organic material as 0.5% wheat straw (C)

NO = 40 kg N as NH<sub>4</sub>NO<sub>3</sub> plus 0.5% wheat straw (C&N)

## Stellenbosch - day 1

5% moisture

| Sample | Area   | ng N2O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) | Sample | Area    | ng N2O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) |
|--------|--------|-----------|--|---|--------|---------|-----------|--|---|
| SAK 1  | 172832 | 1.73      | 0.21   | 205.609   | SCO 1  | 127806  | 1.28      | 0.15   | 152.04  |
| SAK 2  | 192663 | 1.93      | 0.23   | 229.20  | SCO 2  | 162057  | 1.62      | 0.19   | 192.79  |
| SAK 3  | 206469 | 2.06      | 0.25   | 245.63  | SCO 3  | 185540  | 1.86      | 0.22   | 220.73  |
| SAN 1  | 238923 | 2.39      | 0.28   | 284.23  | SCNO 1 |         |           |  |   |
| SAN 2  | 236598 | 2.37      | 0.28   | 281.47  | SCNO 2 | 165391  | 1.65      | 0.20   | 196.76  |
| SAN 3  | 236193 | 2.36      | 0.28   | 280.99  | SCNO 3 | 128812  | 1.29      | 0.15   | 153.24  |
| SAO 1  | 236391 | 2.36      | 0.28   | 281.22  | SDK 1  | 1056660 | 26.17     | 3.11   | 3113.56   |
| SAO 2  | 216076 | 2.16      | 0.26   | 257.05  | SDK 2  | 919383  | 22.77     | 2.71   | 2709.06   |
| SAO 3  | 273962 | 2.74      | 0.33   | 325.92  | SDK 3  | 989649  | 24.51     | 2.92   | 2916.10   |
| SANO 1 | 232152 | 2.32      | 0.28   | 276.18  | SDN 1  | 858338  | 21.26     | 2.53   | 2529.18   |
| SANO 2 | 229347 | 2.29      | 0.27   | 272.84  | SDN 2  | 1272202 | 31.51     | 3.75   | 3748.67   |
| SANO 3 | 236557 | 2.37      | 0.28   | 281.42  | SDN 3  | 967565  | 23.97     | 2.85   | 2851.03   |
| SBK 1  | 181192 | 1.81      | 0.22   | 215.55  | SDO 1  | 1111081 | 27.52     | 3.27   | 3273.91   |
| SBK 2  | 155548 | 1.56      | 0.19   | 185.05  | SDO 2  | 1003533 | 24.86     | 2.96   | 2957.01   |
| SBK 3  | 199389 | 1.99      | 0.24   | 237.20  | SDO 3  | 1148616 | 28.45     | 3.38   | 3384.51   |
| SBN 1  | 152905 | 1.53      | 0.18   | 181.90  | SDNO 1 | 855534  | 21.19     | 2.52   | 2520.92   |
| SBN 2  | 161846 | 1.62      | 0.19   | 192.54  | SDNO 2 | 1050262 | 26.01     | 3.09   | 3094.70   |
| SBN 3  | 133322 | 1.33      | 0.16   | 158.61  | SDNO 3 | 1048304 | 25.97     | 3.09   | 3088.93   |
| SBO 1  | 178547 | 1.79      | 0.21   | 212.41  | SEK 1  | 1010271 | 25.02     | 2.98   | 2976.87   |
| SBO 2  | 167573 | 1.68      | 0.20   | 199.35  | SEK 2  | 1355020 | 33.56     | 3.99   | 3992.70   |
| SBO 3  | 172362 | 1.72      | 0.21   | 205.05  | SEK 3  | 1100424 | 27.26     | 3.24   | 3242.51   |
| SBNO 1 | 138638 | 1.39      | 0.16   | 164.93  | SEN 1  | 1050775 | 26.03     | 3.10   | 3096.22   |
| SBNO 2 | 120333 | 1.20      | 0.14   | 143.15  | SEN 2  | 1074735 | 26.62     | 3.17   | 3166.82   |
| SBNO 3 | 139906 | 1.40      | 0.17   | 166.44  | SEN 3  | 1105593 | 27.38     | 3.26   | 3257.74   |
| SCK 1  | 147766 | 1.48      | 0.18   | 175.79  | SEO 1  | 746858  | 18.50     | 2.20   | 2200.69   |
| SCK 2  | 150424 | 1.50      | 0.18   | 178.95  | SEO 2  | 1167938 | 28.93     | 3.44   | 3441.45   |
| SCK 3  | 174324 | 1.74      | 0.21   | 207.38  | SEO 3  | 1137057 | 28.16     | 3.35   | 3350.45   |
| SCN 1  | 177382 | 1.77      | 0.21   | 211.02  | SENO 1 | 740284  | 18.34     | 2.18   | 2181.32   |
| SCN 2  | 227607 | 2.28      | 0.27   | 270.77  | SENO 2 | 933494  | 23.12     | 2.75   | 2750.64   |
| SCN 3  | 188755 | 1.89      | 0.22   | 224.55  | SENO 3 | 706967  | 17.51     | 2.08   | 2083.15   |



## Stellenbosch - day 4

5% moisture

| Sample | Area   | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | Sample | Area    | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) |
|--------|--------|------------------------|--|--|--------|---------|------------------------|--|---|
| SAK 1  | 112565 | 0.34                   | 0.04   | 40.32  | SCO 1  | 147639  | 0.44                   | 0.05   | 52.89   |
| SAK 2  | 123341 | 0.37                   | 0.04   | 44.18  | SCO 2  | 182279  | 0.55                   | 0.07   | 65.30   |
| SAK 3  | 128523 | 0.39                   | 0.05   | 46.04  | SCO 3  | 177600  | 0.53                   | 0.06   | 63.62   |
| SAN 1  | 136658 | 0.41                   | 0.05   | 48.96  | SCNO 1 | 93763   | 0.28                   | 0.03   | 33.59   |
| SAN 2  | 143403 | 0.43                   | 0.05   | 51.37  | SCNO 2 | 110646  | 0.33                   | 0.04   | 39.64   |
| SAN 3  | 144116 | 0.43                   | 0.05   | 51.63  | SCNO 3 | 147007  | 0.44                   | 0.05   | 52.66   |
| SAO 1  | 156650 | 0.47                   | 0.06   | 56.12  | SDK 1  | 1341550 | 13.42                  | 1.60   | 1595.97   |
| SAO 2  | 165416 | 0.50                   | 0.06   | 59.26  | SDK 2  | 1440199 | 14.40                  | 1.71   | 1713.33   |
| SAO 3  | 115871 | 0.35                   | 0.04   | 41.51  | SDK 3  | 1314778 | 13.15                  | 1.56   | 1564.12   |
| SANO 1 | 183118 | 0.55                   | 0.07   | 65.60  | SDN 1  | 1225544 | 12.26                  | 1.46   | 1457.96   |
| SANO 2 | 156148 | 0.47                   | 0.06   | 55.94  | SDN 2  | 958714  | 9.59                   | 1.14   | 1140.53   |
| SANO 3 | 154776 | 0.47                   | 0.06   | 55.45  | SDN 3  | 1170478 | 11.70                  | 1.39   | 1392.46   |
| SBK 1  | 100849 | 0.30                   | 0.04   | 36.13  | SDO 1  | 1221500 | 12.22                  | 1.45   | 1453.15   |
| SBK 2  | 101649 | 0.31                   | 0.04   | 36.41  | SDO 2  | 1252777 | 12.53                  | 1.49   | 1490.36   |
| SBK 3  | 100424 | 0.30                   | 0.04   | 35.98  | SDO 3  | 1248461 | 12.48                  | 1.49   | 1485.23   |
| SBN 1  | 109241 | 0.33                   | 0.04   | 39.13  | SDNO 1 | 1108529 | 11.09                  | 1.32   | 1318.76   |
| SBN 2  | 118698 | 0.36                   | 0.04   | 42.52  | SDNO 2 | 1282677 | 12.83                  | 1.53   | 1525.93   |
| SBN 3  | 101254 | 0.30                   | 0.04   | 36.27  | SDNO 3 | 1191453 | 11.91                  | 1.42   | 1417.41   |
| SBO 1  | 120177 | 0.36                   | 0.04   | 43.05  | SEK 1  | 1247233 | 12.47                  | 1.48   | 1483.77   |
| SBO 2  | 111674 | 0.34                   | 0.04   | 40.01  | SEK 2  | 1006122 | 10.06                  | 1.20   | 1196.93   |
| SBO 3  | 111664 | 0.34                   | 0.04   | 40.00  | SEK 3  |         |                        |  |   |
| SBNO 1 | 99960  | 0.30                   | 0.04   | 35.81  | SEN 1  | 1375712 | 13.76                  | 1.64   | 1636.61   |
| SBNO 2 | 100438 | 0.30                   | 0.04   | 35.98  | SEN 2  | 1151028 | 11.51                  | 1.37   | 1369.32   |
| SBNO 3 | 94722  | 0.29                   | 0.03   | 33.93  | SEN 3  | 1302838 | 13.03                  | 1.55   | 1549.92   |
| SCK 1  | 110159 | 0.33                   | 0.04   | 39.46  | SEO 1  | 1299190 | 12.99                  | 1.55   | 1545.58   |
| SCK 2  | 82920  | 0.25                   | 0.03   | 29.70  | SEO 2  | 1211560 | 12.12                  | 1.44   | 1441.33   |
| SCK 3  | 108997 | 0.33                   | 0.04   | 39.05  | SEO 3  | 1014956 | 10.15                  | 1.21   | 1207.44   |
| SCN 1  | 100973 | 0.30                   | 0.04   | 36.17  | SENO 1 | 1129218 | 11.29                  | 1.34   | 1343.37   |
| SCN 2  | 113436 | 0.34                   | 0.04   | 40.64  | SENO 2 | 1299988 | 13.00                  | 1.55   | 1546.53   |
| SCN 3  | 108283 | 0.33                   | 0.04   | 38.79  | SENO 3 | 1046694 | 10.47                  | 1.25   | 1245.20   |

Stellenbosch - day 4  
 15% moisture

| Sample | Area   | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) | Sample | Area    | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) |
|--------|--------|------------------------|--|---|--------|---------|------------------------|--|---|
| SAK 1  | 154931 | 0.47                   | 0.06   | 55.50   | SCO 1  | 184631  | 0.56                   | 0.07   | 66.14   |
| SAK 2  | 111955 | 0.34                   | 0.04   | 40.11   | SCO 2  | 215101  | 0.65                   | 0.08   | 77.06   |
| SAK 3  | 152737 | 0.46                   | 0.05   | 54.72   | SCO 3  | 158471  | 0.48                   | 0.06   | 56.77   |
| SAN 1  | 173145 | 0.52                   | 0.06   | 62.03   | SCNO 1 |         |                        |  |   |
| SAN 2  | 190126 | 0.57                   | 0.07   | 68.11   | SCNO 2 | 262907  | 0.79                   | 0.09   | 94.18   |
| SAN 3  | 165977 | 0.50                   | 0.06   | 59.46   | SCNO 3 | 217398  | 0.65                   | 0.08   | 77.88   |
| SAO 1  | 193517 | 0.58                   | 0.07   | 69.32   | SDK 1  | 1059900 | 10.60                  | 1.26   | 1260.91   |
| SAO 2  |        |                        |  |   | SDK 2  | 1039930 | 10.40                  | 1.24   | 1237.15   |
| SAO 3  | 198293 | 0.60                   | 0.07   | 71.04   | SDK 3  | 1039750 | 10.40                  | 1.24   | 1236.93   |
| SANO 1 | 155819 | 0.47                   | 0.06   | 55.82   | SDN 1  | 1124651 | 11.25                  | 1.34   | 1337.94   |
| SANO 2 | 165731 | 0.50                   | 0.06   | 59.37   | SDN 2  | 1158843 | 11.59                  | 1.38   | 1378.61   |
| SANO 3 | 126370 | 0.38                   | 0.05   | 45.27   | SDN 3  | 1286617 | 12.87                  | 1.53   | 1530.62   |
| SBK 1  | 275624 | 0.83                   | 0.10   | 98.74   | SDO 1  | 761229  | 7.61                   | 0.91   | 905.59  |
| SBK 2  | 218451 | 0.66                   | 0.08   | 78.26   | SDO 2  | 988548  | 9.89                   | 1.18   | 1176.02   |
| SBK 3  | 313377 | 0.94                   | 0.11   | 112.26  | SDO 3  | 1133066 | 11.33                  | 1.35   | 1347.95   |
| SBN 1  | 145879 | 0.44                   | 0.05   | 52.26   | SDNO 1 | 1310208 | 13.10                  | 1.56   | 1558.68   |
| SBN 2  | 162338 | 0.49                   | 0.06   | 58.15   | SDNO 2 | 1023233 | 10.23                  | 1.22   | 1217.29   |
| SBN 3  | 100851 | 0.30                   | 0.04   | 36.13   | SDNO 3 | 1121288 | 11.21                  | 1.33   | 1333.94   |
| SBO 1  | 273361 | 0.82                   | 0.10   | 97.93   | SEK 1  | 1389208 | 13.89                  | 1.65   | 1652.67   |
| SBO 2  | 113079 | 0.34                   | 0.04   | 40.51   | SEK 2  | 1117136 | 11.17                  | 1.33   | 1329.00   |
| SBO 3  | 248568 | 0.75                   | 0.09   | 89.05   | SEK 3  | 1076870 | 10.77                  | 1.28   | 1281.09   |
| SBNO 1 | 160542 | 0.48                   | 0.06   | 57.51   | SEN 1  | 1131200 | 11.31                  | 1.35   | 1345.73   |
| SBNO 2 | 163135 | 0.49                   | 0.06   | 58.44   | SEN 2  | 1247786 | 12.48                  | 1.48   | 1484.42   |
| SBNO 3 | 184659 | 0.56                   | 0.07   | 66.15   | SEN 3  | 1001201 | 10.01                  | 1.19   | 1191.08   |
| SCK 1  | 411047 | 1.24                   | 0.15   | 147.25  | SEO 1  | 1088054 | 10.88                  | 1.29   | 1294.40   |
| SCK 2  | 692157 | 2.08                   | 0.25   | 247.95  | SEO 2  | 1553736 | 15.54                  | 1.85   | 1848.40   |
| SCK 3  | 915067 | 2.76                   | 0.33   | 327.81  | SEO 3  | 1147993 | 11.48                  | 1.37   | 1365.71   |
| SCN 1  | 136093 | 0.41                   | 0.05   | 48.75   | SENO 1 | 1124123 | 11.24                  | 1.34   | 1337.31   |
| SCN 2  | 179257 | 0.54                   | 0.06   | 64.22   | SENO 2 | 1339494 | 13.39                  | 1.59   | 1593.52   |
| SCN 3  | 148755 | 0.45                   | 0.05   | 53.29   | SENO 3 | 1244157 | 12.44                  | 1.48   | 1480.11   |

Stellbosch - day 10

5% moisture

| Sample | Area   | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) | Sample | Area    | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) |
|--------|--------|------------------------|--|---|--------|---------|------------------------|--|---|
| SAK 1  | 89389  | 0.99                   | 0.12   | 118.01  | SCO 1  | 178736  | 1.98                   | 0.24   | 235.97  |
| SAK 2  | 112843 | 1.25                   | 0.15   | 148.97  | SCO 2  | 195853  | 2.17                   | 0.26   | 258.56  |
| SAK 3  | 109607 | 1.22                   | 0.14   | 144.70  | SCO 3  | 186086  | 2.07                   | 0.25   | 245.67  |
| SAN 1  | 139292 | 1.55                   | 0.18   | 183.89  | SCNO 1 | 205540  | 2.28                   | 0.27   | 271.35  |
| SAN 2  | 104588 | 1.16                   | 0.14   | 138.08  | SCNO 2 | 217144  | 2.41                   | 0.29   | 286.67  |
| SAN 3  | 126119 | 1.40                   | 0.17   | 166.50  | SCNO 3 | 210407  | 2.33                   | 0.28   | 277.78  |
| SAO 1  | 221103 | 2.45                   | 0.29   | 291.90  | SDK 1  | 479574  | 28.70                  | 3.41   | 3414.35   |
| SAO 2  | 170214 | 1.89                   | 0.22   | 224.72  | SDK 2  | 336990  | 20.17                  | 2.40   | 2399.21   |
| SAO 3  | 215602 | 2.39                   | 0.28   | 284.64  | SDK 3  | 370086  | 22.15                  | 2.63   | 2634.84   |
| SANO 1 | 203932 | 2.26                   | 0.27   | 269.23  | SDN 1  | 536692  | 32.12                  | 3.82   | 3821.00   |
| SANO 2 | 197808 | 2.20                   | 0.26   | 261.14  | SDN 2  | 1405763 | 84.13                  | 10.01  | 10008.38  |
| SANO 3 | 197901 | 2.20                   | 0.26   | 261.27  | SDN 3  | 1229059 | 73.55                  | 8.75   | 8750.33   |
| SBK 1  | 134318 | 1.49                   | 0.18   | 177.33  | SDO 1  | 1178285 | 70.52                  | 8.39   | 8388.85   |
| SBK 2  | 167315 | 1.86                   | 0.22   | 220.89  | SDO 2  | 1325051 | 79.30                  | 9.43   | 9433.75   |
| SBK 3  | 102970 | 1.14                   | 0.14   | 135.94  | SDO 3  | 1071638 | 64.13                  | 7.63   | 7629.57   |
| SBN 1  | 106478 | 1.18                   | 0.14   | 140.57  | SDNO 1 |         |                        |  |   |
| SBN 2  | 118186 | 1.31                   | 0.16   | 156.03  | SDNO 2 | 826303  | 49.45                  | 5.88   | 5882.90   |
| SBN 3  | 105991 | 1.18                   | 0.14   | 139.93  | SDNO 3 | 811865  | 48.59                  | 5.78   | 5780.10   |
| SBO 1  | 107239 | 1.19                   | 0.14   | 141.58  | SEK 1  | 753318  | 45.08                  | 5.36   | 5363.28   |
| SBO 2  | 87179  | 0.97                   | 0.12   | 115.09  | SEK 2  | 870504  | 52.10                  | 6.20   | 6197.59   |
| SBO 3  | 80286  | 0.89                   | 0.11   | 105.99  | SEK 3  | 775629  | 46.42                  | 5.52   | 5522.12   |
| SBNO 1 | 25060  | 0.28                   | 0.03   | 33.08   | SEN 1  | 1057267 | 63.27                  | 7.53   | 7527.25   |
| SBNO 2 | 65233  | 0.72                   | 0.09   | 86.12   | SEN 2  | 1074105 | 64.28                  | 7.65   | 7647.13   |
| SBNO 3 | 39428  | 0.44                   | 0.05   | 52.05   | SEN 3  | 104589  | 6.26                   | 0.74   | 744.63  |
| SCK 1  | 35656  | 0.40                   | 0.05   | 47.07   | SEO 1  | 917082  | 54.88                  | 6.53   | 6529.20   |
| SCK 2  | 39568  | 0.44                   | 0.05   | 52.24   | SEO 2  | 726366  | 43.47                  | 5.17   | 5171.39   |
| SCK 3  | 56268  | 0.62                   | 0.07   | 74.28   | SEO 3  | 1001333 | 59.93                  | 7.13   | 7129.03   |
| SCN 1  | 94622  | 1.05                   | 0.12   | 124.92  | SENO 1 | 1054680 | 63.12                  | 7.51   | 7508.84   |
| SCN 2  | 82382  | 0.91                   | 0.11   | 108.76  | SENO 2 | 1109538 | 66.40                  | 7.90   | 7899.40   |
| SCN 3  | 84421  | 0.94                   | 0.11   | 111.45  | SENO 3 | 1181239 | 70.69                  | 8.41   | 8409.88   |

## Stellenbosch - day 10

15% moisture

| Sample | Area   | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) | Sample | Area    | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) |
|--------|--------|------------------------|--|---|--------|---------|------------------------|--|---|
| SAK 1  | 229363 | 2.55                   | 0.30   | 302.80  | SCO 1  | 316523  | 3.51                   | 0.42   | 417.87  |
| SAK 2  |        |                        |  |   | SCO 2  | 366025  | 4.06                   | 0.48   | 483.22  |
| SAK 3  | 299142 | 3.32                   | 0.39   | 394.92  | SCO 3  | 348833  | 3.87                   | 0.46   | 460.53  |
| SAN 1  | 136971 | 1.52                   | 0.18   | 180.83  | SCNO 1 | 294266  | 3.27                   | 0.39   | 388.49  |
| SAN 2  | 116403 | 1.29                   | 0.15   | 153.67  | SCNO 2 | 280640  | 3.11                   | 0.37   | 370.50  |
| SAN 3  | 195705 | 2.17                   | 0.26   | 258.37  | SCNO 3 | 211731  | 2.35                   | 0.28   | 279.53  |
| SAO 1  | 277383 | 3.08                   | 0.37   | 366.20  | SDK 1  | 898119  | 53.75                  | 6.39   | 6394.19   |
| SAO 2  | 424218 | 4.71                   | 0.56   | 560.05  | SDK 2  | 569822  | 34.10                  | 4.06   | 4056.87   |
| SAO 3  | 212171 | 2.35                   | 0.28   | 280.11  | SDK 3  | 637217  | 38.13                  | 4.54   | 4536.69   |
| SANO 1 | 331065 | 3.67                   | 0.44   | 437.07  | SDN 1  | 1288099 | 77.09                  | 9.17   | 9170.67   |
| SANO 2 | 164526 | 1.83                   | 0.22   | 217.21  | SDN 2  | 1131262 | 67.70                  | 8.05   | 8054.06   |
| SANO 3 | 276948 | 3.07                   | 0.37   | 365.62  | SDN 3  | 887601  | 53.12                  | 6.32   | 6319.31   |
| SBK 1  |        |                        |  |   | SDO 1  | 1064271 | 63.69                  | 7.58   | 7577.12   |
| SBK 2  | 165934 | 1.84                   | 0.22   | 219.06  | SDO 2  | 993012  | 59.43                  | 7.07   | 7069.79   |
| SBK 3  | 169479 | 1.88                   | 0.22   | 223.74  | SDO 3  | 969673  | 58.03                  | 6.90   | 6903.62   |
| SBN 1  | 91728  | 1.02                   | 0.12   | 121.10  | SDNO 1 | 636619  | 38.10                  | 4.53   | 4532.43   |
| SBN 2  | 130551 | 1.45                   | 0.17   | 172.35  | SDNO 2 | 741886  | 44.40                  | 5.28   | 5281.89   |
| SBN 3  | 194245 | 2.16                   | 0.26   | 256.44  | SDNO 3 | 711937  | 42.61                  | 5.07   | 5068.66   |
| SBO 1  | 699881 | 7.77                   | 0.92   | 923.98  | SEK 1  | 1081722 | 64.74                  | 7.70   | 7701.36   |
| SBO 2  | 353274 | 3.92                   | 0.47   | 466.39  | SEK 2  | 1097595 | 65.69                  | 7.81   | 7814.37   |
| SBO 3  | 494872 | 5.49                   | 0.65   | 653.33  | SEK 3  | 1011641 | 60.54                  | 7.20   | 7202.42   |
| SBNO 1 | 132645 | 1.47                   | 0.18   | 175.12  | SEN 1  | 906445  | 54.25                  | 6.45   | 6453.47   |
| SBNO 2 | 130416 | 1.45                   | 0.17   | 172.17  | SEN 2  | 870782  | 52.11                  | 6.20   | 6199.57   |
| SBNO 3 | 142001 | 1.58                   | 0.19   | 187.47  | SEN 3  | 841821  | 50.38                  | 5.99   | 5993.38   |
| SCK 1  | 150077 | 1.67                   | 0.20   | 198.13  | SEO 1  | 918938  | 54.99                  | 6.54   | 6542.41   |
| SCK 2  | 149443 | 1.66                   | 0.20   | 197.29  | SEO 2  | 1165578 | 69.75                  | 8.30   | 8298.38   |
| SCK 3  | 167919 | 1.86                   | 0.22   | 221.69  | SEO 3  | 1032152 | 61.77                  | 7.35   | 7348.45   |
| SCN 1  | 277633 | 3.08                   | 0.37   | 366.53  | SENO 1 | 1245914 | 74.56                  | 8.87   | 8870.33   |
| SCN 2  | 132151 | 1.47                   | 0.17   | 174.46  | SENO 2 |         |                        |  |   |
| SCN 3  | 198241 | 2.20                   | 0.26   | 261.72  | SENO 3 | 1282078 | 76.73                  | 9.13   | 9127.80   |

Bethlehem - day 1  
 5% moisture

| Sample | Area   | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) | Sample | Area    | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) |
|--------|--------|------------------------|--|---|--------|---------|------------------------|--|---|
| BAK 1  | 113743 | 12.12                  | 1.44   | 1441.41   | BCO 1  | 80665   | 8.59                   | 1.02   | 1022.23   |
| BAK 2  |        |                        |  |   | BCO 2  | 103487  | 11.02                  | 1.31   | 1311.44   |
| BAK 3  | 110147 | 11.73                  | 1.40   | 1395.84   | BCO 3  | 75319   | 8.02                   | 0.95   | 954.48  |
| BAN 1  | 109081 | 11.62                  | 1.38   | 1382.33   | BCNO 1 | 155319  | 16.55                  | 1.97   | 1968.28   |
| BAN 2  | 155624 | 16.58                  | 1.97   | 1972.15   | BCNO 2 | 102002  | 10.87                  | 1.29   | 1292.62   |
| BAN 3  | 134179 | 14.29                  | 1.70   | 1700.39   | BCNO 3 | 72250   | 7.70                   | 0.92   | 915.59  |
| BAO 1  | 221278 | 23.57                  | 2.80   | 2804.15   | BDK 1  | 900938  | 46.34                  | 5.51   | 5512.88   |
| BAO 2  | 218780 | 23.31                  | 2.77   | 2772.50   | BDK 2  | 870035  | 44.75                  | 5.32   | 5323.78   |
| BAO 3  | 229682 | 24.47                  | 2.91   | 2910.65   | BDK 3  | 886174  | 45.58                  | 5.42   | 5422.54   |
| BANO 1 | 248555 | 26.48                  | 3.15   | 3149.82   | BDN 1  | 925879  | 47.62                  | 5.67   | 5665.50   |
| BANO 2 | 246633 | 26.27                  | 3.13   | 3125.46   | BDN 2  | 881073  | 45.32                  | 5.39   | 5391.33   |
| BANO 3 | 247953 | 26.41                  | 3.14   | 3142.19   | BDN 3  | 1312157 | 67.49                  | 8.03   | 8029.15   |
| BBK 1  | 185733 | 19.78                  | 2.35   | 2353.71   | BDO 1  | 1076705 | 55.38                  | 6.59   | 6588.41   |
| BBK 2  | 132625 | 14.13                  | 1.68   | 1680.69   | BDO 2  | 1000974 | 51.49                  | 6.13   | 6125.01   |
| BBK 3  | 131934 | 14.05                  | 1.67   | 1671.94   | BDO 3  | 1203925 | 61.92                  | 7.37   | 7366.87   |
| BBN 1  | 147604 | 15.72                  | 1.87   | 1870.52   | BDNO 1 | 1040556 | 53.52                  | 6.37   | 6367.21   |
| BBN 2  | 126475 | 13.47                  | 1.60   | 1602.76   | BDNO 2 | 1165321 | 59.94                  | 7.13   | 7130.65   |
| BBN 3  | 171590 | 18.28                  | 2.17   | 2174.48   | BDNO 3 | 1070251 | 55.05                  | 6.55   | 6548.91   |
| BBO 1  | 141389 | 15.06                  | 1.79   | 1791.76   | BEK 1  | 918968  | 47.27                  | 5.62   | 5623.21   |
| BBO 2  | 141086 | 15.03                  | 1.79   | 1787.92   | BEK 2  | 847297  | 43.58                  | 5.18   | 5184.65   |
| BBO 3  | 138202 | 14.72                  | 1.75   | 1751.37   | BEK 3  | 1106134 | 56.89                  | 6.77   | 6768.48   |
| BBNO 1 | 125983 | 13.42                  | 1.60   | 1596.52   | BEN 1  | 1020587 | 52.49                  | 6.25   | 6245.02   |
| BBNO 2 | 116790 | 12.44                  | 1.48   | 1480.02   | BEN 2  | 729765  | 37.54                  | 4.47   | 4465.47   |
| BBNO 3 | 141678 | 15.09                  | 1.80   | 1795.42   | BEN 3  | 891893  | 45.88                  | 5.46   | 5457.53   |
| BCK 1  | 107295 | 11.43                  | 1.36   | 1359.70   | BEO 1  | 1010219 | 51.96                  | 6.18   | 6181.58   |
| BCK 2  | 132022 | 14.06                  | 1.67   | 1673.05   | BEO 2  | 713363  | 36.69                  | 4.37   | 4365.10   |
| BCK 3  | 138861 | 14.79                  | 1.76   | 1759.72   | BEO 3  | 1006568 | 51.77                  | 6.16   | 6159.24   |
| BCN 1  | 102106 | 10.88                  | 1.29   | 1293.94   | BENO 1 | 997644  | 51.31                  | 6.10   | 6104.63   |
| BCN 2  | 137756 | 14.67                  | 1.75   | 1745.72   | BENO 2 | 1113145 | 57.26                  | 6.81   | 6811.38   |
| BCN 3  | 135725 | 14.46                  | 1.72   | 1719.98   | BENO 3 | 892012  | 45.88                  | 5.46   | 5458.26   |

## Bethlehem - day 1

15% moisture

| Sample | Area   | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) | Sample | Area    | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) |
|--------|--------|------------------------|--|---|--------|---------|------------------------|--|---|
| BAK 1  | 121044 | 12.89                  | 1.53   | 1533.93   | BCO 1  | 107536  | 11.46                  | 1.36   | 1362.75   |
| BAK 2  | 109484 | 11.66                  | 1.39   | 1387.44   | BCO 2  | 101275  | 10.79                  | 1.28   | 1283.41   |
| BAK 3  | 125728 | 13.39                  | 1.59   | 1593.29   | BCO 3  | 108504  | 11.56                  | 1.38   | 1375.02   |
| BAN 1  | 147148 | 15.67                  | 1.86   | 1864.74   | BCNO 1 | 137796  | 14.68                  | 1.75   | 1746.22   |
| BAN 2  | 191869 | 20.44                  | 2.43   | 2431.47   | BCNO 2 | 127898  | 13.62                  | 1.62   | 1620.79   |
| BAN 3  | 159954 | 17.04                  | 2.03   | 2027.02   | BCNO 3 | 117698  | 12.54                  | 1.49   | 1491.53   |
| BAO 1  | 206494 | 22.00                  | 2.62   | 2616.80   | BDK 1  | 203741  | 74.00                  | 8.80   | 8802.93   |
| BAO 2  | 219040 | 23.33                  | 2.78   | 2775.79   | BDK 2  | 255835  | 92.92                  | 11.05  | 11053.72  |
| BAO 3  | 207862 | 22.14                  | 2.63   | 2634.14   | BDK 3  | 250543  | 90.99                  | 10.83  | 10825.07  |
| BANO 1 | 180651 | 19.24                  | 2.29   | 2289.31   | BDN 1  | 450075  | 163.46                 | 19.45  | 19446.14  |
| BANO 2 | 213942 | 22.79                  | 2.71   | 2711.19   | BDN 2  | 388032  | 140.93                 | 16.77  | 16765.49  |
| BANO 3 | 240643 | 25.63                  | 3.05   | 3049.56   | BDN 3  | 387933  | 140.89                 | 16.76  | 16761.21  |
| BBK 1  | 176520 | 18.80                  | 2.24   | 2236.96   | BDO 1  | 1422250 | 516.54                 | 61.45  | 61450.38  |
| BBK 2  | 150855 | 16.07                  | 1.91   | 1911.71   | BDO 2  | 1552056 | 563.69                 | 67.06  | 67058.83  |
| BBK 3  | 144416 | 15.38                  | 1.83   | 1830.12   | BDO 3  | 1445565 | 525.01                 | 62.46  | 62457.74  |
| BBN 1  | 109453 | 11.66                  | 1.39   | 1387.05   | BDNO 1 | 1542624 | 560.26                 | 66.65  | 66651.31  |
| BBN 2  | 128876 | 13.73                  | 1.63   | 1633.19   | BDNO 2 | 1453936 | 528.05                 | 62.82  | 62819.42  |
| BBN 3  | 129005 | 13.74                  | 1.63   | 1634.82   | BDNO 3 | 1452681 | 527.60                 | 62.77  | 62765.19  |
| BBO 1  | 131887 | 14.05                  | 1.67   | 1671.34   | BEK 1  | 1190933 | 432.53                 | 51.46  | 51455.99  |
| BBO 2  | 196508 | 20.93                  | 2.49   | 2490.25   | BEK 2  | 1598147 | 580.43                 | 69.05  | 69050.26  |
| BBO 3  | 120230 | 12.81                  | 1.52   | 1523.62   | BEK 3  | 1371567 | 498.14                 | 59.26  | 59260.54  |
| BBNO 1 |        |                        |  |   | BEN 1  | 1429200 | 519.07                 | 61.75  | 61750.66  |
| BBNO 2 | 129314 | 13.77                  | 1.64   | 1638.74   | BEN 2  | 1342281 | 487.50                 | 58.00  | 57995.20  |
| BBNO 3 | 108456 | 11.55                  | 1.37   | 1374.41   | BEN 3  | 1443064 | 524.10                 | 62.35  | 62349.68  |
| BCK 1  | 169926 | 18.10                  | 2.15   | 2153.39   | BEO 1  | 1392296 | 505.66                 | 60.16  | 60156.17  |
| BCK 2  | 166228 | 17.71                  | 2.11   | 2106.53   | BEO 2  | 1329325 | 482.79                 | 57.44  | 57435.42  |
| BCK 3  | 172454 | 18.37                  | 2.19   | 2185.43   | BEO 3  | 1304402 | 473.74                 | 56.36  | 56358.58  |
| BCN 1  | 145257 | 15.47                  | 1.84   | 1840.77   | BENO 1 | 962323  | 349.50                 | 41.58  | 41578.56  |
| BCN 2  | 161549 | 17.21                  | 2.05   | 2047.23   | BENO 2 | 621362  | 225.67                 | 26.85  | 26846.85  |
| BCN 3  | 142793 | 15.21                  | 1.81   | 1809.55   | BENO 3 | 896812  | 325.71                 | 38.75  | 38748.07  |



Bethlehem - day 4  
 5% moisture

| Sample | Area   | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) | Sample | Area    | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) |
|--------|--------|------------------------|--|---|--------|---------|------------------------|--|---|
| BAK 1  | 125984 | 8.29                   | 0.99   | 986.73  | BCO 1  | 213848  | 14.08                  | 1.67   | 1674.90   |
| BAK 2  | 122476 | 8.06                   | 0.96   | 959.25  | BCO 2  | 183571  | 12.09                  | 1.44   | 1437.76   |
| BAK 3  | 119988 | 7.90                   | 0.94   | 939.77  | BCO 3  | 164722  | 10.84                  | 1.29   | 1290.13   |
| BAN 1  | 125312 | 8.25                   | 0.98   | 981.47  | BCNO 1 | 130545  | 8.59                   | 1.02   | 1022.45   |
| BAN 2  | 123606 | 8.14                   | 0.97   | 968.11  | BCNO 2 | 144812  | 9.53                   | 1.13   | 1134.19   |
| BAN 3  | 131250 | 8.64                   | 1.03   | 1027.97   | BCNO 3 | 143157  | 9.42                   | 1.12   | 1121.23   |
| BAO 1  | 118234 | 7.78                   | 0.93   | 926.03  | BDK 1  | 1328320 | 482.43                 | 57.39  | 57392.00  |
| BAO 2  | 158068 | 10.41                  | 1.24   | 1238.02   | BDK 2  | 1244390 | 451.95                 | 53.77  | 53765.68  |
| BAO 3  | 155797 | 10.26                  | 1.22   | 1220.23   | BDK 3  | 1233244 | 447.90                 | 53.28  | 53284.10  |
| BANO 1 | 90629  | 5.97                   | 0.71   | 709.82  | BDN 1  | 774094  | 281.14                 | 33.45  | 33445.86  |
| BANO 2 | 149141 | 9.82                   | 1.17   | 1168.10   | BDN 2  | 563346  | 204.60                 | 24.34  | 24340.18  |
| BANO 3 | 131143 | 8.63                   | 1.03   | 1027.14   | BDN 3  | 744373  | 270.35                 | 32.16  | 32161.72  |
| BBK 1  | 159369 | 10.49                  | 1.25   | 1248.21   | BDO 1  | 315304  | 114.51                 | 13.62  | 13623.17  |
| BBK 2  | 117592 | 7.74                   | 0.92   | 921.00  | BDO 2  | 546608  | 198.52                 | 23.62  | 23616.99  |
| BBK 3  | 103536 | 6.82                   | 0.81   | 810.91  | BDO 3  | 393016  | 142.74                 | 16.98  | 16980.83  |
| BBN 1  | 47699  | 3.14                   | 0.37   | 373.59  | BDNO 1 | 1677412 | 609.21                 | 72.48  | 72475.02  |
| BBN 2  | 97044  | 6.39                   | 0.76   | 760.07  | BDNO 2 | 1811593 | 657.95                 | 78.27  | 78272.51  |
| BBN 3  | 101376 | 6.67                   | 0.79   | 794.00  | BDNO 3 | 1703591 | 618.72                 | 73.61  | 73606.12  |
| BBO 1  | 92490  | 6.09                   | 0.72   | 724.40  | BEK 1  | 1376791 | 500.03                 | 59.49  | 59486.26  |
| BBO 2  | 117498 | 7.74                   | 0.92   | 920.27  | BEK 2  | 1183026 | 429.66                 | 51.11  | 51114.36  |
| BBO 3  | 97677  | 6.43                   | 0.77   | 765.02  | BEK 3  | 1417525 | 514.83                 | 61.25  | 61246.23  |
| BBNO 1 | 74008  | 4.87                   | 0.58   | 579.64  | BEN 1  | 1403393 | 509.69                 | 60.64  | 60635.63  |
| BBNO 2 | 75105  | 4.94                   | 0.59   | 588.24  | BEN 2  | 1263954 | 459.05                 | 54.61  | 54610.97  |
| BBNO 3 | 72957  | 4.80                   | 0.57   | 571.41  | BEN 3  | 1529355 | 555.44                 | 66.08  | 66078.00  |
| BCK 1  | 72292  | 4.76                   | 0.57   | 566.20  | BEO 1  | 921366  | 334.63                 | 39.81  | 39808.96  |
| BCK 2  | 75821  | 4.99                   | 0.59   | 593.84  | BEO 2  | 1492701 | 542.13                 | 64.49  | 64494.31  |
| BCK 3  | 75478  | 4.97                   | 0.59   | 591.16  | BEO 3  | 930577  | 337.97                 | 40.21  | 40206.93  |
| BCN 1  | 132634 | 8.73                   | 1.04   | 1038.81   | BENO 1 | 1169989 | 424.93                 | 50.55  | 50551.07  |
| BCN 2  | 114932 | 7.57                   | 0.90   | 900.17  | BENO 2 | 938582  | 340.88                 | 40.55  | 40552.80  |
| BCN 3  | 89727  | 5.91                   | 0.70   | 702.76  | BENO 3 | 1104550 | 401.16                 | 47.72  | 47723.69  |

Bethlehem - day 4  
15% moisture

| Sample | Area   | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) | Sample | Area    | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) |
|--------|--------|------------------------|--|---|--------|---------|------------------------|--|---|
| BAK 1  | 192956 | 12.70                  | 1.51   | 1511.27   | BCO 1  | 209634  | 13.80                  | 1.64   | 1641.89   |
| BAK 2  | 173649 | 11.43                  | 1.36   | 1360.05   | BCO 2  | 196111  | 12.91                  | 1.54   | 1535.98   |
| BAK 3  | 149627 | 9.85                   | 1.17   | 1171.91   | BCO 3  | 198412  | 13.06                  | 1.55   | 1554.00   |
| BAN 1  | 176000 | 11.59                  | 1.38   | 1378.46   | BCNO 1 | 202044  | 13.30                  | 1.58   | 1582.45   |
| BAN 2  | 183009 | 12.05                  | 1.43   | 1433.36   | BCNO 2 | 196541  | 12.94                  | 1.54   | 1539.35   |
| BAN 3  | 183754 | 12.10                  | 1.44   | 1439.20   | BCNO 3 | 203863  | 13.42                  | 1.60   | 1596.69   |
| BAO 1  | 133607 | 8.80                   | 1.05   | 1046.43   | BDK 1  | 203741  | 74.00                  | 8.80   | 8802.93   |
| BAO 2  | 192318 | 12.66                  | 1.51   | 1506.27   | BDK 2  | 255835  | 92.92                  | 11.05  | 11053.72  |
| BAO 3  | 187885 | 12.37                  | 1.47   | 1471.55   | BDK 3  | 250543  | 90.99                  | 10.83  | 10825.07  |
| BANO 1 | 157962 | 10.40                  | 1.24   | 1237.19   | BDN 1  | 450075  | 163.46                 | 19.45  | 19446.14  |
| BANO 2 | 97901  | 6.45                   | 0.77   | 766.78  | BDN 2  | 388032  | 140.93                 | 16.77  | 16765.49  |
| BANO 3 | 165969 | 10.93                  | 1.30   | 1299.90   | BDN 3  | 387933  | 140.89                 | 16.76  | 16761.21  |
| BBK 1  | 62479  | 4.11                   | 0.49   | 489.35  | BDO 1  | 1422250 | 516.54                 | 61.45  | 61450.38  |
| BBK 2  | 108306 | 7.13                   | 0.85   | 848.27  | BDO 2  | 1552056 | 563.69                 | 67.06  | 67058.83  |
| BBK 3  | 121194 | 7.98                   | 0.95   | 949.21  | BDO 3  | 1445565 | 525.01                 | 62.46  | 62457.74  |
| BBN 1  | 117143 | 7.71                   | 0.92   | 917.49  | BDNO 1 | 1542624 | 560.26                 | 66.65  | 66651.31  |
| BBN 2  | 97565  | 6.42                   | 0.76   | 764.15  | BDNO 2 | 1453936 | 528.05                 | 62.82  | 62819.42  |
| BBN 3  | 116154 | 7.65                   | 0.91   | 909.74  | BDNO 3 | 1452681 | 527.60                 | 62.77  | 62765.19  |
| BBO 1  | 112315 | 7.39                   | 0.88   | 879.67  | BEK 1  | 1190933 | 432.53                 | 51.46  | 51455.99  |
| BBO 2  | 117609 | 7.74                   | 0.92   | 921.14  | BEK 2  | 1598147 | 580.43                 | 69.05  | 69050.26  |
| BBO 3  | 84523  | 5.56                   | 0.66   | 662.00  | BEK 3  | 1371567 | 498.14                 | 59.26  | 59260.54  |
| BBNO 1 | 129782 | 8.54                   | 1.02   | 1016.48   | BEN 1  | 1429200 | 519.07                 | 61.75  | 61750.66  |
| BBNO 2 | 151110 | 9.95                   | 1.18   | 1183.52   | BEN 2  | 1342281 | 487.50                 | 58.00  | 57995.20  |
| BBNO 3 | 116705 | 7.68                   | 0.91   | 914.06  | BEN 3  | 1443064 | 524.10                 | 62.35  | 62349.68  |
| BCK 1  | 150209 | 9.89                   | 1.18   | 1176.46   | BEO 1  | 1392296 | 505.66                 | 60.16  | 60156.17  |
| BCK 2  | 169555 | 11.16                  | 1.33   | 1327.99   | BEO 2  | 1329325 | 482.79                 | 57.44  | 57435.42  |
| BCK 3  | 136583 | 8.99                   | 1.07   | 1069.74   | BEO 3  | 1304402 | 473.74                 | 56.36  | 56358.58  |
| BCN 1  | 139534 | 9.19                   | 1.09   | 1092.86   | BENO 1 | 962323  | 349.50                 | 41.58  | 41578.56  |
| BCN 2  | 90508  | 5.96                   | 0.71   | 708.88  | BENO 2 | 621362  | 225.67                 | 26.85  | 26846.85  |
| BCN 3  |        |                        |  |   | BENO 3 | 896812  | 325.71                 | 38.75  | 38748.07  |



Bethlehem - day 10

5% moisture

| Sample | Area   | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) | Sample | Area    | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) |
|--------|--------|------------------------|--|---|--------|---------|------------------------|--|---|
| BAK 1  | 139765 | 9.20                   | 1.09   | 1094.67   | BCO 1  | 138936  | 9.15                   | 1.09   | 1088.17   |
| BAK 2  | 131475 | 8.66                   | 1.03   | 1029.74   | BCO 2  | 140216  | 9.23                   | 1.10   | 1098.20   |
| BAK 3  | 148387 | 9.77                   | 1.16   | 1162.19   | BCO 3  | 146455  | 9.64                   | 1.15   | 1147.06   |
| BAN 1  | 149410 | 9.84                   | 1.17   | 1170.21   | BCNO 1 | 165427  | 10.89                  | 1.30   | 1295.66   |
| BAN 2  | 141702 | 9.33                   | 1.11   | 1109.84   | BCNO 2 | 140758  | 9.27                   | 1.10   | 1102.44   |
| BAN 3  | 150502 | 9.91                   | 1.18   | 1178.76   | BCNO 3 | 143581  | 9.45                   | 1.12   | 1124.55   |
| BAO 1  | 102582 | 6.75                   | 0.80   | 803.44  | BDK 1  | 1345408 | 88.58                  | 10.54  | 10537.49  |
| BAO 2  | 104765 | 6.90                   | 0.82   | 820.54  | BDK 2  | 1118238 | 73.62                  | 8.76   | 8758.25   |
| BAO 3  | 132513 | 8.72                   | 1.04   | 1037.87   | BDK 3  | 1016794 | 66.94                  | 7.96   | 7963.72   |
| BANO 1 | 180600 | 11.89                  | 1.41   | 1414.49   | BDN 1  | 1305861 | 85.97                  | 10.23  | 10227.75  |
| BANO 2 | 185314 | 12.20                  | 1.45   | 1451.41   | BDN 2  | 1094591 | 72.06                  | 8.57   | 8573.04   |
| BANO 3 |        | 0.00                   |  |   | BDN 3  | 1159600 | 76.34                  | 9.08   | 9082.20   |
| BBK 1  | 177905 | 11.71                  | 1.39   | 1393.39   | BDO 1  | 1110107 | 73.09                  | 8.69   | 8694.57   |
| BBK 2  | 156584 | 10.31                  | 1.23   | 1226.40   | BDO 2  | 882585  | 58.11                  | 6.91   | 6912.57   |
| BBK 3  | 165146 | 10.87                  | 1.29   | 1293.45   | BDO 3  | 1102336 | 72.57                  | 8.63   | 8633.70   |
| BBN 1  | 93304  | 6.14                   | 0.73   | 730.77  | BDNO 1 | 689406  | 45.39                  | 5.40   | 5399.56   |
| BBN 2  | 149381 | 9.83                   | 1.17   | 1169.98   | BDNO 2 | 810441  | 53.36                  | 6.35   | 6347.53   |
| BBN 3  | 107124 | 7.05                   | 0.84   | 839.02  | BDNO 3 | 589133  | 38.79                  | 4.61   | 4614.20   |
| BBO 1  | 130012 | 8.56                   | 1.02   | 1018.28   | BEK 1  | 1028483 | 67.71                  | 8.06   | 8055.27   |
| BBO 2  | 168391 | 11.09                  | 1.32   | 1318.87   | BEK 2  | 754387  | 49.67                  | 5.91   | 5908.50   |
| BBO 3  | 152636 | 10.05                  | 1.20   | 1195.47   | BEK 3  | 877725  | 57.79                  | 6.87   | 6874.51   |
| BBNO 1 | 180930 | 11.91                  | 1.42   | 1417.08   | BEN 1  | 1003832 | 66.09                  | 7.86   | 7862.20   |
| BBNO 2 | 138816 | 9.14                   | 1.09   | 1087.23   | BEN 2  | 943783  | 62.14                  | 7.39   | 7391.88   |
| BBNO 3 | 133452 | 8.79                   | 1.05   | 1045.22   | BEN 3  | 943408  | 62.11                  | 7.39   | 7388.95   |
| BCK 1  | 101881 | 6.71                   | 0.80   | 797.95  | BEO 1  | 800587  | 52.71                  | 6.27   | 6270.35   |
| BCK 2  | 77674  | 5.11                   | 0.61   | 608.36  | BEO 2  | 801294  | 52.75                  | 6.28   | 6275.88   |
| BCK 3  | 100391 | 6.61                   | 0.79   | 786.28  | BEO 3  | 894303  | 58.88                  | 7.00   | 7004.35   |
| BCN 1  | 85619  | 5.64                   | 0.67   | 670.58  | BENO 1 | 879151  | 57.88                  | 6.89   | 6885.67   |
| BCN 2  | 73532  | 4.84                   | 0.58   | 575.92  | BENO 2 | 896187  | 59.00                  | 7.02   | 7019.10   |
| BCN 3  | 45328  | 2.98                   | 0.36   | 355.02  | BENO 3 | 835883  | 55.03                  | 6.55   | 6546.79   |

## Bethlehem - day 10

15% moisture

| Sample | Area   | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) | Sample | Area    | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) |
|--------|--------|------------------------|--|---|--------|---------|------------------------|--|---|
| BAK 1  | 192575 | 12.68                  | 1.51   | 1508.28   | BCO 1  | 150784  | 9.93                   | 1.18   | 1180.97   |
| BAK 2  | 141872 | 9.34                   | 1.11   | 1111.17   | BCO 2  | 151753  | 9.99                   | 1.19   | 1188.56   |
| BAK 3  | 185190 | 12.19                  | 1.45   | 1450.44   | BCO 3  | 132205  | 8.70                   | 1.04   | 1035.45   |
| BAN 1  | 157759 | 10.39                  | 1.24   | 1235.60   | BCNO 1 | 130110  | 8.57                   | 1.02   | 1019.05   |
| BAN 2  | 164345 | 10.82                  | 1.29   | 1287.18   | BCNO 2 | 177128  | 11.66                  | 1.39   | 1387.30   |
| BAN 3  |        | 0.00                   |  |   | BCNO 3 | 132792  | 8.74                   | 1.04   | 1040.05   |
| BAO 1  | 149608 | 9.85                   | 1.17   | 1171.76   | BDK 1  | 919825  | 60.56                  | 7.20   | 7204.24   |
| BAO 2  | 153645 | 10.12                  | 1.20   | 1203.38   | BDK 2  | 994837  | 65.50                  | 7.79   | 7791.75   |
| BAO 3  | 152577 | 10.05                  | 1.20   | 1195.01   | BDK 3  | 1094641 | 72.07                  | 8.57   | 8573.43   |
| BANO 1 | 181745 | 11.97                  | 1.42   | 1423.46   | BDN 1  | 1148394 | 75.61                  | 8.99   | 8994.44   |
| BANO 2 | 181692 | 11.96                  | 1.42   | 1423.05   | BDN 2  | 1095657 | 72.13                  | 8.58   | 8581.39   |
| BANO 3 | 180453 | 11.88                  | 1.41   | 1413.34   | BDN 3  | 1227942 | 80.84                  | 9.62   | 9617.47   |
| BBK 1  | 58938  | 3.88                   | 0.46   | 461.61  | BDO 1  | 1124709 | 74.05                  | 8.81   | 8808.93   |
| BBK 2  | 24956  | 1.64                   | 0.20   | 195.46  | BDO 2  | 1069000 | 70.38                  | 8.37   | 8372.61   |
| BBK 3  | 41619  | 2.74                   | 0.33   | 325.97  | BDO 3  | 1101703 | 72.53                  | 8.63   | 8628.74   |
| BBN 1  | 192786 | 12.69                  | 1.51   | 1509.94   | BDNO 1 | 938985  | 61.82                  | 7.35   | 7354.31   |
| BBN 2  | 162180 | 10.68                  | 1.27   | 1270.22   | BDNO 2 | 1199147 | 78.95                  | 9.39   | 9391.94   |
| BBN 3  | 189431 | 12.47                  | 1.48   | 1483.66   | BDNO 3 | 1062248 | 69.93                  | 8.32   | 8319.72   |
| BBO 1  | 134663 | 8.87                   | 1.05   | 1054.71   | BEK 1  | 803426  | 52.89                  | 6.29   | 6292.58   |
| BBO 2  | 117894 | 7.76                   | 0.92   | 923.37  | BEK 2  | 1092625 | 71.93                  | 8.56   | 8557.64   |
| BBO 3  | 115992 | 7.64                   | 0.91   | 908.47  | BEK 3  | 825239  | 54.33                  | 6.46   | 6463.43   |
| BBNO 1 | 103400 | 6.81                   | 0.81   | 809.85  | BEN 1  | 933199  | 61.44                  | 7.31   | 7308.99   |
| BBNO 2 | 111930 | 7.37                   | 0.88   | 876.66  | BEN 2  | 1005677 | 66.21                  | 7.88   | 7876.65   |
| BBNO 3 | 126774 | 8.35                   | 0.99   | 992.92  | BEN 3  | 758603  | 49.94                  | 5.94   | 5941.52   |
| BCK 1  |        |                        |  |   | BEO 1  | 826231  | 54.40                  | 6.47   | 6471.20   |
| BCK 2  | 100391 | 6.61                   | 0.79   | 786.28  | BEO 2  | 865491  | 56.98                  | 6.78   | 6778.69   |
| BCK 3  | 92416  | 6.08                   | 0.72   | 723.82  | BEO 3  | 869835  | 57.27                  | 6.81   | 6812.71   |
| BCN 1  | 95737  | 6.30                   | 0.75   | 749.83  | BENO 1 | 844492  | 55.60                  | 6.61   | 6614.22   |
| BCN 2  | 107000 | 7.04                   | 0.84   | 838.04  | BENO 2 | 946625  | 62.32                  | 7.41   | 7414.14   |
| BCN 3  | 94143  | 6.20                   | 0.74   | 737.35  | BENO 3 | 855555  | 56.33                  | 6.70   | 6700.87   |

## Pretoria - day 1

## 5% moisture

| Sample | Area   | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) | Sample | Area   | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) |
|--------|--------|------------------------|--|---|--------|--------|------------------------|--|---|
| PAK 1  | 294680 | 9.39                   | 1.12   | 1116.90   | PCO 1  | 207032 | 6.60                   | 0.78   | 784.70  |
| PAK 2  | 208122 | 6.63                   | 0.79   | 788.83  | PCO 2  | 296545 | 9.45                   | 1.12   | 1123.97   |
| PAK 3  | 255723 | 8.15                   | 0.97   | 969.25  | PCO 3  | 242041 | 7.71                   | 0.92   | 917.39  |
| PAN 1  | 171404 | 5.46                   | 0.65   | 649.66  | PCNO 1 | 183289 | 5.84                   | 0.69   | 694.71  |
| PAN 2  | 185840 | 5.92                   | 0.70   | 704.38  | PCNO 2 | 277142 | 8.83                   | 1.05   | 1050.43   |
| PAN 3  | 218867 | 6.97                   | 0.83   | 829.56  | PCNO 3 | 179734 | 5.73                   | 0.68   | 681.23  |
| PAO 1  | 254031 | 8.09                   | 0.96   | 962.84  | PDK 1  |        |                        |  |   |
| PAO 2  | 225468 | 7.18                   | 0.85   | 854.58  | PDK 2  | 512471 | 16.33                  | 1.94   | 1942.38   |
| PAO 3  | 267354 | 8.52                   | 1.01   | 1013.33   | PDK 3  | 505717 | 16.11                  | 1.92   | 1916.78   |
| PANO 1 | 213452 | 6.80                   | 0.81   | 809.03  | PDN 1  | 157594 | 5.02                   | 0.60   | 597.32  |
| PANO 2 | 279373 | 8.90                   | 1.06   | 1058.89   | PDN 2  | 305975 | 9.75                   | 1.16   | 1159.72   |
| PANO 3 | 268781 | 8.56                   | 1.02   | 1018.74   | PDN 3  | 349663 | 11.14                  | 1.33   | 1325.30   |
| PBK 1  | 107350 | 3.42                   | 0.41   | 406.88  | PDO 1  | 459797 | 14.65                  | 1.74   | 1742.74   |
| PBK 2  | 103338 | 3.29                   | 0.39   | 391.67  | PDO 2  | 438902 | 13.98                  | 1.66   | 1663.54   |
| PBK 3  | 104091 | 3.32                   | 0.39   | 394.53  | PDO 3  | 346361 | 11.04                  | 1.31   | 1312.79   |
| PBN 1  | 114805 | 3.66                   | 0.44   | 435.14  | PDNO 1 | 349218 | 11.13                  | 1.32   | 1323.62   |
| PBN 2  | 105879 | 3.37                   | 0.40   | 401.31  | PDNO 2 | 387944 | 12.36                  | 1.47   | 1470.40   |
| PBN 3  | 105951 | 3.38                   | 0.40   | 401.58  | PDNO 3 | 393773 | 12.55                  | 1.49   | 1492.49   |
| PBO 1  | 102888 | 3.28                   | 0.39   | 389.97  | PEK 1  | 397809 | 12.67                  | 1.51   | 1507.79   |
| PBO 2  | 132718 | 4.23                   | 0.50   | 503.03  | PEK 2  | 284213 | 9.06                   | 1.08   | 1077.23   |
| PBO 3  | 101294 | 3.23                   | 0.38   | 383.93  | PEK 3  | 308368 | 9.82                   | 1.17   | 1168.79   |
| PBNO 1 | 103583 | 3.30                   | 0.39   | 392.60  | PEN 1  | 396157 | 12.62                  | 1.50   | 1501.53   |
| PBNO 2 | 103121 | 3.29                   | 0.39   | 390.85  | PEN 2  | 350658 | 11.17                  | 1.33   | 1329.07   |
| PBNO 3 | 105759 | 3.37                   | 0.40   | 400.85  | PEN 3  | 277972 | 8.86                   | 1.05   | 1053.58   |
| PCK 1  | 101525 | 3.23                   | 0.38   | 384.80  | PEO 1  | 359848 | 11.46                  | 1.36   | 1363.91   |
| PCK 2  | 121433 | 3.87                   | 0.46   | 460.26  | PEO 2  | 227771 | 7.26                   | 0.86   | 863.30  |
| PCK 3  | 101287 | 3.23                   | 0.38   | 383.90  | PEO 3  | 308513 | 9.83                   | 1.17   | 1169.33   |
| PCN 1  | 157677 | 5.02                   | 0.60   | 597.63  | PENO 1 | 45631  | 1.45                   | 0.17   | 172.95  |
| PCN 2  | 126236 | 4.02                   | 0.48   | 478.46  | PENO 2 | 30421  | 0.97                   | 0.12   | 115.30  |
| PCN 3  | 135675 | 4.32                   | 0.51   | 514.24  | PENO 3 | 52394  | 1.67                   | 0.20   | 198.59  |

Pretoria - day 1  
 15% moisture

| Sample | Area   | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) | Sample | Area   | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) |
|--------|--------|------------------------|--|---|--------|--------|------------------------|--|---|
| PAK 1  | 237382 | 7.56                   | 0.90   | 899.73  | PCO 1  | 200238 | 6.38                   | 0.76   | 758.95  |
| PAK 2  | 224780 | 7.16                   | 0.85   | 851.97  | PCO 2  | 219986 | 7.01                   | 0.83   | 833.80  |
| PAK 3  | 235527 | 7.50                   | 0.89   | 892.70  | PCO 3  | 244986 | 7.81                   | 0.93   | 928.55  |
| PAN 1  | 192373 | 6.13                   | 0.73   | 729.14  | PCNO 1 | 272680 | 8.69                   | 1.03   | 1033.52   |
| PAN 2  | 222844 | 7.10                   | 0.84   | 844.63  | PCNO 2 | 195588 | 6.23                   | 0.74   | 741.32  |
| PAN 3  | 206878 | 6.59                   | 0.78   | 784.11  | PCNO 3 | 204843 | 6.53                   | 0.78   | 776.40  |
| PAO 1  | 379258 | 12.08                  | 1.44   | 1437.47   | PDK 1  | 477254 | 15.21                  | 1.81   | 1808.90   |
| PAO 2  | 424600 | 13.53                  | 1.61   | 1609.33   | PDK 2  | 429593 | 13.69                  | 1.63   | 1628.26   |
| PAO 3  | 406328 | 12.95                  | 1.54   | 1540.08   | PDK 3  | 349666 | 11.14                  | 1.33   | 1325.31   |
| PANO 1 | 416786 | 13.28                  | 1.58   | 1579.71   | PDN 1  | 412012 | 13.13                  | 1.56   | 1561.62   |
| PANO 2 | 418723 | 13.34                  | 1.59   | 1587.06   | PDN 2  | 421206 | 13.42                  | 1.60   | 1596.47   |
| PANO 3 | 308673 | 9.83                   | 1.17   | 1169.94   | PDN 3  | 400764 | 12.77                  | 1.52   | 1518.99   |
| PBK 1  | 130167 | 4.15                   | 0.49   | 493.36  | PDO 1  | 263577 | 8.40                   | 1.00   | 999.02  |
| PBK 2  | 101192 | 3.22                   | 0.38   | 383.54  | PDO 2  | 272812 | 8.69                   | 1.03   | 1034.02   |
| PBK 3  | 125133 | 3.99                   | 0.47   | 474.28  | PDO 3  | 359800 | 11.46                  | 1.36   | 1363.72   |
| PBN 1  | 108891 | 3.47                   | 0.41   | 412.72  | PDNO 1 | 251025 | 8.00                   | 0.95   | 951.44  |
| PBN 2  | 102786 | 3.27                   | 0.39   | 389.58  | PDNO 2 | 257841 | 8.21                   | 0.98   | 977.28  |
| PBN 3  | 106605 | 3.40                   | 0.40   | 404.06  | PDNO 3 | 211397 | 6.74                   | 0.80   | 801.24  |
| PBO 1  | 106851 | 3.40                   | 0.40   | 404.99  | PEK 1  | 512075 | 16.31                  | 1.94   | 1940.88   |
| PBO 2  | 109561 | 3.49                   | 0.42   | 415.26  | PEK 2  | 635543 | 20.25                  | 2.41   | 2408.85   |
| PBO 3  | 136205 | 4.34                   | 0.52   | 516.25  | PEK 3  | 600609 | 19.14                  | 2.28   | 2276.45   |
| PBNO 1 | 162010 | 5.16                   | 0.61   | 614.05  | PEN 1  | 454214 | 14.47                  | 1.72   | 1721.57   |
| PBNO 2 | 159971 | 5.10                   | 0.61   | 606.33  | PEN 2  | 320265 | 10.20                  | 1.21   | 1213.88   |
| PBNO 3 | 163006 | 5.19                   | 0.62   | 617.83  | PEN 3  | 404430 | 12.89                  | 1.53   | 1532.88   |
| PCK 1  | 133922 | 4.27                   | 0.51   | 507.59  | PEO 1  | 710010 | 22.62                  | 2.69   | 2691.10   |
| PCK 2  | 170203 | 5.42                   | 0.65   | 645.11  | PEO 2  | 724019 | 23.07                  | 2.74   | 2744.20   |
| PCK 3  | 175468 | 5.59                   | 0.67   | 665.06  | PEO 3  | 736652 | 23.47                  | 2.79   | 2792.08   |
| PCN 1  | 153236 | 4.88                   | 0.58   | 580.80  | PENO 1 | 241832 | 7.70                   | 0.92   | 916.60  |
| PCN 2  | 167524 | 5.34                   | 0.63   | 634.95  | PENO 2 | 340518 | 10.85                  | 1.29   | 1290.64   |
| PCN 3  | 143756 | 4.58                   | 0.54   | 544.87  | PENO 3 | 208446 | 6.64                   | 0.79   | 790.06  |

Pretoria - day 4  
5% moisture

| Sample | Area   | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) | Sample | Area   | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) |
|--------|--------|------------------------|--|---|--------|--------|------------------------|--|---|
| PAK 1  | 152094 | 4.85                   | 0.58   | 576.47  | PCO 1  | 137534 | 4.38                   | 0.52   | 521.29  |
| PAK 2  | 140321 | 4.47                   | 0.53   | 531.85  | PCO 2  | 155663 | 4.96                   | 0.59   | 590.00  |
| PAK 3  | 139569 | 4.45                   | 0.53   | 529.00  | PCO 3  | 135023 | 4.30                   | 0.51   | 511.77  |
| PAN 1  | 154542 | 4.92                   | 0.59   | 585.75  | PCNO 1 | 208832 | 6.65                   | 0.79   | 791.52  |
| PAN 2  | 151998 | 4.84                   | 0.58   | 576.11  | PCNO 2 | 170059 | 5.42                   | 0.64   | 644.56  |
| PAN 3  | 142467 | 4.54                   | 0.54   | 539.98  | PCNO 3 | 162110 | 2.18                   | 0.26   | 259.62  |
| PAO 1  | 190208 | 6.06                   | 0.72   | 720.93  | PDK 1  | 241015 | 7.68                   | 0.91   | 913.50  |
| PAO 2  | 171894 | 5.48                   | 0.65   | 651.52  | PDK 2  | 349141 | 11.12                  | 1.32   | 1323.32   |
| PAO 3  | 164022 | 5.23                   | 0.62   | 621.68  | PDK 3  | 380834 | 12.13                  | 1.44   | 1443.45   |
| PANO 1 | 151579 | 4.83                   | 0.57   | 574.52  | PDN 1  | 395045 | 12.59                  | 1.50   | 1497.31   |
| PANO 2 | 164406 | 5.24                   | 0.62   | 623.14  | PDN 2  | 339328 | 10.81                  | 1.29   | 1286.13   |
| PANO 3 | 195520 | 6.23                   | 0.74   | 741.07  | PDN 3  | 398458 | 12.69                  | 1.51   | 1510.25   |
| PBK 1  | 96943  | 3.09                   | 0.37   | 367.44  | PDO 1  | 269524 | 8.59                   | 1.02   | 1021.56   |
| PBK 2  | 127592 | 4.07                   | 0.48   | 483.60  | PDO 2  | 320578 | 10.21                  | 1.22   | 1215.06   |
| PBK 3  | 113888 | 3.63                   | 0.43   | 431.66  | PDO 3  | 309530 | 9.86                   | 1.17   | 1173.19   |
| PBN 1  | 124066 | 3.95                   | 0.47   | 470.24  | PDNO 1 | 331226 | 10.55                  | 1.26   | 1255.42   |
| PBN 2  | 119698 | 3.81                   | 0.45   | 453.68  | PDNO 2 | 426229 | 13.58                  | 1.62   | 1615.51   |
| PBN 3  | 108227 | 3.45                   | 0.41   | 410.21  | PDNO 3 | 319338 | 10.17                  | 1.21   | 1210.36   |
| PBO 1  | 190049 | 6.05                   | 0.72   | 720.33  | PEK 1  | 241723 | 7.70                   | 0.92   | 916.19  |
| PBO 2  | 113578 | 3.62                   | 0.43   | 430.49  | PEK 2  | 242374 | 7.72                   | 0.92   | 918.65  |
| PBO 3  | 97847  | 3.12                   | 0.37   | 370.86  | PEK 3  | 238282 | 7.59                   | 0.90   | 903.14  |
| PBNO 1 | 108132 | 3.45                   | 0.41   | 409.84  | PEN 1  | 451574 | 14.39                  | 1.71   | 1711.57   |
| PBNO 2 | 101421 | 3.23                   | 0.38   | 384.41  | PEN 2  | 384796 | 12.26                  | 1.46   | 1458.46   |
| PBNO 3 | 90878  | 2.90                   | 0.34   | 344.45  | PEN 3  | 335424 | 10.69                  | 1.27   | 1271.33   |
| PCK 1  | 114487 | 3.65                   | 0.43   | 433.93  | PEO 1  | 318935 | 10.16                  | 1.21   | 1208.84   |
| PCK 2  | 120542 | 3.84                   | 0.46   | 456.88  | PEO 2  | 487934 | 15.55                  | 1.85   | 1849.38   |
| PCK 3  | 105316 | 3.36                   | 0.40   | 399.17  | PEO 3  | 413326 | 13.17                  | 1.57   | 1566.60   |
| PCN 1  | 158941 | 5.06                   | 0.60   | 602.42  | PENO 1 | 276695 | 8.82                   | 1.05   | 1048.74   |
| PCN 2  | 180171 | 5.74                   | 0.68   | 682.89  | PENO 2 | 441554 | 14.07                  | 1.67   | 1673.59   |
| PCN 3  | 172423 | 5.49                   | 0.65   | 653.52  | PENO 3 | 435597 | 13.88                  | 1.65   | 1651.01   |

Pretoria - day 4  
 15% moisture

| Sample | Area   | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) | Sample | Area   | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) |
|--------|--------|------------------------|--|---|--------|--------|------------------------|--|---|
| PAK 1  | 170163 | 5.42                   | 0.64   | 644.96  | PCO 1  | 167185 | 5.33                   | 0.63   | 633.67  |
| PAK 2  | 167564 | 5.34                   | 0.64   | 635.11  | PCO 2  | 197619 | 6.30                   | 0.75   | 749.02  |
| PAK 3  | 165202 | 5.26                   | 0.63   | 626.15  | PCO 3  | 185100 | 5.90                   | 0.70   | 701.57  |
| PAN 1  | 168220 | 5.36                   | 0.64   | 637.59  | PCNO 1 | 171323 | 5.46                   | 0.65   | 649.35  |
| PAN 2  | 217807 | 6.94                   | 0.83   | 825.54  | PCNO 2 | 187639 | 5.98                   | 0.71   | 711.19  |
| PAN 3  | 182909 | 5.83                   | 0.69   | 693.27  | PCNO 3 | 194476 | 6.20                   | 0.74   | 737.11  |
| PAO 1  | 228841 | 7.29                   | 0.87   | 867.36  | PDK 1  | 416955 | 13.28                  | 1.58   | 1580.35   |
| PAO 2  | 267862 | 8.53                   | 1.02   | 1015.26   | PDK 2  | 390327 | 12.44                  | 1.48   | 1479.43   |
| PAO 3  | 247222 | 7.88                   | 0.94   | 937.03  | PDK 3  | 407608 | 12.99                  | 1.54   | 1544.93   |
| PANO 1 | 244805 | 7.80                   | 0.93   | 927.87  | PDN 1  | 581898 | 18.54                  | 2.21   | 2205.53   |
| PANO 2 | 240364 | 7.66                   | 0.91   | 911.03  | PDN 2  | 318104 | 10.13                  | 1.21   | 1205.69   |
| PANO 3 | 247440 | 7.88                   | 0.94   | 937.85  | PDN 3  | 345991 | 11.02                  | 1.31   | 1311.38   |
| PBK 1  | 126900 | 4.04                   | 0.48   | 480.98  | PDO 1  | 318538 | 10.15                  | 1.21   | 1207.33   |
| PBK 2  | 136584 | 4.35                   | 0.52   | 517.68  | PDO 2  | 344910 | 10.99                  | 1.31   | 1307.29   |
| PBK 3  | 130028 | 4.14                   | 0.49   | 492.84  | PDO 3  | 367940 | 11.72                  | 1.39   | 1394.58   |
| PBN 1  | 127645 | 4.07                   | 0.48   | 483.80  | PDNO 1 | 432210 | 13.77                  | 1.64   | 1638.17   |
| PBN 2  | 134852 | 4.30                   | 0.51   | 511.12  | PDNO 2 | 431342 | 13.74                  | 1.63   | 1634.88   |
| PBN 3  | 140739 | 4.48                   | 0.53   | 533.43  | PDNO 3 | 379768 | 12.10                  | 1.44   | 1439.41   |
| PBO 1  | 183496 | 5.85                   | 0.70   | 695.49  | PEK 1  | 323440 | 10.30                  | 1.23   | 1225.91   |
| PBO 2  | 262474 | 8.36                   | 0.99   | 994.84  | PEK 2  | 387964 | 12.36                  | 1.47   | 1470.47   |
| PBO 3  | 165301 | 5.27                   | 0.63   | 626.53  | PEK 3  | 297888 | 9.49                   | 1.13   | 1129.06   |
| PBNO 1 | 163461 | 5.21                   | 0.62   | 619.55  | PEN 1  | 284149 | 9.05                   | 1.08   | 1076.99   |
| PBNO 2 | 184003 | 5.86                   | 0.70   | 697.41  | PEN 2  | 354064 | 11.28                  | 1.34   | 1341.98   |
| PBNO 3 | 169533 | 5.40                   | 0.64   | 642.57  | PEN 3  | 253519 | 8.08                   | 0.96   | 960.89  |
| PCK 1  | 139863 | 4.46                   | 0.53   | 530.11  | PEO 1  | 548881 | 17.49                  | 2.08   | 2080.38   |
| PCK 2  | 182199 | 5.80                   | 0.69   | 690.58  | PEO 2  | 451580 | 14.39                  | 1.71   | 1711.59   |
| PCK 3  | 163620 | 5.21                   | 0.62   | 620.16  | PEO 3  | 474065 | 15.10                  | 1.80   | 1796.81   |
| PCN 1  | 202707 | 6.46                   | 0.77   | 768.31  | PENO 1 | 571623 | 18.21                  | 2.17   | 2166.58   |
| PCN 2  | 209082 | 6.66                   | 0.79   | 792.47  | PENO 2 | 510757 | 16.27                  | 1.94   | 1935.89   |
| PCN 3  | 206816 | 6.59                   | 0.78   | 783.88  | PENO 3 | 544640 | 17.35                  | 2.06   | 2064.31   |



Pretoria - day 10

5% moisture

| Sample | Area   | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) | Sample | Area    | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) |
|--------|--------|------------------------|--|---|--------|---------|------------------------|--|---|
| PAK 1  | 229086 | 7.30                   | 0.87   | 868.29  | PCO 1  | 123019  | 3.92                   | 0.47   | 466.27  |
| PAK 2  | 204684 | 6.52                   | 0.78   | 775.80  | PCO 2  | 188154  | 5.99                   | 0.71   | 713.15  |
| PAK 3  | 223026 | 7.11                   | 0.85   | 845.32  | PCO 3  | 165052  | 5.26                   | 0.63   | 625.58  |
| PAN 1  | 186074 | 5.93                   | 0.71   | 705.26  | PCNO 1 | 126696  | 4.04                   | 0.48   | 480.21  |
| PAN 2  | 215359 | 6.86                   | 0.82   | 816.26  | PCNO 2 | 157753  | 5.03                   | 0.60   | 597.92  |
| PAN 3  | 221352 | 7.05                   | 0.84   | 838.97  | PCNO 3 | 159547  | 5.08                   | 0.60   | 604.72  |
| PAO 1  | 171144 | 5.45                   | 0.65   | 648.67  | PDK 1  | 1241100 | 39.54                  | 4.70   | 4704.05   |
| PAO 2  | 168868 | 5.38                   | 0.64   | 640.05  | PDK 2  | 1477959 | 47.09                  | 5.60   | 5601.80   |
| PAO 3  | 168278 | 5.36                   | 0.64   | 637.81  | PDK 3  | 1461078 | 46.55                  | 5.54   | 5537.82   |
| PANO 1 | 166857 | 5.32                   | 0.63   | 632.43  | PDN 1  | 1472952 | 46.93                  | 5.58   | 5582.82   |
| PANO 2 | 188127 | 5.99                   | 0.71   | 713.04  | PDN 2  | 1311567 | 41.79                  | 4.97   | 4971.14   |
| PANO 3 | 190818 | 6.08                   | 0.72   | 723.24  | PDN 3  | 1330071 | 42.38                  | 5.04   | 5041.27   |
| PBK 1  | 140096 | 4.46                   | 0.53   | 531.00  | PDO 1  | 1414754 | 45.07                  | 5.36   | 5362.24   |
| PBK 2  | 135698 | 4.32                   | 0.51   | 514.33  | PDO 2  | 1453764 | 46.32                  | 5.51   | 5510.10   |
| PBK 3  | 131553 | 4.19                   | 0.50   | 498.62  | PDO 3  | 1393833 | 44.41                  | 5.28   | 5282.94   |
| PBN 1  | 119027 | 3.79                   | 0.45   | 451.14  | PDNO 1 | 1495127 | 47.63                  | 5.67   | 5666.87   |
| PBN 2  | 126960 | 4.04                   | 0.48   | 481.21  | PDNO 2 | 1487888 | 47.40                  | 5.64   | 5639.43   |
| PBN 3  | 120586 | 3.84                   | 0.46   | 457.05  | PDNO 3 | 1348897 | 42.98                  | 5.11   | 5112.63   |
| PBO 1  | 123380 | 3.93                   | 0.47   | 467.64  | PEK 1  | 52585   | 1.68                   | 0.20   | 199.31  |
| PBO 2  | 122559 | 3.90                   | 0.46   | 464.53  | PEK 2  | 1199422 | 38.21                  | 4.55   | 4546.08   |
| PBO 3  | 145524 | 4.64                   | 0.55   | 551.57  | PEK 3  | 59978   | 1.91                   | 0.23   | 227.33  |
| PBNO 1 | 135278 | 4.31                   | 0.51   | 512.73  | PEN 1  | 1327248 | 42.29                  | 5.03   | 5030.57   |
| PBNO 2 | 129568 | 4.13                   | 0.49   | 491.09  | PEN 2  | 55548   | 1.77                   | 0.21   | 210.54  |
| PBNO 3 | 93686  | 2.98                   | 0.36   | 355.09  | PEN 3  | 1234956 | 39.35                  | 4.68   | 4680.76   |
| PCK 1  | 137395 | 4.38                   | 0.52   | 520.76  | PEO 1  | 1381780 | 44.02                  | 5.24   | 5237.26   |
| PCK 2  | 140852 | 4.49                   | 0.53   | 533.86  | PEO 2  | 50839   | 1.62                   | 0.19   | 192.69  |
| PCK 3  | 132047 | 4.21                   | 0.50   | 500.49  | PEO 3  | 54331   | 1.73                   | 0.21   | 205.93  |
| PCN 1  | 161943 | 5.16                   | 0.61   | 613.80  | PENO 1 | 1238877 | 39.47                  | 4.70   | 4695.63   |
| PCN 2  | 156144 | 4.97                   | 0.59   | 591.82  | PENO 2 | 51040   | 1.63                   | 0.19   | 193.45  |
| PCN 3  | 175252 | 5.58                   | 0.66   | 664.25  | PENO 3 | 57520   | 1.83                   | 0.22   | 218.01  |

Pretoria - day 10  
15% moisture

| Sample | Area   | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) | Sample | Area   | ng N <sub>2</sub> O/ml | N <sub>2</sub> O-N (ng g <sup>-1</sup> h <sup>-1</sup> ) | N <sub>2</sub> O-N (ng kg <sup>-1</sup> h <sup>-1</sup> ) |
|--------|--------|------------------------|--|---|--------|--------|------------------------|--|---|
| PAK 1  | 138199 | 4.40                   | 0.52   | 523.81  | PCO 1  | 166879 | 5.32                   | 0.63   | 632.51  |
| PAK 2  | 143367 | 4.57                   | 0.54   | 543.39  | PCO 2  | 156118 | 4.97                   | 0.59   | 591.72  |
| PAK 3  | 142027 | 4.52                   | 0.54   | 538.31  | PCO 3  | 175347 | 5.59                   | 0.66   | 664.61  |
| PAN 1  | 159554 | 5.08                   | 0.60   | 604.75  | PCNO 1 | 199092 | 6.34                   | 0.75   | 754.60  |
| PAN 2  | 153100 | 4.88                   | 0.58   | 580.28  | PCNO 2 | 181440 | 5.78                   | 0.69   | 687.70  |
| PAN 3  | 152960 | 4.87                   | 0.58   | 579.75  | PCNO 3 | 187696 | 5.98                   | 0.71   | 711.41  |
| PAO 1  | 153794 | 4.90                   | 0.58   | 582.91  | PDK 1  | 416955 | 13.28                  | 1.58   | 1580.35   |
| PAO 2  | 177078 | 5.64                   | 0.67   | 671.17  | PDK 2  | 390327 | 12.44                  | 1.48   | 1479.43   |
| PAO 3  | 160516 | 5.11                   | 0.61   | 608.39  | PDK 3  | 407608 | 12.99                  | 1.54   | 1544.93   |
| PANO 1 | 167886 | 5.35                   | 0.64   | 636.33  | PDN 1  | 581898 | 18.54                  | 2.21   | 2205.53   |
| PANO 2 | 154588 | 4.93                   | 0.59   | 585.92  | PDN 2  | 318104 | 10.13                  | 1.21   | 1205.69   |
| PANO 3 | 159578 | 5.08                   | 0.60   | 604.84  | PDN 3  | 345991 | 11.02                  | 1.31   | 1311.38   |
| PBK 1  | 109037 | 3.47                   | 0.41   | 413.28  | PDO 1  | 318538 | 10.15                  | 1.21   | 1207.33   |
| PBK 2  | 117568 | 3.75                   | 0.45   | 445.61  | PDO 2  | 344910 | 10.99                  | 1.31   | 1307.29   |
| PBK 3  | 127313 | 4.06                   | 0.48   | 482.55  | PDO 3  | 367940 | 11.72                  | 1.39   | 1394.58   |
| PBN 1  | 122515 | 3.90                   | 0.46   | 464.36  | PDNO 1 | 432210 | 13.77                  | 1.64   | 1638.17   |
| PBN 2  | 127451 | 4.06                   | 0.48   | 483.07  | PDNO 2 | 431342 | 13.74                  | 1.63   | 1634.88   |
| PBN 3  | 119297 | 3.80                   | 0.45   | 452.16  | PDNO 3 | 379768 | 12.10                  | 1.44   | 1439.41   |
| PBO 1  | 137593 | 4.38                   | 0.52   | 521.51  | PEK 1  | 323440 | 10.30                  | 1.23   | 1225.91   |
| PBO 2  | 134593 | 4.29                   | 0.51   | 510.14  | PEK 2  | 387964 | 12.36                  | 1.47   | 1470.47   |
| PBO 3  | 128129 | 4.08                   | 0.49   | 485.64  | PEK 3  | 297888 | 9.49                   | 1.13   | 1129.06   |
| PBNO 1 | 115603 | 3.68                   | 0.44   | 438.16  | PEN 1  | 284149 | 9.05                   | 1.08   | 1076.99   |
| PBNO 2 | 122514 | 3.90                   | 0.46   | 464.36  | PEN 2  | 354064 | 11.28                  | 1.34   | 1341.98   |
| PBNO 3 | 115865 | 3.69                   | 0.44   | 439.15  | PEN 3  | 253519 | 8.08                   | 0.96   | 960.89  |
| PCK 1  | 166431 | 5.30                   | 0.63   | 630.81  | PEO 1  | 548881 | 17.49                  | 2.08   | 2080.38   |
| PCK 2  | 172597 | 5.50                   | 0.65   | 654.18  | PEO 2  | 451580 | 14.39                  | 1.71   | 1711.59   |
| PCK 3  | 161943 | 5.16                   | 0.61   | 613.80  | PEO 3  | 474065 | 15.10                  | 1.80   | 1796.81   |
| PCN 1  | 198662 | 6.33                   | 0.75   | 752.97  | PENO 1 | 571623 | 18.21                  | 2.17   | 2166.58   |
| PCN 2  | 192146 | 6.12                   | 0.73   | 728.28  | PENO 2 | 510757 | 16.27                  | 1.94   | 1935.89   |
| PCN 3  | 192200 | 6.12                   | 0.73   | 728.48  | PENO 3 | 544640 | 17.35                  | 2.06   | 2064.31   |