STUDIES ON THE NITRIFICATION INHIBITOR,"NITRAPYRIN" [2-CHLORO-6(TRICHLOROMETHYL) PYRIDINE] IN RELATION TO AVAILABILITY AND TRANSFORMATION OF NITROGEN IN SOIL

by

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This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and to the best of my knowledge contains no copy or paraphrase of material previously published or written by any other person except where due reference is made in the text of the thesis.

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ABSTRACT

Nitrification inhibitors have been claimed to offer a means of achieving better control of the delivery of applied fertilizer nitrogen to crops. However, considerable variability in reported usefulness of nitrification inhibitors indicates a need for further research on their effectiveness under different agricultural conditions.

A series of experiments was conducted to examine effects of the nitrification inhibitor nitrapyrin [2-chloro 6-(trichloromethyl) pyridine] on soil N availability and transformation and the influence of certain factors (soil pH, relative mobility, temperature etc.,) on its effectiveness.

Because urea is rapidly becoming most favoured on a world scale attention was focussed on this as a form of fertilizer N. After selecting a suitable soil type a field experiment was set-up to compare the N supplying capacity of urea to that of other common commercial N fertilizers and to evaluate N utilization efficiency of a test crop (barley cv. Triumph). Urea compared unfavourably with ammonium nitrate and calcium nitrate but was better than ammonium sulphate in terms of plant growth and yield on the acid soil used thus warranting improvement practices viz. the use of lime as a soil amendment and/or nitrification inhibitor as a fertilizer amendment.

The effect of liming on certain N transformation processes and on the effectiveness of "nitrapyrin" was studied in the glasshouse and the laboratory. Liming promoted urease activity, nitrification and potential denitrification of soil whereas nitrapyrin significantly reduced nitrification. Liming reduced the effectiveness of nitrification inhibition probably because of faster recovery of nitrifiers in limed soil. The importance of considering the total duration of inhibition in establishing inhibitor effectiveness was underlined. Nitrapyrin also had a marked effect on production of nitrous oxide by denitrification.

There was no visible phytotoxic effects of nitrapyrin nor was there any measurable effect on plant height or top dry matter production of barley during 45 days of growth in the glasshouse. However, liming resulted in better plant growth.

The relative mobility of nitrapyrin compared to urea and ammonium-N may be a critical factor in determining its effectiveness as an inhibitor. In leaching column experiments, it was found that most of the added urea moved with the wetting front while the rest was hydrolysed. Added ammonium-N moved more slowly but at a sufficent speed to effect separation from nitrapyrin. Nitrapyrin scarcely moved at all. Measured effects of temperature, soil moisture and air flow on persistence of nitrapyrin indicated that it is likely to persist longer in cool, moist soils exposed to minimal air flows.

Nitrapyrin application had little or no effect on the growth of barley (cv. Triumph) in a field experiment in which, amongst other treatments, a single application of urea plus nitrapyrin was compared with a split application of the same

amount of urea without nitrapyrin. Higher yields were obtained with split application of urea. Liming enhanced vegetative growth but this was not reflected uniformly in final yield of dry matter or grain.

In conclusion, although control over nitrification was obtained by use of nitrapyrin under glasshouse/laboratory conditions its use could not be justified under field conditions experienced during this study. Field management practices such as liming and the method of nitrapyrin application may affect the performance of the inhibitor.

CHAPTER ONE.

1. General Introduction

Nitrogen is a widely used fertilizer element that is considered as the most important plant nutrient after the three basic elements carbon, hydrogen and oxygen. The common fertilizers that supply nitrogen are urea, ammonium nitrate, ammonium sulphate, anhydrous ammonia and calcium nitrate, of which urea is the most popular in world agriculture. However, not all the nitrogen added to soil is utilized or utilizable by plants. It is said that "no other nutrient requires as much attention and no other brings greater rewards for wise management than nitrogen" (Olson and Kurtz, 1982). Hence, agricultural scientists and others must continue their search for more efficient management techniques for individual soils and crops if available resources are to be optimized .

The work presented in this thesis has been carried out on transformation of urea N added to soil. Emphasis has been placed on the nitrification process since this exclusively converts ammonium into nitrate N. Change in the oxidative state of N during nitrification may significantly affect the soil N budget. For example, when nitrate is formed, it may be easily lost from the soil/plant system by leaching or denitrification whereas positively charged ammonium in most soils may be held by the cation exchange complex (clay minerals and organic matter) restricting its movement. Nitrate leaching is not only agronomically undesirable but may also result in pollution of waterways causing environmental problems. The amount of N available to crops may also be reduced by denitrification. On the other hand, if a large amount of nitrate remains in the soil and is absorbed by plants, it may accumulate in some plants to levels toxic to humans and livestock.

In view of these agronomically and environmentally unfavorable consequences associated with nitrate in soil it is suggested that retaining N as ammonium rather than nitrate, atleast during early stages of plant growth, may be beneficial. Nevertheless, several common agricultural practices may enhance nitrate formation in soil. For example, increased aeration following tillage and increased soil pH following liming of acid soil may stimulate nitrification. In the present study, the effect of liming an acid soil on urea-N transformation and the effectiveness of using a nitrification inhibitor to control nitrifier activity under such circumstances have been investigated. Certain factors that determine the effectiveness of nitrification inhibition have also been studied in order to understand the observed variability in effectiveness between incubation and field experiments.

An introduced high-yielding barley variety (cv. Triumph) was used as the test plant to measure N availability to plants and N use efficiency (NUE). Use of barley as

the test crop in this study may be further justified by the fact that N is a major determinant of malting quality (moderate N content in grain) and forage production (high N content in vegetative parts) and any information to assist regulation of N uptake by the plant should be useful.

The site of field work reported in this thesis was a duplex soil with an acid sandy loam topsoil and poorly drained sandy clay subsoil becoming neutral to alkaline at depth (Holz, pers. comm.) on the University of Tasmania Farm, Cambridge.

The main objectives were:

a. to investigate the effect of liming on Urea N transformation and on the ratio of ammonium to nitrate-N in soil;

b. to study the effectiveness of the nitrification inhibitor, nitrapyrin;

c. to evaluate nitrogen use efficiency, growth and yield of a high yielding barley cultivar as affected by application of nitrification inhibitor and lime;

d. to study some of the factors influencing the effectiveness of nitrapyrin in controlling nitrification;

In pursuit of these objectives, work was conducted as follows:

a. screening of soils to select an acid soil with a significant nitrification potential following urea application to facilitate study of the effect of nitrification inhibitor.

b. comparison of urea with some common nitrogenous fertilizers in terms of N use efficiency and plant growth performance.

c. study of lime and nitrapyrin effects on soil N transformation and ammonium to nitrate N ratio.

d. measurement of crop growth response to lime and/or nitrapyrin application, to detect any significant toxic effects.

e. investigation of factors that affect nitrification inhibitor activity in relation to observed variability in the effectiveness of nitrapyrin.

CHAPTER TWO

2. Literature Review

2.1 Soil N Transformation

A complex network of biochemical reactions, collectively termed "N transformations", determines the forms of N present in soils. Some terms used in this review, to describe these reactions are defined below.

Nitrogen mineralization- Conversion of nitrogen from an organic to an inorganic form as a result of heterotrophic microbial activity.

Ammonification- The processes by which organic nitrogenous compounds are converted enzymatically to ammonium e.g. hydrolysis of urea by urease.

Urea hydrolysis and urease activity- The process by which urea-N enters the soil N transformation cycle and the enzyme activity responsible for that process respectively.

Nitrogen immobilization- The conversion of nitrogen from inorganic to organic forms by microbes.

Nitrogen assimilation/uptake- Utilization of inorganic nitrogen by higher plants.

Nitrification- Biological oxidation of ammonium to nitrite and then to nitrate.

Denitrification - The biological reduction of nitrate and nitrite to gaseous nitrogen either as molecular nitrogen or as an oxide of nitrogen.

Depending on the extent of these reactions, both the composition of soil N and the availability of N to plants will vary. Although a comprehensive review of published work on the individual processes is beyond the scope of this thesis, it is appropriate to emphasize some findings relevant to the present study. Since the present work is concerned mainly with availability of ammonium- and nitrate-N, the nitrification process and the means by which nitrate-N is lost from a soil-plant system, this review is restricted to urea hydrolysis, nitrification, denitrification, nitrate leaching and ammonia volatilization with emphasis on effects of liming/pH and nitrification inhibitors on these processes.

2.1.1 Forms of soil nitrogen and plant availability/ utilization

Forms of nitrogen in soils can be categorized broadly as organic or inorganic. The composition of these two categories of N varies in relation to the history of land use, vegetation, topography, cultural practices, climate, soil parent material, and subsequent pedogenic processes (Haynes, 1986a). Over 90% of the N in the surface layer in terrestrial ecosystems occurs in organic forms (Stevenson, 1982a). The rest exists mainly in about seven different inorganic forms (nitrates, nitrites, ammonia, exchangeable and nonexchangeable ammonium, dinitrogen gas, and nitrous oxide).

2.1.1.1. Organic N: Organic N has been categorized into two main fractions i.e. acid-insoluble N and acid-hydrolysable N. The products of acid hydrolysis of the latter fraction include ammonia-N, amino acid-N, amino sugar-N and "hydrolysable unknown-N" (HUN) (Kowalenko, 1978; Stevenson, 1982a). The relative proportion of these forms of N in agricultural soils is affected by climate, vegetation, and agricultural practices (Sowden et al., 1977) such as tillage, irrigation and liming.

When mineralization takes place organic N declines but the decline is not spread uniformly over all the fractions (Ladd and Paul, 1973). Research on the dynamic nature of organic N in soil indicates that there is an "internal cycling of N" (Stevenson, 1982b) in soil. Two basic features of this cycling are the transfer of nitrogenous metabolites of microorganisms into stable humus forms and their mineralization to inorganic mineral forms that can in turn be utilized by micro-organisms to again form a part of the microbial biomass (immobilization).

Recognition of the simultaneous occurrence of mineralization and immobilization processes has led to the introduction of the term "Mineralization Immobilization Turnover" (MIT) (Campbell, 1978). The magnitude and direction of MIT depends mainly on the ratio of substrate energy (carbon) to nitrogen (C/N) in the organic matter undergoing decomposition. During decomposition carbon is used as the proton donor and N as a cell constituent by heterotrophic microflora. Therefore, the C/N ratio is an approximate indicator of the favoured direction of MIT. If the C/N ratio is high, C is released as Carbon dioxide while N is retained in organic forms and net immobilization results. If the C/N ratio is low, net mineralization occurs. This brief description is an excessively simplified account of a rather complex process.

Organic N in soil is fairly stable but is not inert. It can be considered as a temporary reservoir in which the N is protected for short term from N losses. As immobilized N becomes available in the plant use at some stage via mineralization, the use of the term "immobilization losses" in the same sense as ammonia volatilization and denitrification by Hendrickson et al., (1987) may be inappropriate.

2.1.1.2. Inorganic N: This includes ionic and molecular forms of N in the soil solution and mineral N bonded/sorbed to soil colloids. The main forms of inorganic N include ammonium (exchangeable ammonium, 'fixed' ammonium), ammonia (gaseous and aqueous), nitrite and nitrate (usually in the aqueous form), other oxidized forms of nitrogen (e.g. hydroxylamine, nitric oxide, nitrous oxide and nitrogen dioxide), azides, and molecular N. Ammonium and nitrate are the two agronomically important constituents of the inorganic-N pool.

Ammonium-N and ammonia can be held by soil colloids (clay minerals and organic matter) thereby restricting its movement relative to free-moving nitrate and nitrite in the soil solution. However, as Black and Waring (1976a-c) demonstrated,

some soils (e.g. krasnozems) have enough anion exchange capacity to restrict the movement of nitrate-N as well.

Two types of ammonium binding by clay minerals have been described i.e. exchangeable and nonexchangeable (Nommik, 1965a). Non-exchangeable forms are also described as "fixed" since they are not readily released to soil solution. The following order of decreasing ammonium fixing capacity for different clay minerals has been reported:

vermiculite, illite (may or may not fix ammonium depending on the degree of weathering beidellite, montmorillonite, (does not fix under moist conditions, while kaolinite does not fix ammonium (Stevenson, 1982).

The ammonium-fixing capacity of a soil significantly influences mineral N cycling and availability of N for plants and microbes. Several workers have studied the availability of fixed ammonium to plants (Bower, 1951 and Kowalenko, 1978). Results suggest that soils rich in vermicullite show the lowest availability of fixed N while montmorillonite shows the highest availability amongst the 2:1 clays tested. However, it is interesting to note that Freney (1964), suggested that ammonium fixation in soil was largely a laboratory phenomenon due to the use of extractants containing potassium ions which trap exchangeable ammonium or to the use of reagents that decompose organic nitrogen compounds.

Exchangeable ammonium-N is held at negatively charged sites on soil colloids and is replaceable by other cations (Ca and H). The organic fraction also contributes to the total cation exchange capacity of a soil. The fixation of ammonium or ammonia by organic matter takes place by dissociation of -COOH groups of organic acids and -OH groups of phenolic compounds giving rise to negatively charged sites (Mortland and Wolcott, 1965; Nommik & Vahatras, 1982). Findings also indicate the presence of additional binding forces, e.g. physical sorption of ammonia by coordination involving metal cations such as Cu^{2+} and Fe^{3+}(Mortland, 1966) and by hydrogen bonding (Jensen, 1973). As fixation by adsorption to organic matter usually occurs to a significant degree only in soils containing high levels of organic matter (Nommik and Nilsson, 1963) and has little relevance to the work presented in this thesis, this aspect will not be discussed further.

Cation exchange and ammonium fixation capacities of soils are important in restricting leaching and volatilization losses following addition of fertilizer N as ammonium or urea. Loss of ammonium-N can be significant in soils with very low CEC.

On the other hand retention of ammonium by soil may affect N utilization by plants. There is little information on the effects of ammonium fixing capacity of soil on N utilization by plants (Nommik and Vahatras, 1982). Axley & Legg (1960) after a series of experiments reported that plant uptake of N from soil to which ammonium

capacity unless sufficient K was present to block the release of ammonium. Walsh and Murdock (1963) also stated that ammonium fertilizers should not be applied with K fertilizers to soils having high fixing capacities. Black and Waring (1972) showed that virtually all recently fixed ammonium in krasnozems was completely available to plants only when these soils were cropped successively. They also confirmed that native clay-fixed ammonium was normally unavailable to plants as reported earlier by Martin et al., (1970).

The interaction between the availability of ammonium and nitrate N in soil and plant "preference" for either of these forms may affect the results of experiments carried out to determine the effects of different forms of mineral N on plant growth. Hageman (1980), and Hocking et al., (1984), confirmed the earlier observations of Arnon (1937) and Hewitt (1970), that most plant species grow better when supplied with nitrate-N than with ammonium-N. Nevertheless for some plants grown in solution cultures or under other controlled conditions, ammonium has been shown to be the preferred source of nitrogen (Watson, 1986). Hageman (1984), in a later review, concluded that this question of preference remains unanswered largely because of technical difficulties in evaluation, such as inherent differences in the behaviour of the two ions in soil, the effect of companion ions and pH changes accompanying their absorption. Also, it is not clear whether this so called "preference" of plants growing in soil for one particular form of N has anything to do with the plant's physiological capability to select N or whether it is just the apparent availability of the form of N. This is because in most arable soils added ammonium-N is rapidly nitrified before utilization by plants or while nitrate-N remains completely available to plants.

Nitrate and nitrite (the latter in much lower amounts in most agricultural soils) are the two major oxidized forms of inorganic N in soil. The presence of these forms is mainly via nitrification of ammonium released from fertilizers and/or organic matter, addition of nitrate-containing fertilizers, and some minor amounts via irrigation and rainfall (Wetselaar and Hutton, 1972). Other minor N oxides (nitrous oxide, nitric oxide) are formed as intermediates of nitrification and/or denitrification processes.

2.1.2. Urea hydrolysis and urease activity in soils

Hydrolysis of urea can be considered as the first step of a series of reactions that occur following addition of urea to soil. Urea added to soil undergoes rapid hydrolysis due to the presence of an enzyme urease (urea amido-hydrolase, EC 3.5.1.5). Although some authors have suggested a slow non-biological breakdown of urea (Chin and Kroontje, 1963; Raison & McGarity, 1978) others have disagreed (Rachhpal-Singh & Nye, 1984) suggesting that if such non-enzymatic degradation of urea did occur it would be of little significance.

Urease is found in most surface crop residues and is produced by a wide range of microorganisms (Voss, 1984). It may occur naturally either as an endocellular or extracellular form (Mulvaney & Bremner, 1981). In the extracellular environment urease may resist degradation by formation of complexes with soil clay and organic matter fractions (Theng, 1979; Boyd and Mortland,1985).

2.1.2.1. Factors affecting urease activity : The level of urease activity varies from one soil type to another. However, results of work on factors affecting urease activity cannot be compared directly as different assay methods have been employed and results have been expressed in a variety of units. Mulvaney and Bremner (1981) attempted such a comparison with limited success.

Despite differences in methodology, many observations indicate that urease activity is positively correlated with soil organic matter content (Dalal, 1975; Rao, 1977; Zantua et al.,1977; Myers and McGarity, 1968). The effects of other soil factors such as pH, total N, CEC, particle size distribution and moisture content and also type of vegetation, on urease activity are not clear. The present review has been restricted mainly to findings on the effects of added urea, soil pH, and liming on urease activity and on the stability of urease in soils.

Overrein and Moe (1967) showed that hydrolysis of urea followed first order kinetics. Subsequently, Dalal (1975), Douglas and Bremner (1971) and Zantua and Bremner (1977) showed that urease activity increased paralleled to urea concentration only until the enzyme present was saturated by the amount of urea added. Thereafter the rate was independent of substrate urea concentration. Laidler and Hoare (1949) and recently Rachhapal-Singh and Nye (1984) reported that when urea concentration was increased further, urease activity may even decrease. After ruling out the possibility of product inhibition of urease, they attributed the decline in urease activity to some form of substrate inhibition. Monreal et al., (1986) reported partial urease inhibition following increase in substrate urea concentration beyond 5mM and complete inhibition at 80 mM.

In discussing enzyme stability in soil, Burns (1982) stated that generally, exocellular enzymes may be immobilized on organo-mineral complexes. He referred to a review by Theng (1979) on studies of the interaction of urease with clay particles that indicated H-bonding and ion-dipole co-ordination between the enzyme and organo-mineral complexes. Studies by O'Toole & Morgan (1984) confirmed these findings. This would mean that some of the bonding is pH-dependent and that soil reaction could be expected to influence enzyme activity. This may partly explain the pH-dependence of urease activity observed by other workers (Silva and Perera, 1971; Rao, 1977). However, Dalal (1975) and Zantua and Bremner (1977) found no significant correlation between urease activity and soil pH. Even among the reports

that support pH-dependence of urease activity there are considerable differences in the pH optima observed. Hoffmann (1963) and Pettit et al., (1976) reported an optimum pH range of 6.5-7.0, while maximum activity at pH 8.8-9.0 has been recorded by Tabatabai and Bremner (1972) May and Douglas (1976), and Rao (1977). Investigations by Rachhapal-Singh and Nye (1984) recently supported the lower pH range for maximum activity when they found that the maximum rate of enzyme-mediated reaction occurred in the range pH 6.0-6.8. However, they agreed that this need not necessarily indicate the optimum pH for activity because the Michaelis constant (Km) and the inhibition constant (Ki) of enzyme reactions are also affected by pH.

Reported responses of urease activity to liming vary. Moe (1967) and Peltser (1972) found a significant decrease in urease activity due to liming whereas Zantua and Bremner (1977) reported the opposite. Later Mulvaney and Bremner (1981) dismissed Peltser's findings as due to defective experimental technique but the observation by Moe was supported recently by Kumar and Wagenet (1984), when they concluded that addition of up to 8% Calcium carbonate resulted in a considerable decrease in urease activity in their soil. Earlier, Galstyan (1958) observed that calcareous soils could have low urease activity. He attributed this to a possible detrimental effect of Ca^{2+} on urease-producing microorganisms, a hypothesis supported by Carter (1986) who found that lime mixed with gypsum had a deleterious effect on soil microbial biomass activity. The differences in observations reported in the literature, underline the need for further research to clarify possible effects of pH changes and associated ions in liming materials on urease activity.

Since soil urease is predominantly microbial in origin factors that promote microbial growth can result in increases in the level of urease in soils. Agricultural inputs such as fertilizers (Zantua and Bremner, 1977) pesticides (Cerevelli et al., 1975), heavy metals (Bremner and Douglas, 1971) and organic matter are known to influence levels of urease activity. No significant effect on urease activity due to nitrification inhibitors has been reported (Mulvaney and Bremner, 1981; Voss, 1984).

However urea hydrolysis is affected by another group of chemicals known as urease inhibitors. They have given promising results in delaying urea hydrolysis and thereby reducing ammonia loss (Voss, 1984). However, the loss of ammonia following application of ammonium sulphate cannot be controlled by this technique (Fillery and De Datta, 1986). On the other hand, Hendrickson et al.,(1987) suggested that addition of the urease inhibitor "PPD" (phenyl phosphorodimaidate) with urea may promote immobilization of N thus reducing the availability of N for plant uptake.

2.1.3. Nitrification

Microbially mediated conversion of ammonium into nitrate is known as biological nitrification. Recent reviews on nitrification include those by Focht & Verstraete (1977); Belser (1979); Schmidt (1982) Haynes (1986c) and Prosser (1986a).

2.1.3.1. Autotrophic and heterotrophic processes in the soil environment:

There are two different metabolic processes of biological nitrate formation. These are heterotrophic nitrification and autotrophic nitrification. The autotrophic process predominates in most agricultural soils and is therefore the main topic of this review. However autotrophic nitrification is rather low in strongly acid soils, where the heterotrophic process is the major means of nitrate formation.

Activity of heterotrophic nitrifiers was first reported by Mishustin in 1926, while autotrophic nitrification was reported as early as the 1890's by Winogradski. and has received greater attention (Duggins, 1984). This is partly because of the earlier belief that heterotrophic nitrification occurred only in vitro. However, several reports have been published lately emphasizing its importance in natural systems. Focht & Verstraete (1977) found the rate of heterotrophic nitrification is 10^3-10^4 times slower than that of autotrophic nitrification under optimal conditions. Van de Dijk & Troelstra in 1980, using an approach proposed by Focht and Verstraete (1977), demonstrated the activity of heterotrophic nitrifiers in an acid heath soil (pH 4.3). The predominance of heterotrophic nitrification in acid soils was later confirmed by Duggins (1984) and Adams (1986).

Unlike autotrophic nitrifiers that oxidize ammonium independently of organic nitrogenous compounds, heterotrophic nitrifiers depend on the oxidation of organic amino compounds or other organic intermediates containing N for their energy requirements (Verstraete and Alexander, 1972; Focht and Verstraete, 1977). Addition of ammonium had no effect and sometimes even inhibited heterotrophic nitrification (Focht and Verstraete, 1977). However, there are few reports that indicate the presence of an inorganic pathway for heterotrophic nitrification (Kilham, 1986). At some stage partly oxidized inorganic N compounds are incorporated into organic molecules via enzymic activity (e.g. glutamine synthetase converts glutamic acid and hydroxylamine in the presence of Mg ions and ATP into glutamyl hydroxamic acid as shown by Waelsch, 1972). This latter example of ability to use inorganic N may imply that heterotrophic nitrifiers either are a diverse population or that some heterotrophs are capable of adapting to utilize inorganic N

when available under suitable conditions such as acid pH. However, experiments with nitrification inhibitors have shown that heterotrophic nitrifiers are generally unaffected by inhibitor activity implying that heterotrophs use organic N sources for the most part (Prosser, 1986b).

The autotrophic nitrifiers fall into two categories: primary nitrifiers and secondary nitrifiers. Primary nitrifiers include Nitrosomonas and Nitrobacter spp. responsible for the production of nitrite and nitrate respectively. All other microorganisms capable of autotrophic nitrification are grouped as secondary nitrifiers e.g. ammonium oxidizers such as Nitrosolobus, Nitrosospira, and Nitrosococcus and nitrite oxidizers such as Nitrospina, and Nitrococcus. The term "secondary autotrophic nitrifiers" is used for organisms that appear to be present in far fewer numbers than the primary nitrifiers and have narrower optimum temperature and pH ranges for growth (Watson, 1974). It may be noted that Bhuiya and Walker (1977), suggested that Nitrosomonas may not be the major organism responsible for conversion of fertilizer ammonium to nitrite, but there is a need for further evidence to fully substantiate this suggestion. The primary nitrifiers oxidize ammonium to nitrite via several intermediate compounds. However, during nitrification, nitrite does not accumulate in soil unless the soil pH is too high (more than pH 7.5-8.0, Wetsellar, pers. commun) for growth of Nitrobacter species that rapidly oxidize nitrite into nitrate. Incidentaly, this latter oxidation is much faster than the oxidation of ammonium to nitrite which is therefore the rate limiting reaction.

Use of carbon dioxide as the major carbon source by nitrifying bacteria, (except for certain strains of Nitrobacter), requires an exceptionally high energy expenditure. Nearly 80% of total autotrophic energy produced by oxidation of ammonium or nitrite is used in fixing C from carbon dioxide (Forrest and Walker, 1971). This is the reason for slow growth rates of nitrifiers, with minimum doubling time of 7hrs for ammonium oxidizers and 13 hrs for nitrite oxidizers (Wood, 1986).

In using ammonium and nitrite as their sole energy source nitrifiers produce several specific intermediate products such as hydroxylamine, nitric oxide and nitrous oxide. For production and handling of these compounds, nitrifiers possess several specific enzymes (such as ammonium monooxygenase, hydroxylamine oxidoreductase) and special cytoplasmic structures (Prior and Dalton, 1985). Scientists have targeted these specific nitrification enzymes and/or the ability to produce them, in their attempts to develop specific nitrification inhibitors.

2.1.3.2. Effect of soil pH and natural inhibition of nitrification: The main factors that affect nitrification include, soil reaction (pH), temperature, ammonium concentration, level of oxygen and carbon dioxide, and presence of natural and/or added inhibitory agents (Focht and Verstraete, 1977).

Nitrification has been reported at pH levels as low as 3.9 and as high as 11.2 (Focht and Verstraete, 1977). However the growth rate of autotrophic nitrifiers declines dramatically when soil pH is decreased (Schimdt, 1982). Plant growth and agricultural practices such as the addition of chemical fertilizers, liming, drainage and flooding can affect soil pH thereby influencing the nitrification process. On the other hand, because of narrow species diversity and the relatively simple reaction pathway, change in a single environmental factor like pH may have a measurable and predictable affect on autotrophic nitrification (Watson, 1974). Although most of the factors mentioned above can have significant effects on the nitrification process, the present review deals mainly with the effect of soil pH and liming on nitrification and factors inhibiting the process.

Growth of nitrifiers is most favoured in the pH range 7.0 to 8.0 and ceases below 5.0-4.5 or above 10-11 (Focht and Verstraete, 1977). Nevertheless, Schimdt (1982), commenting on reported differences in pH limits for nitrification described this as "...a long standing anomaly that is no closer to resolution than it was in 1932 when Waksman reviewed the literature to note that nitrification ceased at pH 3.9-4.0 and the nitrifying bacteria isolated from acid soils may be somewhat adapted to such soils." According to recent works the formation of nitrate-N in strongly acid soils can be attributed to heterotrophic nitrification (Focht and Verstraete, 1977, Belser, 1979; Duggins, 1984; Adams, 1986). The two processes can be discriminated easily because the heterotrophic process will diminish when soil pH is increased while the autotrophic process steadily increases until the environment is mildly alkaline ($pH \geq 8$).

Morill and Dowson (1967) characterized four basic types of autotrophic nitrification patterns based on nitrifier response to soil pH. The first type occurred in soils with pH of 7.9 in which ammonium was oxidized rapidly with accumulation of nitrite, while the nitrite was oxidized only after most of the ammonium disappeared; in the second type (pH 6.4), the oxidation of both ammonium and nitrite was rapid with no accumulation of nitrite. In type 3 (pH 5.4) the pattern was similar to type 2 but the rate of each process was markedly low; in the fourth type (pH 5.1)there was no detectable nitrate formation. The inhibition at the two extremes of pH has been attributed to ammonia (in alkaline soils) and undissociated nitrous acid (in acid soils), each being toxic to Nitrobacter. More recent work suggests that, a soil with an apparently unfavorable bulk pH, may still allow nitrification to proceed due to more suitable pH of the microsite (soil/fertilizer/microbe) environment. It is reported that microsite pH levels of 7.5-8.0 are optimal for nitrification while nitrite accumulation can occur when microsite pH level exceeds 8 (Hauck, 1984).

In addition to the pH effect, increase in salinity associated with application of mineral fertilizer can affect nitrification as can addition of lime and inorganic pesticides (McClung and Frankenberger, 1985). However, in their experiments, the salt

concentrations that gave significant inhibition of nitrification were around 15-20dS m-1 or more (depending on the salt used, i.e. NaCl, $CaCl_2$, or Na_2SO_4) which are extremely high in comparison with levels found in most arable soils. Such high salt concentrations may cause extremely high microsite osmotic pressures that may not be tolerable by soil microflora. Martikainen (1985), investigating the effect of urea, ammonium sulphate and potassium sulphate on nitrification in a forest soil(pH 4.7) showed that when these fertilizers were applied at recommended rates, the two salts; ammonium sulphate and pottasium sulphate but urea inhibited nitrification. He concluded that this inhibition was due to decreased soil pH rather than an osmotic effect.

Effects of liming on nitrification have been well documented and generally indicate that autotrophic nitrification can increase markedly following lime application (Adams and Martin, 1984). Pang et al., (1975) showed that increase in nitrification following liming was due to increase in soil nitrifier population with increased pH. They used two nitrifying soils, one with a much higher nitrifier population than the other, to demonstrate that while increased soil pH resulted in increased nitrification in both soils nitrification in the soil with the lower nitrifier population remained considerably below that for the soil with the originally higher nitrifier population. It has been reported that liming of some acid soils was followed initially by a decline in nitrification followed by a subsequent increase in rate of nitrate formation (Weier and Gilliam, 1986). They attributed the initial decline to inhibition of the heterotrophic nitrification process with increasing soil pH. The initial decline occurred because autotrophic nitrification in the initial stages had not picked-up sufficiently to compensate for the reduction in heterotrophic nitrate formation.

Nitrification has been shown to be affected also by root exudates, metabolic by-products and decomposition products of organic residues via inhibitory effects on nitrifiers (Clark and Paul, 1970). Inhibition of nitrification in some natural environments such as forest floors (Olson and Reiners, 1983) peat, grassland soils (Robinson, 1963), and soils of tea plantations (Krishnapillai, 1979; Wickramasinghe et al., 1985) indicates the presence of naturally occurring environmental factors that inhibit the growth of nitrifiers (Keeney, 1980; 1986a). It is suggested that in addition to the acid soil conditions of environments such as pine forest floors and tea plantation soils, polyphenolic compounds in these soils may inhibit nitrification (Krishnapillai, 1979; Lodhi & Killinbeck, 1980; Olson & Reiners, 1983; Baldwin et al., 1983). However other workers have found no such effects in polyphenol-rich soil subjected to incubation (Sivapalan et al., 1985). Although no definite mechanism has been proposed for inhibition by polyphenolic compounds, the observation of Azhar et al., (1986 a,b &c) that nitrite formed during nitrification could bind with polyphenols, thus preventing its use by autotrophic nitrifiers, may offer an explanation. Lensi et al.

(1986) tried a different approach to explain the absence or low rate of nitrification in a forest soil by proposing that the litter layer on the forest floor could be a barrier for oxygen diffusion thus creating anaerobic conditions unfavorable for nitrifiers. However this explanation will not hold for tea plantations since there is no significant ground cover to prevent free aeration (pers. experience). Further evidence of nitrification inhibition by natural products comes from the works of Sahrawat (1980) who found that neem cake extracted from Kranjin seeds had an inhibitory effect.

Also, high levels of ammonia sometimes found in soil can be toxic to nitrite oxidizers, and will result in accumulation of nitrite (Boon & Lauderlot, 1962; Watson, 1971). High nitrate concentrations, on the other hand can non-competitively inhibit nitrite oxidation by Nitrobacter (Boon and Lauderlot, 1962). When nitrite accumulates, it could become toxic to ammonium oxidizers thus completely inhibiting the nitrification process. This inhibition may be reversed when sufficient oxygen and carbon dioxide is available for Nitrobacter activity (Fliermans and Schmidt, 1975).

Autotrophs could be indirectly affected by the C/N ratio of the environment. A high C/N ratio may suppress nitrification by favouring heterotrophic N immobilizers and ammonifiers that are better competitors for ammonium N in soil (Jansson, 1958).

2.1.3.3. Consequences of nitrification and needs to control the process: Nitrate nitrogen may be removed from soil by leaching, denitrification or by plant absorption. Leaching and denitrification have been identified as major processes by which N may be lost from the most plant/soil system. Although, plant uptake of nitrate is generally considered as beneficial, high levels of soil nitrate N may lead to excess plant uptake resulting in excess vegetative growth stimulated by nitrate-N at the expense of reproductive development. Excess soil nitrate-N has been found to decrease phosphorous and sulphur absorption while ammonium increased phosphorus uptake in wheat (Nielson et al., 1967). Furthermore, nitrate leaching, in addition to being uneconomical and environmentally unfavorable, causes acidification of soils since the removal of anion is accompanied by equivalent cation, mainly Ca (also Mg and Na). In contrast, ammonium-N is held in the cation exchange complex of most soils and is much less subject to loss by leaching. As most plants have no apparent preference for either ammonium or nitrate (Hageman, 1980) it would seem that regulation of nitrification is the most logical way to avoid the adverse consequences of excess uptake and/or loss of soil nitrate.

Although the factors that affect nitrification can be altered to delay or prevent the nitrification process, the use of nitrification-inhibitory chemicals is considered as one of the most effective and direct ways of preventing excess nitrate formation (Amberger, 1983). A wide range of chemicals capable of inhibiting nitrification has been discovered (Hauck, 1972) and many have been developed for commercial

application since 1967. In addition many agricultural chemicals used for other purposes (e.g. fertilizers and pesticides) have been found to inhibit one or more steps of the nitrification process (Bundy and Bremner, 1973). However before discussing work on nitrification inhibitors it is appropriate to consider the salient aspects of N losses and their consequences since the primary objective of using these chemicals is to reduce such losses.

2.1.4. N losses from plant-soil systems

Nitrogen losses occur when soil nitrogen is moved beyond the reach of roots or shoots. Nitrogen is lost from soil by escape of gaseous forms (e.g. nitrous oxide & dinitrogen and ammonia), leaching and run-off, and removal via harvested plant products. Although some workers (Hendrickson et al., 1987) have described immobilization of N as a loss such temporary or long term unavailability of mineral N due to phenomena such as ammonium fixation on clay minerals and biological/chemical immobilization of N will not be considered as losses in this review since nitrogen is not lost from the plant/soil system.

2.1.4.1 Gaseous loss of N: Recently Freney & Simpson (1983) edited a compilation of reviews of work carried out on gaseous forms of N loss. Other recent reviews include those by Haynes & Sherlock (1986), Nelson (1982), and Terman (1979).

Denitrification: The biochemistry of the denitrification process and factors affecting it have been investigated and reviewed by Focht and Verstraete (1977), Firestone (1982) and Fillery (1983). A short review by Colbourn & Dowdell (1984), dealt with effects of agronomic factors on denitrification and summarized salient developments from 1976 to 1983.

Early workers considered denitrification only as a nitrate reduction process yielding gaseous nitrogen for completion of the nitrogen cycle. This is one reason why denitrification received less attention than other N transformation processes. However increased use of nitrogenous fertilizer has prompted scientists to study the process from different perspectives. For example, nitrous oxide evolved as a result of incomplete denitrification contributes to depletion of the stratospheric ozone layer (Crutzen and Ehhalt, 1975).

Fewson & Nicholas (1961) divided biological nitrate reduction into two forms, i.e. nitrate assimilatory and nitrate dissimilatory processes. Denitrification is a nitrate dissimilatory process by which nitrogenous gases are formed. Early reports identified most denitrifying bacteria as facultative anaerobes (Simpson & Freney, 1974). More recent studies have shown that most of these bacteria are aerobes with few exceptions

such as some Bacillus spp. which unable to grow by fermentation are capable of anaerobic respiration, utilizing nitrate as an alternative electron acceptor (Fillery, 1983). It has been confirmed that denitrification occurs mainly under restricted oxygen supply or under completely anaerobic conditions (De Datta, 1981). As for many other microbially-mediated processes, denitrification is also influenced by environmental factors (e.g. pH, temperature, aeration/moisture) and introduced factors (e.g. lime, agricultural chemicals). However since the species diversity of denitrifiers is greater than that of nitrifiers (Fillery, 1983) their overall responses to changing environmental factors may not be as marked as those involving nitrifiers.

Nitrite is the immediate product of nitrate reduction in both nitrate-respiring and denitrifying bacteria. While nitrate-respiring bacteria will not reduce nitrite further denitrifying bacteria continue to do so (Focht and Verstraete, 1977). During the process nitric oxide and nitrous oxide are produced as intermediates. Under strongly to mildly acidic conditions nitrite decomposes rapidly but may accumulate under alkaline conditions (Vancleemput and Baert, 1984). Although nitrite rarely accumulates in agricultural soils there is evidence for its accumulation under particular conditions, such as high ammonium concentrations with increased soil pH (Wetselaar, pers. comm.), low temperature, higher moisture content and depleted oxygen and available C supply (McGarity and Myers, 1968).

Although several soil factors such as organic matter content, temperature, and moisture/aeration status are known to influence denitrification only those factors directly related to the present project have been considered. These factors include level of nitrification/nitrate concentration in soil and soil pH.

The early belief that denitrification was independent of nitrate concentration has changed in the light of subsequent findings showing that denitrification obeyed first-order kinetics (Balakrishnan & Eckenfelder, 1969; Starr and Parlange, 1975). In fact, the concentration of nitrate may influence the selection of different denitrifying bacteria that become active in a particular environment. Blackmer and Bremner (1978) showed that high nitrate concentration may inhibit the reduction of nitrous oxide to gaseous nitrogen thus altering the end product ratio of denitrification. Since nitrification is the major, if not the only means of nitrate build-up in soils, the control of nitrification in a soil with high denitrification potential is a logical method of controlling potential N losses.

The common denitrifying bacteria have pH optima similar to those of general bacterial flora (i.e. in the pH range 5-9 with peak activity between 7-7.5)(Focht and Verstraete, 1977; Firestone, 1982). Generally, denitrification is slower in acid soils (<pH 5) than in near neutral soils (>pH 6) (Adams and Martin, 1984). However,change in soil pH appears to affect denitrification products more than the organism's activities. For example, Nommik (1956) observed that nitrous oxide

production is greater at lower pH (pH <7) while Wijler and Delwich (1954) reported that Nitrous oxide reduction was less below pH 6.0. They also found Nitric oxide to be the dominant product below pH 5.0. Cooper & Smith (1963) and Fillery (1979) found no significant effect due to soil pH on denitrification from soils of different native pH values and from soils of long-term liming trials.

In addition to biological denitrification, (inorganic) chemical denitrification in soils is also known (Smith & Chalk, 1980, Chalk and Smith, 1983). Although not directly related to the work reported in this thesis, the occurance of inorganic chemical denitrification emphasizes the importance of inhibiting nitrification at a stage before nitrite is formed. This is because nitrites formed during nitrification (and/or biological denitrification) may undergo chemical reactions to form gaseous nitrogenous compounds via a side-tracking process described as "chemo-denitrification". This has been found to occur when oxidation of nitrite by Nitrobacter is inhibited by ammonia (Bundy & Bremner, 1974a). The usual pH range of this inhibition is between 7.00 and 8.00. However, it may be noted that although these two authors suggested that nitrite accumulation was a prerequisite to chemo-denitrification, Smith & Chalk (1980) showed this was not necessarily a prerequisite.

Because of the pH dependence of the reaction, liming will reduce chemo-denitrification in acid soils. Earlier Van cleemput and Patrick (1974) reported that the level of nitrate-N was reduced during chemo-denitrification and the rate of reduction increased with decreasing soil pH because more H ions were available to combine chemically with nitrate to form nitrogen and water (Adams and Martin, 1984). Therefore, it appears that liming may reduce the potential loss of N due to chemo-denitrification. However, it should be noted that there appears to be no general agreement about the exact mechanism(s) of chemo-denitrification.

Application of agricultural chemicals to soil may also change microsite pH, composition of soil microbial population and soil N levels. For example, addition of ammonium fertilizer to a warm wet soil that subsequently became waterlogged was followed by substantial denitrification losses (Wetselaar et al., 1973). Freney et al. (1985) demonstrated that urea applied in irrigation water to a commercial sunflower crop on a calcareous, cracking clay soil resulted in substantial loss of N, presumably by denitrification. Further evidence for increased denitrification following nitrogen fertilizer application in field comes from, Craswell (1978) and Saffigna et al.(1984a) who showed that losses ranging from 6 to 30 % of the applied fertilizer-N occurred due to denitrification. Later workers found that highest losses resulted from calcium nitrate application when compared to urea or ammonium sulphate. However, Dowdell and Webster (1984) found no significant denitrification N losses with calcium nitrate compared to ammonium nitrate and urea, when added to field lysimeters cropped with cut grass swards. These variations may have resulted due to differences in factors

such as soil pH, organic matter content, denitrifying population and the type of crop use.

Denitrification may be either retarded or inhibited by agricultural chemicals such as nitrification inhibitors (Hauck 1972 and Mills and McElhannon 1984), insecticides, herbicides and fungicides (Yeoman and Bremner, 1985 a,b). Although the significance of denitrification inhibitory compounds has not been highlighted in the literature, agricultural systems involving flooded field conditions (e.g. rice fields with poor drainage), where ammonia volatilization loss is high or where nitrite accumulates (e.g. in alkaline conditions) or in a situation similar to one described by Freney et al. (1985) where high denitrification loss was recorded, the use of denitrification inhibitors together with nitrate fertilizers may be beneficial. Such chemicals should be specific enough to inhibit the nitrate reduction step rather than nitrite reduction. The possible development of denitrification inhibitors has been discussed recently by Hauck (1983) who expressed doubts on the practicalities of using such chemicals to control the activity of denitrifying organisms in the field. He argued that only nitrite and nitrate close to the added inihibitor would be protected against denitrification. Nitrate that moved out of the inhibitor's influence would be subject to denitrification. He proposed that a denitrification inhibitor applied as a soil fumigant might solve this problem. However, it may be noted that this and other problems such as the need to find chemicals that are specific to denitrifiers, non-toxic to beneficial fauna and flora, resistant to degradation and economical to use, are common to the development of most agrochemicals. Even if these requirements were satisfied, Hauck (1983) thought that any direct chemical control of denitrification in soil would be very difficult because of the diversity of soil denitrifiers (Payne, 1981). For the same reason, Keeney (1986a) found it difficult to accept results that demonstrated inhibition of denitrification (e.g.Rolston and Cerevelli, 1980; McElhannon and Mills, 1981; Mills and McElhannon, 1984 and McElhannon et al., 1984) by pesticides, herbicides and nitrification inhibitors. However, recent finding that Nitrosomonas can also denitrify nitrite (that may form due to partial denitrification of nitrate added to soil) to nitrous oxide (both in poorly aerated and aerobic soils), and may contribute significantly to nitrous oxide emission from soil (Poth and Focht, 1985; Blackmer et al., 1980; Goodroad and Keeney, 1984; Davidson et al., 1986), support the possibility of nitrification inhibitor mediated reduction of gaseous N loss.

Nitrification: Further justification for inhibition of nitrification as a means of reducing N losses comes from the fact that N can be lost as gaseous products even during nitrification. Nitrite formed as an intermediate compound during nitrification may accumulate under alkaline conditions (Focht and Verstrate, 1977). Such nitrite instead of being further oxidized by nitrifiers, may be reduced to nitrous oxide.

Davidson et al. (1986) and Poth and Focht (1985) pointed out that nitrous oxide production from nitrification and denitrification could occur simultaneously in the same soil aggregate due to microsite differences in redox potential. They found that nitrification-mediated Nitrous oxide production was more prominent on well-drained sites with an ambient supply of nitrate whereas denitrification mediated nitrous oxide production took precedence in poorly drained sites of the same area.

Ammonia loss: Although ammonia loss is outside the primary concern of this thesis the literature on this aspect is covered briefly because it will be the main form of N loss if N is retained as ammonium rather than nitrate. Emphasis has been given to reports dealing with the effects of pH, urease activity, lime and nitrification inhibitors.The different forms of ammonium and ammonia take part in the following equilibria:

$$NH_4^+(\text{fixed}) = NH_4^+ \text{ (exchange.)} = NH_3 \text{ (aq)} = NH_3 \text{ (adsorbed,aqueous, gas)}$$

Loss of ammonia by volatilization depends on the rate of ammonia production and the rapidity of its removal. The rate of production of ammonia is a direct function of the level of ammonium in the soil solution while its rate of removal is determined in part by the extent of diffusion of gaseous ammonia into the atmosphere.

Increased soil pH promotes the conversion of ammonium-N to ammonia-N and its subsequent volatilization (Ryan et al., 1981; Fillery et al., 1986a,b). Ammonia loss from acid soils is usually negligible or absent. Hence any amendment that decrease soil acidity will encourage ammonia production. Avnimelech and Laher (1977), after studying the relative importance of initial soil pH on ammonia volatilization from ammonium salts applied to alkaline soils, concluded that initial soil pH was an important factor only when the soil buffer capacity was high or when the concentration of ammonium in the soil solution was high. At a given soil buffer capacity, ammonia volatilization increased with increasing soil pH. On the other hand, as ammonia volatilization proceeded, soils gradually became more acidic and ammonia production was reduced. Hence ammonia volatilization was more prolonged in soils of higher than those of lower base saturation.

Several workers have shown that the pH in the immediate environment (i.e. microsite pH) of the applied fertilizer may be more important than the overall soil pH. This may explain substantial ammonia volatilization losses from acid soils fertilized with urea (Sherlock & Goh, 1984; Black et al., 1985). The pH effect on ammonia volatilization is directly linked to the practice of liming and urea addition to agricultural soils. The low potential for ammonia volatilization losses in an acid soil may significantly increase following these two practices unless other measures to reduce

ammonia levels are introduced. Introduction or retention of organic matter (to increase CEC), deep placement of fertilizers, timing of application, and irrigation immediately after fertilizer placement are some of the measures known to reduce potential ammonia losses (Myers et al., 1961); Terman, 1979).

2.1.4.2 Leaching loss of N: Leaching may be the only or major mechanism of N loss from certain plant-soil systems involving sandy or sandy loam soils protected from wind erosion. When the net movement of water and solutes is downward (more commonly with a lateral component), it is called leaching whereas surface movement is termed runoff.

Generally, nitrate and nitrite ions are lost more rapidly by leaching or runoff than ammonium ions that are held in the exchange complex of soil. Some N fertilizers like urea can also be removed quite rapidly. On the other hand nitrates may be held by non-specific electrostatic adsorption (Black and Waring, 1976 a,b,c). This has been explained as being due to anion adsorption capacity of some sesquioxidic soils with positively charged sites that result from iron and aluminium oxides and 1:1 clay minerals (e.g. kaolinite and allophane).

Numerous attempts have been made to predict nitrate movement with varying success due to the interaction of many unpredictable factors such as heterogenity of soil texture and physical structure. Reviews on this topic include those by Wild and Cameron (1980), Soil Science Society of America (1983), Addiscot and Wagenet (1985), and Cameron and Haynes (1986).

Factors affecting N leaching losses: These factors can be categorized into two groups:

(i) those influencing the levels of nitrate in soil (e.g. nitrification and denitrification in relation to other N transformation processes, fertilizer application, crops grown, climatic conditions); and

(ii) those factors which determine the movement of water such as climatic conditions, soil properties, land management and irrigation practices (Cameron and Haynes, 1986). For leaching losses to take place, one or more factors from both categories need to be operative.

Where excess water drains from the soil after infiltration the net effect of nitrification, denitrification and nitrate uptake capacity of crops (and anion exchange capacity in some soils, Black and Waring, 1976a,b,&c) determines the extent of nitrate leaching losses. Many workers have found that in certain ecosystems (e.g. acid forest floors, and in grassland soils) where nitrification is naturally inhibited due to acid conditions or anaerobic conditions, or by the presence of natural inhibitory compounds, nitrate leaching losses are either absent or negligible (Robinson, 1963; Martikainen, 1985; Adams, 1986). When soil acidity is the reason for low

nitrification potential liming may result in a significant increase in autotrophic nitrification rate resulting in higher nitrate levels (Nyborg and Hoyt, 1978; Adams and Martin, 1984).

Rapid adsorption of nitrate by plants helps to maintain low nitrate levels and leaching losses. The extent of leaching from the time of fertilizer application to commencement of rapid plant uptake of N is of critical importance for better N economy. When excessive leaching cannot be controlled easily, particularly when crops are grown in predominantly sandy textured soils under high rainfall, artificially delaying nitrate formation until plants are able to take up N rapidly would help to restrict N leaching losses.

Soil properties such as soil texture (Hoyt et al., 1977), hydraulic conductivity and organic matter content (Neilsen and McKenzie, 1977) that directly influence water infiltration and soil permeability also have an important effect on nitrate leaching.

Methods of studying leaching losses: Wild and Cameron (1980) presented a detailed review of methods used in studies of leaching losses. Some of these methods include:

Field soil sampling. Soil specimens are collected periodically from different depths and analyzed for ammonium, nitrate and moisture content so that movement of these constituents with time can be determined. However, the spatial variability of nitrate levels in field soils makes interpretation of the data from such studies a difficult task (Broadbent and Carlton, 1978).

Lysimeter studies. Different types of lysimeters have been used for leaching studies some of which are ideal for the study of nitrate movements through soil. Limitations such as container edge effects, variable aeration conditions and preferential drainage pathways cause problems in interpretation of data.

Catchment studies. These provide an integrated evaluation of leaching losses (plus surface run-off losses) from strictly defined catchment areas. This method is suitable for soils with less permeable lower horizons (e.g. duplex soils).

Laboratory column studies Undisturbed soil columns are essentially similar to mini-lysimeters and have the same limitations for leaching studies. Artificially packed soil columns are useful in studies of the movement of N through designedly uniform soil systems relatively free of preferential water paths or when results need to be related to a particular type of soil material with 'foreign matter' (such as coarse fragments and undecomposed vegetative parts) excluded. There may be technical difficulties in avoiding subsurface waterlogging and in achieving uniform aeration within the columns and some of these difficulties and technical solutions have been discussed in detail in Chapter 7.

2.1.4.3 Control of N losses and the use of nitrification inhibitors: Agronomic and environmental consequences of poor management of fertilizer N are well documented (Wetselaar, 1974; Keeney, 1982, Hauck, 1983). In view of the many reports that show rapid nitrogen loss by ammonia volatilization, denitrification and leaching, there is little doubt of the need for research to improve N fertilizer efficiency. Excess N in the environment has also been shown to have a direct effect on plant growth [e.g. ammonium, nitrite and nitrate toxicities (Goyal and Huffiker, 1984)] and on the environment [(e.g. nitrate-induced soil acidity, pollution and eutrophication of water ways, acid rains and depletion of stratospheric ozone (Stanford and Legg, 1984)) with indirect effects on human & livestock health. These disastrous consequences have stimulated research for means to reduce N losses from soil and to realize optimum crop yields.

Because of the greater mobility of nitrate-N in soils a common technique is to conserve soil N as ammonium. However the agronomic success of such action is influenced by site-specific factors (like CEC, N transformation, the extent of leaching losses) including the preference of plants to absorb N as ammonium or nitrate. A general answer to this latter question of plant preference is no nearer than it was a century ago. Hageman (1980) reviewed work on the effect of these two N forms on different crops. Some plants prefer ammonium while others prefer nitrate but generally most plants absorb more ammonium during earlier growth, shifting "preference" to nitrate at later stages of growth. It remains unclear whether such a shift is due to changing levels of availability of those N forms in soil during the growing season or to an actual change in plant preference. (see Section 2.1.1, page 7)

If there is indeed no specific preference for one form of N over the other it is apparent that conserving soil N as ammonium rather than nitrate would be beneficial for reasons discussed previously. Nitrification inhibitors offer a promising way of conserving N as ammonium and many workers have reported increased N retention in soil following treatment with nitrification inhibitors (Meisinger, 1980; Amberger, 1983). However, reports on their effectiveness in improving crop performances are highly variable (see Appendix 1, Prasad et al., 1971; Slangen and Kerkhoff, 1984). This is probably a result of variable inhibitor performance due to different soil and environmental factors, different techniques employed to evaluate inhibitor performance, and differences in active nitrifying organisms in soils (Section 2.3. of this chapter).

2.2. Effect of liming on plant growth and N transformation

Lime ameliorates soils by neutralizing acidity. The change in acidity can bring about changes to biological activities and chemical reactions such as N transformation and bonding of ions which will directly affect plant growth and the soil microflora.

2.2.1. Effect of liming on plant growth

Application of lime to an agricultural soil serves the dual purpose of correcting pH associated problems, and supplying Ca (and/or Mg) to crops.

Significant responses in plant dry matter production, N, P, and K uptake, improved root growth and yield increases following lime application have been reported (Mendham and Russell, 1987; Dalal, 1986; Pinkerton and Simpson, 1986). It is common to attribute such crop responses to lime-induced pH changes. However, soil pH per se has little direct effect on plant growth as most plants are known to tolerate pH levels beyond the critical soil pH levels reported in field experiments (Jarvis, 1984; Temple-Smith, 1985). The effects are rather due to pH-induced changes in soil chemical and biochemical activities that determine the availability of various elements.

Lime is also believed to have direct affects on Al uptake (and toxicity) (Jackson, 1967; Huett and Menary, 1979,1980) while others have shown that lime primarily affects the activity of microorganisms that are closely associated with plant growth or nutrient transformations (Adams and Martin, 1984; Jarvis, 1984).

2.2.2. Effect of liming on N transformation :

Research on the various aspects of the effect of liming on N transformation has been reviewed by Jackson (1967) and Adams and Martin (1984). All pH-dependent microbial activities that determine the fate of N in soil may change as a result of lime induced pH increase (Adams and Martin, 1984). The effect of Ca on such activities was also found to be significant (Jarvis, 1984; Carter, 1986).

Mineralization and Immobilization of N: The effect of liming on N-mineralization has been discussed by several authors (Harmsen & van Shreven 1955; Nyborg and Hoyt, 1978) These workers support the observation that N-mineralization in acid soils generally increases with liming. Nyborg and Hoyt (1978), for example, observed that liming of acid soils (with pH ranging between 4-5.6) to pH 6.7 almost doubled mineralization of N in the short term.

The inhibition of mineralization of organic N in acid soils has been attributed to a combination of hydrogen and aluminium toxicities and calcium deficiency all of which may be ameliorated by liming (Isirimah and Keeney, 1973; Adams and Martin, 1984). The link between reduction of active aluminium by liming and increased N-mineralization is further supported by the fact that acidic soils with high organic matter (e.g. peat) have high mineralization rates: active aluminium in these soils is either absent or 'fixed' by organic matter (Chew et al., 1976). Nyborg and Hoyt (1978) however, found that a lime-induced increase in mineralization was only temporary, although no explanation was given. On the other hand there is limited evidence

indicating that N-mineralization may not respond to liming (Ivarson, 1977) while Weier and Gilliam (1986) reported that adding a small quantity of lime tended to decrease mineralization in some soils with high organic matter content, as reported earlier by Waring and Gilliam (1983).

Nitrification : As discussed previously, nitrification in strongly acid soils tends to be more heterotrophic than autotrophic. When pH is increased heterotrophic nitrifier activity may decline while autotrophic nitrification starts to increase. However, liming of strongly acidic soils may result in an initial decline in overall nitrification. This may be because the reduction of nitrification due to decreased heterotrophic activity is not fully compensated by an increase in autotrophic nitrification (Gilliam and Waring, 1980; Weier and Gilliam, 1986). Failure of liming to increase nitrification may indicate a lack of autotrophic bacteria (Yavinder-Singh and Beauchamp, 1986) since in general, autotrophic nitrification does tend to increase with addition of lime (Adams and Martin, 1984) when other factors are favourable.

Denitrification : Microbial denitrification may be much slower in very acidic soils (pH 5) than in near-neutral soils (pH 6) (Adams and Martin, 1984). Focht and Verstraete (1977) concluded that the optimum pH for denitrification was around 7.0 to 7.5. This means that liming of acid soils can stimulate denitrifying bacteria under anaerobic conditions provided other environmental factors such as, temperature and nitrate-N are not limiting. Chemo-denitrification may also be significant at low soil pH (van Cleemput and Patrick, 1974). However, Gilliam and Gambrell (1978) found that low soil pH may be less inhibitory even to denitrification when there is a supply of easily decomposable organic matter.

The ratio of Nitrous oxide to gaseous nitrogen seems to depend on soil pH (Balasubramanian and Kanehiro, 1976; Bremner and Blackmer, 1978) and the proportion of nitrous oxide increases with decrease in soil pH (Reuss and Smith, 1965). Since the pH change has little effect on total gas production (nitrous oxide and gaseous nitrogen) it is suggested that effect of pH must be immediate rather than due to a population shift. It may be that nitrous oxide reductase is quite sensitive to pH or that decomposition to nitrous oxide of an NO intermediate may be promoted by acidity (Firestone, 1982).

One obvious way to reduce denitrification losses is to inhibit nitrification and maintain N as ammonium at least until plants are sufficiently mature to utilize nitrate-N rapidly.

2.3. Nitrification inhibitors in N management and agricultural production

2.3.1. Introduction

Nitrification inhibitors are recommended primarily to reduce loss of nitrate from soil by leaching and denitrification. Other benefits include lower nitrate levels in

vegetable crops and decreased nitrate contamination of ground and surface waters (Keeney, 1982). The extensive literature on nitrification inhibitors testifies to the interest in this approach as a means of controlling N losses (Goring, 1972; Huber et al., 1977; Meisinger 1980; Hauck, 1980,1983,1984; Slangen and Kerkhoff, 1984; Keeney, 1986a). However, despite the large volume of research, proof of agronomic benefits remains inconclusive.

At present six different nitrification inhibitors are used commercially (Appendix 2) while many more have been patented in several countries and await commercial development (Slangen and Kerkoff, 1984; Powell, 1986). Farmers in USA, Japan, W. Germany, USSR, UK and some other European countries use one or other of these compounds as a fertilizer amendment. Field research on the use of nitrification inhibitors is being conducted in several other countries including Australia, India, Thailand and Philippines.

As pointed out by Hauck (1972) an ideal nitrification inhibitor must satisfy several primary requirements (Appendix 3):

- high specificity for the target microorganisms (ammonium oxidizing bacteria) while being non-toxic to other soil microflora, crops or associated fauna.
- similar mobility to ammonium ions or ammonium-producing fertilizer molecules (Bock et al., 1981; Rodgers & Ashworth, 1982).
- an adequate period, (persistence) at least until the crop has grown sufficiently to utilize soil N rapidly.
- a satisfactory cost/benefit ratio.

Although Hauck (1972) did not include ease of storage and application as significant requirements in his review later work by Keeney (1986a) has underlined the importance of solubility in water and volatility of the chemical, implying that storage and ease of application are important. However, none of the available chemicals meets all of these requirements. Sahrawat and Keeney (1985), have outlined perspectives for future research and development of nitrification inhibitors with these requirements in view.

Preliminary screening tests have resulted in the identification of a number of nitrification inhibitory compounds [e.g. Bundy & Bremner (1973)]. Only some of them have shown the required degree of specificity for commercial use. Compounds with more general bactericidal action may destroy the beneficial microbial population while compounds that specifically inhibit the activity of Nitrobacter spp. (e.g.acetylenic compounds) are also not suitable as their use may allow nitrite to build up in soil.

Despite the fact that mobility is considered a major factor in the effectiveness of nitrification inhibitors there has been very little research on this aspect. If the inhibitor is highly miscible with water and/or is not tightly sorbed by soil components then it

may be rapidly leached from zone of fertilizer application. Bock et al., (1981) found that the inhibitor dicyandiamide (DCD) was so mobile that it was rapidly separated from the ammonium with which it was applied. Nitrapyrin, on the other hand, has a low solubility in water (Goring, 1962a), and is sorbed by organic matter (Hendrickson et al., 1979b; 1987). Hendrickson et al., (1978a) and Briggs (1975) showed that nitrapyrin had lower apparent mobility. In contrast, Landua (1976) reported that nitrapyrin moved more rapidly than ammonium during leaching of artificially packed soil columns.

From consideration of many results a question may arise regarding the optimum mobility of inhibitors. i.e. whether the mobility of inhibitor should be relatively greater or less than that of ammonium or equal to it? Probably there is no general answer since mode of action and persistence of the inhibitor will directly influence inhibitor efficiency. If an inhibitor remains effective after sorption on to soil colloids its consequent low mobility may not be disadvantageous. On the other hand, if an inhibitor moves more slowly than ammonium-N, nitrifiers in lower layers may convert most of ammonium-N into nitrate-N before the inhibitor reaches these sites. This would imply that soil texture, factors that influence ammonium movement such as soil colloid fraction, and hydraulic conductivity may be important determinants of the overall effectiveness of a nitrification inhibitor.

In earlier studies the effectiveness of nitrification inhibitors was determined by comparing amounts of nitrite and nitrate produced under inhibitor treatments with those of untreated controls. The equation used by Bundy & Bremner (1973) for this purpose was

[(C-S)/C]x100=% inhibition

Where C is the amount of nitrite and nitrate in the control, and S the amount of nitrite and nitrate in the treated soil specimen.

Later, Sahrawat (1980) proposed a different equation using nitrification rate rather than nitrate-N concentration to calculate percent inhibition:

[(N1-N2) / N1] x 100= % inhibition

where N1 is the percent nitrification rate in the control and N2-the percent nitrification rate in the treated specimen.

Percent nitrification rate was calculated using the equation:
[(nitrite+nitrate)-N/(ammonium+nitrite+nitrate)-N]x100. As this second approach considers the ammonium level in soil as well as nitrification rate it has received wider acceptance.

Some workers, when evaluating the effects of various factors on nitrification inhibitors, have measured only the nitrification rate over a short incubation period. It may be argued that the measure of "net effectiveness" should also consider the total duration of inhibition (persistence) as a factor since it is important that inhibition

should be effective at least until the crop has grown sufficiently to absorb most of the nitrate-N resulting from increasing nitrification (Hauck, 1972).

2.3.2. Nitrapyrin and other commercial nitrification inhibitors:

Although the nitrification-inhibitory property of certain chemicals was known in the early part of this century, no recognition of their agronomic value was recorded until the early 1960's when Goring (1962a,b) highlighted the usefulness of nitrapyrin [(2 chloro 6(trichloromethyl) pyridine] as an effective nitrification inhibitor. This led to the subsequent development of this chemical as a commercial product by Dow Chemicals, USA, in 1967, under the trade name N-Serve. Since then several other nitrification retarding chemicals have been discovered and patented (Appendix 2).

To date, the amount of research on nitrapyrin far exceeds that on other chemicals. Dicyandiamide (DCD), etradiazole (ED) and aminotriazole(ATC) are among the other compounds that have received some attention and the search for new compounds is continuing with the screening of common agro-chemicals such as fungicides (Wainwright & Pugh, 1973), herbicides, insecticides (Reddy et al., 1984), salts (Golden et al.., 1981) as well as natural products (Sahrawat ,1981).

In view of the large volume of literature on nitrapyrin and also because it is the chemical used in the present study, further discussion has been restricted to it. Thus, the term "nitrification inhibitor" (NI) has been used synonymously with nitrapyrin unless another specific name is given.

2.3.3. Mode of inhibitory action of nitrapyrin

There has been surprisingly little research on the mode of action of nitrification inhibitors in comparison with the amount of work on other aspects of nitrification inhibition such as evaluating the critical concentration of inhibitor and effect of external factors on inhibitor effectiveness.

Hauck (1980) in a review of the literature on possible modes of action of nitrification inhibitors suggested that any one or more of the following could be involved:

a. the creation of an unfavourable micro-environment for nitrifiers;
b. the stimulation of growth of competitive microorganisms;
c. the disruption of membranes and the changing of cell ultra structure;
d. interference with the reductive assimilation of carbon dioxide, or other metabolic activity common to autotrophs;
e. blocking or inactivation of enzyme systems involved in nitrification.

Various chemicals with inhibitory characteristics, such as allylthiourea, potassium ethyl xanthate, acetylene and potassium cyanide have been found to exhibit one or more of the above modes of action (Lees, 1963; Quastel, 1965) but most of

them lacked the specificity to act only on ammonium oxidizers.

Mechanisms proposed to explain the probable mode of action of compounds specifically inhibitory to ammonium oxidizers include:

a. chelation of Cu of cytochrome oxidase involved in ammonia oxidation (Campbell & Aleem, 1965; Hooper and Terry, 1973).

b. binding of enzymes or proteins involved in the oxidation of ammonium (Hooper & Terry, 1973).

Nitrapyrin has been shown to interfere with metabolic pathways in organisms responsible for nitrification (Campbell & Aleem, 1965; Hooper and Terry, 1973), apparently by chelation of Cu co-enzymes of ammonia monooxygenase. This enzyme catalyses the oxidation of ammonia to hydroxylamine during nitrification. The inhibition could be reduced (as much as 45-70%, or even completely) by addition of Cu^{2+} (34.5ppm) to the medium. However, Powell & Prosser (1986a) observed that the addition of Cu^{2+} (046ppm) enhanced rather than reversed inhibition by nitrapyrin. They attributed this anomaly to a "probable" difference in the effect of the type of copper salt added and to differences in the time span over which the experiments were conducted (the earlier researchers did not specify the type of Cu salt used but Powell and Prosser used $CuSO_4$).

Powell and Prosser (1986a,b), referring to an earlier observation by Powell (1985), stated that "....higher concentrations of nitrapyrin are required for inhibition of a laboratory strain of N.europea in soil than for equivalent inhibition in liquid culture." However, Rodgers & Ashworth (1982) earlier reported a result contrary to this finding. Both groups used the same Skinner and Walker (1961) culture medium. Powell and Prosser (1986a,b) explained their results as due to chelation of nitrapyrin with Cu ions bound to cation exchange sites which reduced the amount of active inhibitor concentration compared to liquid culture. They also suggested variable sensitivity of different strains of bacteria to nitrapyrin reported earlier by Belser and Schmidt (1981) as a probable cause for observed differences. If the first explanation is accepted it is difficult to see why Cu added (0.046ppm) with nitrapyrin to a nitrifying liquid medium enhanced the inhibition (Powell and Prosser, 1986a). Rodgers and Ashworth (1982) suggested that soil particles provided a surface for interaction of inhibitor and bacteria which was absent in liquid culture. It is clear that further investigation is needed to clarify the extent to which nitrapyrin is capable of chelating with Cu and the effect of Cu on the nitrification process.

Salvas and Taylor (1980) noted that nitrapyrin behaved similarly to inhibitors of methanogenesis such as DDT and Chloroform and proposed that the trichloromethyl moiety was the functional group responsible for nitrification inhibition. To support this proposition they drew attention to the absence of this group in chloropicolonic acid, the hydrolysis product of nitrapyrin, which was

reported by several workers to be non-inhibitory (Goring, 1962a; Laskowsky, 1972). However, Powell and Prosser (1985) reported that both nitrapyrin and chloropicolonic acid inhibited the growth of exponentially growing cultures of N.europaea but chloroform did not. The differences in results have been attributed to possible differences in strains of bacteria used and the difference in period of incubation in the two experiments.

Attempts have been made by some workers to determine whether the mode of action is bacteriocidal or bacteriostatic (Underhill & Prosser, 1985). According to Hooper and Terry (1973) nitrapyrin was bacteriocidal even in field soil but Goring (1962a) and Laskowski and Bidlack (1977) concluded that it was bacteriostatic. Rodgers and Ashworth (1982) observed a bacteriocidal effect when nitrapyrin was added to a nitrifying culture in-vitro but thought it inappropriate to consider the inhibitor as either purely bacteriocidal or bacteriostatic because it showed bacteriostatic properties when applied to field soil. Much work remains to be done to identify the factors that determine bacteriocidal and/or bacteriostatic properties and the critical concentrations involved. (See the Addendum of this thesis).

2.3.4. Secondary factors affecting the activity of nitrapyrin

In addition to the primary requirements (factors) discussed in section 2.3.1. such as specificity, mobility, persistence (Hauck, 1972), and solubility (Keeney 1986), there are secondary factors that may influence inhibition via primary factors (see Appendix 3). The secondary factors can be further categorized into soil factors and other environmental factors for ease of discussion.

Soil factors: Many workers have investigated the effects of soil characteristics on nitrapyrin activity (Goring, 1962; Hendrickson et al., 1978a,b; Slangen and Kerkhoff, 1984). There is general agreement that nitrapyrin used at recommended rates (0.56kg/ha) inhibits soil nitrification under most soil conditions but the period of effectiveness varies. Goring (1962b) observed that success rates of nitrapyrin-mediated inhibition in pot experiments were greater than in the field. He attributed this to a more intimate mixing of ammonium, inhibitor and soil particles in a limited soil volume thus increasing the efficiency of the chemical. Furthermore, in pots or columns, leached N is no longer available while in the field, N leached to lower horizons may be utilized later due to upward movement during dry periods or if the plant has a capacity for deep rooting.

Organic matter content, moisture, and pH are among the other main soil factors causing variations in activity of nitrapyrin applied to different soils (Goring, 1962b). It was found that the need for higher concentrations of nitrapyrin with increasing organic matter content was attributable to sorption and decomposition of nitrapyrin (Hendrickson et al., 1987). Lewis and Stefanson (1975) found that it was not only

organic matter content but also C:N ratio and near neutral pH conditions of soil which reduced effectiveness of nitrapyrin. Hendrickson and Keeney (1978a), Briggs (1975) and Chancy and Kamprath (1980, 1987) also found that increased organic matter caused rapid decline in inhibition by nitrapyrin. It seems that sorption of nitrapyrin by organic matter is a probable reason for reduced effectiveness (Sahrawat, et al., 1987). Some workers have suggested that adsorption of nitrapyrin onto clay particles cannot be expected to occur as nitrapyrin is uncharged (Goring 1962a; Gurthi and Bomke, 1981). However, Powell and Prosser's,(1986b) suggestion that nitrapyrin could chelate with Cu ions associated with clay minerals implies that amount and type of clay mineral may affect the activity of nitrapyrin.

Increasing soil moisture has been found to increase the hydrolysis of nitrapyrin (Hendrickson and Keeney, 1979a,b; McCall and Swann, 1978; Briggs, 1975) whilst nitrification potential was increased at moisture contents up to 60-70% of field capacity (Linn and Doran, 1986). Therefore the net effect of nitrapyrin may be reduced. At higher moisture contents, even though nitrification may be reduced, the efficacy of the inhibitor may be further reduced due to rapid movement of ammonium and urea away from the nitrapyrin.

According to Goring (1962b) increased soil pH promotes nitrification thus reducing the net effect of nitrapyrin. This means that a higher concentration of nitrapyrin is necessary at higher soil pH to achieve the same relative level of nitrification inhibition (i.e. for a similar period of time; see the schematic diagram in Appendix 4). Subsequent investigations by Hendrickson et al.,(1978a,b), Laskowski and Bidlack (1977), Bundy and Bremner (1973) and Sims and MacKown (1987) have supported this conclusion. However, Hendrickson and Keeney (1979a) showed that reduced effectiveness of nitrapyrin at high pH is not due to hydrolysis of nitrapyrin. On further investigation, Hendrickson and Keeney (1979b) found that 'effectiveness' of nitrapyrin increased with increasing pH, and attributed the difference in their results to those of others as being due to:

(i) the adoption of a technique which resulted in a stable population of nitrifiers before nitrapyrin was added.

(ii) the higher susceptibility of nitrifiers at high pH reported by other workers being a result of rapid recovery of nitrifiers that masked the apparent initially low rate of nitrification.

(iii) the possible existence of different nitrifying populations (other than the common Nitrosomonas spp.) that were more susceptible to nitrapyrin at higher pH .

It appears the increased inhibition (or the decreased nitrification rate) noted by Hendrickson and Keeney (1979b) was limited to the initial period of incubation only and further research is necessary to determine the duration of this effect which would be critical to practical use of nitrapyrin (see Appendix 4). Further clarification would

be desirable in view of the fact that Hendrickson and Keeney work assumed a reasonably uniform, near optimal nitrifier population in soils with different pH values following 10 days incubation with 50 mg/g of N added as diammonium ortho phosphate. They also reported that nitrification rates in these soils were linear at least up to 12 days and assumed a steady state. However, in view of the differences in pH, it is unclear whether equal nitrifier populations with similar nitrification capacity could be established (Pang et al., 1975) simply by incubating with an ammonium salt over a similar time period (see Section 2.1.3).

If a steady state of nitrification was achieved due to different nitrifier strains dominating at different pH levels, as shown later by Belser and Schimdt, (1981), then what was being compared was not the effectiveness of nitrapyrin but the response of different strains to nitrapyrin at their different pH optima.

Hendrickson and Keeney (1979b) reported that at pH 4.7 the presence of nitrapyrin hardly affected nitrification during the whole period of incubation. However, heterotrophic nitrification may have been dominant at such low pH and is known to be tolerant of nitrapyrin (Duggins, 1984) so that direct comparison of nitrification inhibition at low soil pH with that at high soil pH may be questionable. On the other hand, even if autotrophic nitrification was dominant over the whole pH range, from the practical point of view the total period of inhibition should also be an important factor in interpreting the effectiveness of the inhibitor. Nitrification recovered rapidly at higher pH (6.6 and 7.2) and more nitrate was produced at the end of incubation. Thus the interpretation of the initial drop in nitrification rate as an indication of lowered "effectiveness" may be inappropriate.

Nevertheless, Hendrickson and Keeney's (1979b) results indicated an important characteristic of nitrification inhibition, namely that if a soil of higher pH supported a larger autotrophic nitrifying population than one of lower pH the initial inhibition by nitrapyrin would have been much greater because a larger active population would have been inactivated (Appendix 4). But the effect may be only short term as nitrifiers will recover more rapidly at higher than at lower pH in the acid range.

Other factors: Among other factors, climatic conditions such as wind speed and temperature have been shown to affect the persistence of nitrapyrin in soils (Goring, 1962b; McCall and Swann, 1978; Briggs, 1975). These factors and the low vapor pressure of nitrapyrin may result in high volatilization losses. However Kalillo et al., (1980, 1982) reported an exceptionally high half-life for nitrapyrin (4 months) applied to soils at an experimental site compared to previously reported figures of 50 days in soil high in organic matter or 28 days in soil with low organic matter (Briggs, 1975).

Laskowski (1972) reported that the rate of hydrolysis of nitrapyrin doubled with each 5°C increase in temperature, suggesting a half-life of 8 days at 25°C.

Touchton et al., (1979) also found that increasing temperature from 10 °C to more than 20 °C markedly reduced the half-life of nitrapyrin. Interestingly, they found that the half-life of ammonium was extended well beyond the nitrapyrin half-life at all temperatures, while without an inhibitor the ammonium half-life was generally less than that of nitrapyrin. Hendrickson and Keeney (1979a) found a longer half-life (up to 1000 days at 4 °C and 10 days at 25 °C and suggested that temperature was more important than moisture content and organic matter in determining the persistence of nitrapyrin.

The nitrifier population (strain, species or genus of ammonium oxidizing autotrophic bacteria) dominant in the soil, also seems to be an important factor in the effectiveness of nitrapyrin (Belser and Schmidt, 1977; Hendrickson and Keeney, 1979b). However, few studies have been published on the critical concentrations of nitrapyrin necessary for effective inhibition of different strains and species, or even genera of nitrifying bacteria. This probably could be one of the most vital reasons for the variability of nitrapyrin effectiveness reported in the literature.

2.3.5. Use of nitrification inhibitors in crop production

The effects of nitrification inhibitors on the yield and performance of crops under field conditions has been reviewed by Slangen and Kerkhoff (1984), Prasad et al., (1971), Nelson & Huber (1980), and Onken (1980).

Although nitrapyrin has been shown consistently to be an effective nitrification inhibitor, nitrapyrin application has not always been followed by desired yield and N efficiency improvements (Appendix 1). Even positive results have been highly variable and effectiveness appears to depend on many factors such as soil characteristics, active nitrifier strains, climatic conditions, crops grown and N management. Some workers that have reported no yield or N use efficiency improvement following inhibitor application have given no, or insufficient, details of changes in soil N content, native soil N levels, soil texture etc. Such information would be important as the original soil N levels may be sufficient to supply the total plant N requirement thus removing the possibility of response or there may not be significant loss of nitrate-N from the soil used due to poor drainage, and/or low denitrification potential to show a significant advantage in retaining N as ammonium. For instance, Aydeniz et al., (1976) found that barley responded to application of nitrapyrin while vetch and maize did not. Such observations as this could be due to differences in N requirement by crops. Support for the need of supplementary information together with results on crop performance comes from Swaider (1985) who reported positive yield responses to nitrapyrin application at low N fertilizer rates but not at higher rates.

Results obtained by some workers following nitrapyrin application to different crops

under different conditions are summarized in Appendix 1. This table shows the considerable variation in response to nitrapyrin, even with the same crop.

2.3.6. Need for further research on use of nitrapyrin under different environmental conditions

It is clear that several aspects related to the factors affecting nitrapyrin are still not understood thoroughly. Especially in view of the considerable variability in crop performance following nitrapyrin-amended fertilizer application (Appendix 1) further research on the influence of external factors on activity of nitrapyrin and also on the mechanism of inhibition are warranted. Points highlighted in the review that are relevant to the work presented in this thesis are summarized in the following paragraphs.

Agricultural practices such as liming may result in changes in soil N transformation. These changes may promote N losses by nitrate leaching and/or denitrification. However, research results vary considerably and the final outcome appears to be controlled by interaction of various soil factors unique to the environments in which the experiments were conducted. Nevertheless the use of nitrification inhibitor (eg nitrapyrin) as a fertilizer amendment has been proposed as an effective measure to prevent nitrate formation under conditions of potentially high N losses. However, the results obtained after using nitrapyrin also vary greatly and it is clear that more investigations are necessary to understand these variations.

It appears that the effectiveness of nitrapyrin depends on three different types of factors. viz, chemical and physical characteristics of nitrapyrin, soil and other environmental factors, and the active form of nitrifying population (size and type). However, there is a lack of agreement on the influence of these factors on nitrapyrin effectiveness. The effect of soil pH or the secondary changes brought about in soil following soil pH changes on nitrapyrin effectiveness is one such issue that needs further clarification. Therefore, for better understanding of the inhibition process it would be useful to carry out experiments on effectiveness of inhibitors on different types of nitrifying populations subjected to varying environmental factors.

The relative mobility and persistence of the chemical, under different environmental conditions are also important criteria that may influence nitrapyrin effectiveness. Here, too, the results vary, underlining the need to study possible interactions of primary and secondary factors on effectiveness of nitrapyrin in different environments and under different agricultural practices (e.g. method of application, irrigation etc.,).

Use of nitrapyrin is supposedly ineffective against all other microflora except autotrophic ammonium oxidizers and is also supposed to be non-toxic to higher plants. However, some workers have reported changes in denitrification and soil

respiration indicating an apparent effect on heterotrophic microorganisms while some toxic symptoms on plant seedlings have also been reported. Information on these aspects although of considerable importance for wider practical applications is inadequate.

Application of nitrapyrin with N fertilizers has not always yielded desired improvements in crop performance. It could be that the amount of fertilizer-N added in such studies is sufficient to meet the plant N needs. Introduction of nitrification inhibitors may therefore not have any effect on N use efficiency (NUE). On the other hand if plants prefer a particular form of nitrogen (either ammonium or nitrate) and the maximum yields are achievable only by supplying N in that form, the attempt to supply N in the less prefered form will only reduce N use efficiency and final yields (Luckman, pers. commun. 1988). However, it is not easy to establish which form is preferentially absorbed by plants under given soil conditions. Nevertheless, it is possible to compare a particular type of fertilizer-N with other forms (e.g. urea, ammonium sulphate ammonium nitrate or calcium nitrate) in terms of NUE or crop growth. Such comparison would help to indicate any need for the amendment of less efficient fertilizers with nitrification inhibitors.

CHAPTER THREE

3. General Materials and Methods

3.1 Soil sampling and analysis:

3.1.1 Soil site description:

Field experiments were conducted on the University of Tasmania Farm near Cambridge, Tasmania, 20km north-east of Hobart at 42 50 S latitude (Appendix 5). This area is included in the Hobart sheets of the TASMANIA Geological survey and of the Reconnaissance Soil Map of Tasmania (Loveday, 1955). The scale of these surveys is too small to show the full diversity of lithology and soils. An important occurrence of unconsolidated Tertiary clays was not recorded in the geological survey nor as a parent rock in the reconnaissance soil survey. However, the author had access to more detailed information on soil occurrence (Beattie et al., unpubl.) and to current research on principles of soil occurrence (Holz, pers. comm.) which facilitated the choice of site for field experimentation. This area has been farmed for at least 150 years and its recent history is one of intensive cereal cropping. Some relevant climatic data for the cultivation seasons 1986/87 and 1987/88 are shown in Appendix 6

A map showing the location of field experiments and sampling sites from which specimens were collected for analysis and use in laboratory and glasshouse experiments is shown Appendix 5. Plate 1 shows the site of experimentation and its topography including the slight slope of the field. A hardsetting strongly duplex soil (Northcorte, Dy 5.21) occurs at this site above Tertiary clays. A grey-brown acid sandy loam rests abruptly on a very slowly permeable mottled yellow-brown clay subsoil becoming neutral to alkaline with depth. The cultivated layer contained medium levels of bicarbonate-extractable phosphorus and exchangeable potassium for the nutrition of cereal crops. These and other relevant soil characteristics are given in Table 3.1 and were obtained using methods listed in Table 3.2. Published methods have not been described in detail except where some modification was necessary for the present application.

3.1.2 Collection of soil samples:

Soil specimens from field plots for experiments described in Chapters 5 and 9 were collected from 0-150mm depth, using a 25mm stainless steel tube sampler. Three core specimens were collected randomly from each plot and mixed well before subsampling to obtain the desired quantity of soil in aluminium moisture boxes. Soil was kept in a cooler box (with ice packs) during transport to the laboratory.

A bulk sample (0-150mm) was collected on 5th. May 1986, from an area immediately adjacent to the experimental site and used for all laboratory and glasshouse experiments other than experiment 1. Soil from four randomly selected

(2 x 2m) quadrats was collected by shovel, to a depth of 150 mm, mixed and air dried in the glass house. When sufficiently dry, the soil was passed through a 2mm sieve and stored at 2°Cuntil needed. All visible plant debris was removed by hand.

3.2 Plant material collection and analysis

Unless otherwise specified growth measurements were based on samples collected from 0.25 m^2 quadrats selected randomly within the plots. The crop was cut just above the ground surface and transported in polythene bags to the laboratory for analysis. Samples not processed on the day of collection were stored at 4°C.

Final yields were based on mechanical harvesting of the remaining area of the plot (plate 2, page 54) except in the experiment described in Chapter 7 where yields were based on quadrat sampling.

The following plant material analyses were carried out in order to determine N use efficiency and plant growth performance.

-Total N, as described by Chapman and Pratt (1961).

-Dry matter production, by drying at 70 °C for 72 hrs in a forced draught oven.

-Leaf area, using a Paton Electronic Light Planimeter developed in conjunction with CSIRO.

-Percent light interception using a pyranometer as described in experiment 7.

-Plant height (at harvesting) as the length of stem from ground surface to panicle base.

3.3 Data analysis.

Plant and soil data have been expressed on an oven-dry basis unless otherwise specified. Details of experimental design, treatments, and statistical analysis are discussed separately for each experiment. All statistical analyses were carried out on Apple IIe and Macintosh SE computers using suitable statistical soft-ware packages (ANOVA II ™ or NWA STATPAK™, STATVIEW™). Most of the figures produced in the text were drawn using the CRICKET GRAPH™ software package on a Macintosh SE.

Table 3.1: Characteristics of the soil

Soil character	Depth (mm) 0-150	150-300	300-500	500-750	750-1000
pH	5.3	6.8	7.3	7.9	8.3
EC (ms/cm)	0.107	0.093	0.153	0.236	0.254
Bulk density(g/cm^3)	1.29	1.57	1.68	1.75	na
Moisture characteristics (MPa)					
(at 0.03 MPa)	28.33	35.5	60.9	62.0	na.
(at 0.1 MPa)	16.4	26.7	46.35	45.6	na.
Org. C (as at5/6/86)	1.98	1.79	0.45	0.09	0.02
Tot N % (as at5/6/86)	0.168	0.078	0.055	0.041	0.042
Mineral N (2M KCl extractable)					
ammo.-N(mg/g soil)	0.17	0.12	0.13	0.11	0.12
nitr.-N(mg/g soil)	0.06	0.09	0.06	0.03	0.03
Particle size fractions (per cent)					
coarse sand	16.4	14.1	12.8	20.5	18.5
fine sand	64.5	50.3	45.1	42.5	44.5
silt	6.5	4.0	6.5	8.5	10.5
clay[1]	14.0	38.1	36.2	29.1	27.8

1. X-ray diffractograms showing types of clay minerals present are given in Appendix 7.

Table 3.2 : Methods used for soil analysis

Determination/analysis	References
Soil particle size analysis:	Loveday (1974)
Clay type	Xray diffraction method(Appendix 7)
Bulk density of field samples	(Loveday, 1974).
Field capacity (moisture content at 0.03 mPa)	Peters (1965).
Total N (salicylic acid/$Na_2 S_2 O_3$ modification of Kjeldhal method)	Bremner (1982).
Mineral N (ammo. and nitrate)	Bremner (1982).
Urea N	Tabatabai & Bremner (1972).
Urease activity (buffer method)	Douglas and Bremner (1971).
Mineralizable N	Waring and Bremner (1964).
Denitrification potential	Modified after Sarachandra (1978)
Soil respiration	Modified after Anderson (1982),chapt.6
Estimate of CO_2 and N_2O	Thermal conductivity gas chromatography (Appendix 8)
Estimate of nitrapyrin concentration in soil	Chapter 8
Oxidizable organic C	Walkley and Black C:Piper (1974)
Soil profile moisture content (CPN503DR Hydroprobe)	Neutron probe manual (1984)

Plate 1: The Site selected for field experiments conducted during 1986/87 and 1987/88 seasons.

Plate 2: Mechanical harvesting of plots at the end of 1986/87 season

CHAPTER FOUR

4. Selection of soil for nitrogen transformation studies

4.1. Introduction

In view of the objectives of this project it was necessary to find a soil having an active native nitrifier population to justify the use of nitrification inhibitor (nitrapyrin) and to study the interaction of nitrification inhibitor with liming on nitrate production.

The addition of fertilizer urea to soils can trigger several microbially-mediated nitrogen transformation reactions such as urea hydrolysis, nitrification, immobilization and denitrification of the added N. As the nitrification process is related to other urea-N transformation reactions it is appropriate to characterize this process in conjunction with these other reactions.

In order to identify soil material suitable for further studies the effect of urea addition on urease activity and nett nitrification was investigated using the cultivated layer of four soil types. These materials were also characterized in terms of potential denitrification and soil respiration because these properties should be useful indicators of potential denitrification losses and heterotrophic microbial activity respectively.

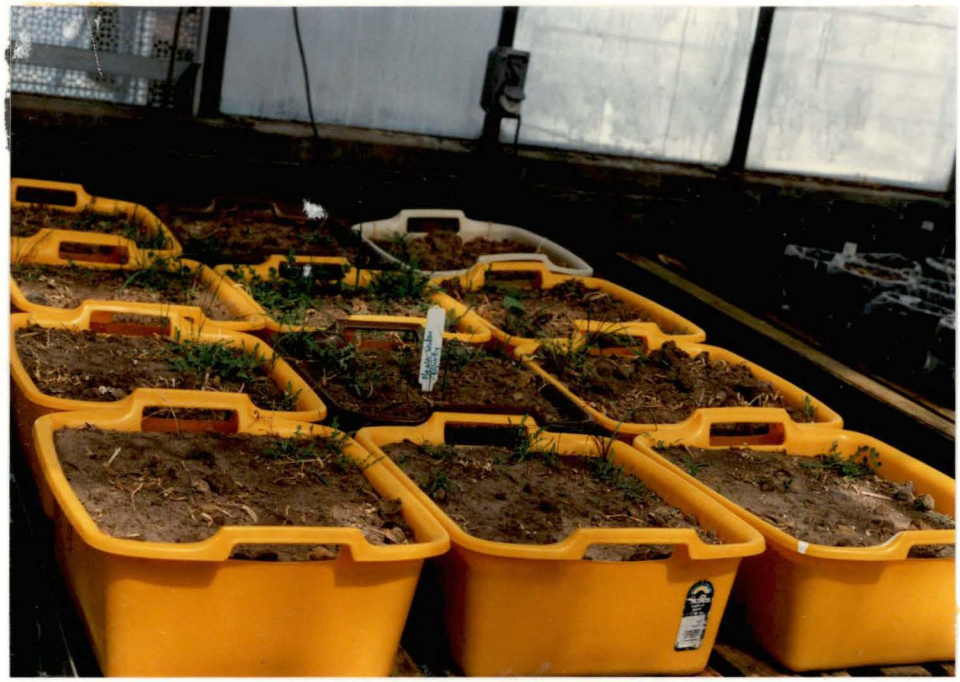
4.2. Materials and methods

Soil blocks (30 x 30 x 20cm, Plate 4.1) were collected in duplicate from sites representing four soil types (Appendix 5).

Soils 1 and 2 were sandy loams while soil 3 was a self-mulching, light clay and soil 4 was a sandy clay loam. The first three soils have formed on unconsolidated Tertiary clays with interca lated sandy members. Soil four has formed in colluvium of mixed origin including material derived from Permian mudstone, Triassic sandstone and Jurassic dolerite. Soils 1,2 and 4 are strongly duplex with a clay B2 horizon 15-20 cm beneath the surface. soil 3 is a uniform to gradational cracking clay. Soils 1,2 and 3 carried pasture sown during the previous season while soil 4 carried wheat. Sites 1 and 3 were on east facing 7% slopes while 2 and 4 were on 2% slopes. Short periods of waterlogging of the topsoils of the duplex soils were observed occasionally due to perching of drainage water above the slowly permeable clay B2 horizon.

Undisturbed soil blocks were used to minimize "handling effects" (Zantua & Bremner, 1975; Ogunkunle and Beckett, 1988). The size of the soil blocks was such as to allow sequential subsampling without interference effects. A metal

Plate 3: Soil Blocks used for experiment described in Chapter 3.



frame made of four (30cm x 30cm) aluminium plates (18 gauge) was driven into the soil (relatively free of stones). The blocks were lifted by removing the surrounding soil and inserting another plate beneath the block. Blocks were placed in square plastic containers (Plate 4.1) with several 1.5cm diameter drainage holes cut in the bottom. After transporting to the glasshouse vegetation was removed with minimum disturbance to the soil surface and the blocks were then left for one week to equilibrate with the glasshouse environment (15°C). During this period the blocks were regularly moistened with distilled water to a weight equivalent to 70-80% of measured field capacity (Loveday, 1974).

Table 4.1: Soil Characteristics of four soil types collected from the University farm.

soil	pH	CEC*	Org. C	Total N	soil texture (0-150mm)
1	5.3	6.26	1.98	0.16	Sandy loam
2	5.8	4.77	1.86	0.18	Sandy loam
3	6.2	7.03	2.48	0.26	Self mulching light clay
4	5.2	6.72	2.26	0.24	Sandy clay loam

*-CEC as mmol($Ca^{+}_{0.5}$)kg^{-1}/100 g soil.

After equilibration, the surface soil (1cm) of each block was removed and urea was added to the surface at the rate of 100 ug urea-N/g soil (calculated using the field bulk densities). The surface soil was then replaced and eight rectangular micro-plots (120mm x 60mm) were marked in the surface of each block, allowing a 30mm buffer zone around the periphery.

Sampling was carried out at intervals over a period of 60 days. The day before fertilizer treatment was recorded as day 0. A 30mm diameter PVC tube was used to withdraw core samples which were air dried sufficiently to pass through an international 2mm round-holed sieve before analysis.The blocks were watered to field capacity at five-day intervals during the experiment.

Mineral nitrogen was determined using the Kjeltec apparatus (Tecactor Instruments) as described by Keeney and Nelson (1982) with 0.2g of Devarda's alloy added to include nitrite-N (if any) and nitrate-N.

Urease activity was determined after 5 days of fertilization by the method of Douglas and Bremner (1971) except that the technique was modified by omission of added urea (Bremner, 1982) to determine urea-N remaining at 6 DAF.

Nitrification activity and rate was estimated as the change in nitrate-N concentration with time. Since the use of nett nitrate-N to determine nitrification rate

can underestimate actual nitrification because part of the nitrate may be immobilized or denitrified (Schimdt, 1982) the change in ammonium-N was also considered.

Soil respiration was measured on 5g (O.D.) soil specimens at 2 days after fertilizer addition (DAF) which were sieved before addition to 125ml glass bottles. 1.0g of dextrose was added to each bottle as a source of carbohydrate before the air inside the bottle was replaced with moistened carbon dioxide-free air. Bottles were sealed with rubber septa and incubated at 25°C for 8 hrs. Assay of carbon dioxide production was performed at two-hour intervals by thermal conductivity gas chromatography (Appendix 8).

For determination of **denitrification potential** 5g (O.D.) of soil was placed in 125ml bottles and flooded by addition of 15ml of distilled water. The gas phase was replaced with nitrogen. Then acetylene, scrubbed in water to remove any acetone vapor present, a contaminant that otherwise would provide an excellent C source for denitrifiers (Keeney, 1986) was injected into each bottle to give 10% gas volume after first removing an equivalent volume of nitrogen. The bottles were then incubated at 20°C with assay of nitrous oxide production at specified time intervals by thermal conductivity gas chromatography. The incubation was terminated after 248 hours.

3.3. Results and Discussion

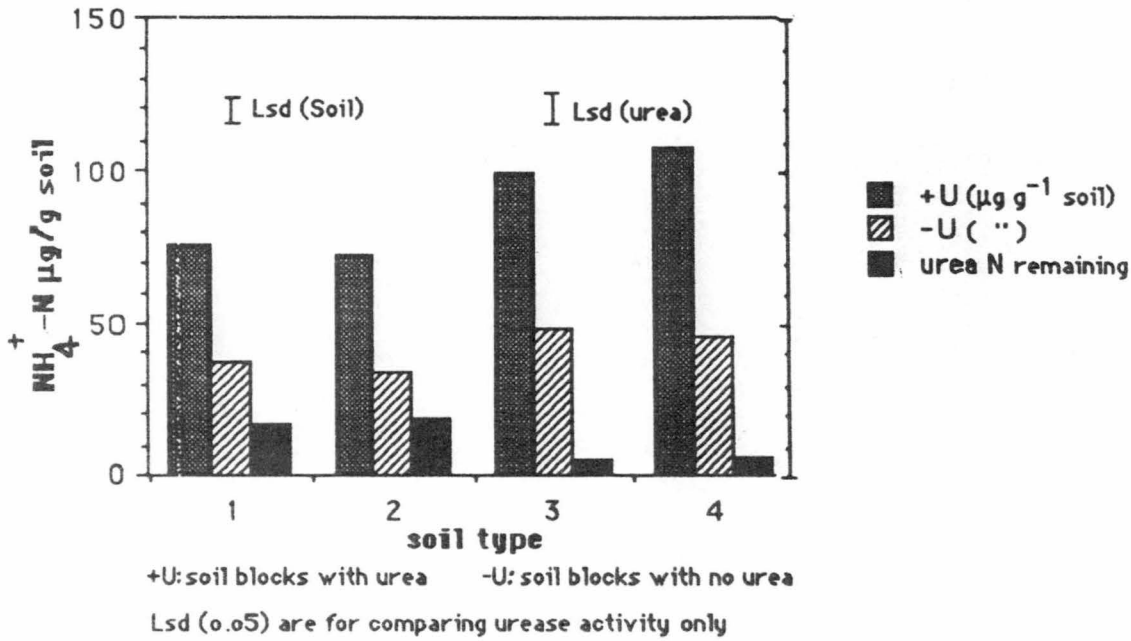
Urease activity. All four soil types showed a significant increase in urease activity (Fig. 4.1) following addition of urea. This indicated that all four soil types had populations of urease-producing microorganisms capable of rapid response to added urea. Soil specimens tested for urea-N at 6 DAF showed some unhydrolyzed urea-N (Appendix 9) suggesting that substrate concentration was not limiting in any of the soils at the time of sampling, and therefore that the levels of urease activity of the four soils could be compared directly. As long as the soils are in a supposedly dynamic state of activity with respect to urease-producing organisms the problems of systems with declining urease production can be avoided.

Urease activities were significantly higher in soil type 3 and 4 than in the other two types (Fig 4.1). This could have been due to a higher level of organic matter (decomposed and otherwise) present in these two soils, supporting a greater microbial population capable of producing urease, or to stabilization of an increased level of urease on organic matter (Mortland, 1986) or perhaps both mechanisms may have been involved.

When urea is rapidly hydrolyzed substantial NH_4^+-N production can occur within a short period of time leading to increased nitrification, immobilization and/or ammonia volatilization loss.

Figure 4.1 Urease activity (5DAF) and urea-N remaining in four soil types (6 DAF)

DAF-days after fertilizer application



(See Appendix 9 for data)

Nitrification. Application of urea resulted in significantly different nitrate-N production in each of the four soils when compared to respective controls that did not receive urea (Figure 4.2a). The data and analysis of variance for each sampling time are given in Appendix 10.

The increase in nitrate-N at 5 DAF was significant only for soil 2 indicating an immediate "take-off" in nitrification when compared to other soil types. However, at 10 DAF the nitrate-N levels in all soils had increased significantly, indicating a progressive nitrification process. Continued incubation of soils 2 and 3 did not result in increased nitrate-N beyond the levels measured at 20 DAF. Soils 1 and 4 showed continued nett nitrate-N production until about 40 DAF after which a slight decline was measured.

The apparent stabilization of nitrate-N from 20 or 40 to 60 DAF could have been due to a decline in ammonium-N available for nitrification or due to the activity of different nitrifying populations as suggested by Bhuiya and Waker,(1977), Belser and Schmidt, (1981) and Wiere and Gilliam, (1986). On the other hand, since the estimated level of nitrate-N reflects nett level of nitrate-N, the possibility remains that denitrification, immobilization of nitrate or decline in level of substrate ammonium-N (Figure 4.2b and Appendix 10) could have influenced the estimates.

Because of these possibilities for the later levelling-off in nitrate-N, evaluation of the different soils for nitrification rate was based on "nett production of nitrate N" (difference between urea-treated soil and untreated soil) until peak production levels were reached.

The four soils showed different nitrification rates (Figure. 4.2C). Soil 2 had the highest nitrification rate (7.459 ug/g/day) whilst soil 4 had the lowest (0.979 ug/g/day). Soils 1 and 3 had similar nitrification rates (1.926 and 1.877 ug/g/day respectively). When Morill and Dowson (1967) found different nitrification rates/types they commented on the variable influence of other N transformation processes. These included (1) immobilization of nitrate, (2) immobilization of ammonium, the latter reducing substrate concentration for nitrifiers, (3) denitrification and (4) possible differences in nitrifying and/or denitrifying populations amongst different soil types (Chapter 2, Section 2.1.3).

Nitrate immobilization can be considered the least significant factor in nett nitrification rate since microbes preferentially utilize ammonium to nitrate, especially over a short time period as in this experiment and when ammonium is available abundantly. Little or no loss of nitrate by leaching was likely under controlled watering. Any nitrate losses may therefore be attributed to denitrification. However, since the soil blocks were not subject to anaerobic conditions the extent of denitrification must have been minimal. Hence, soil factors,

Fig. 4.2a Nitrate recovered from four soil types described in Table 4.1

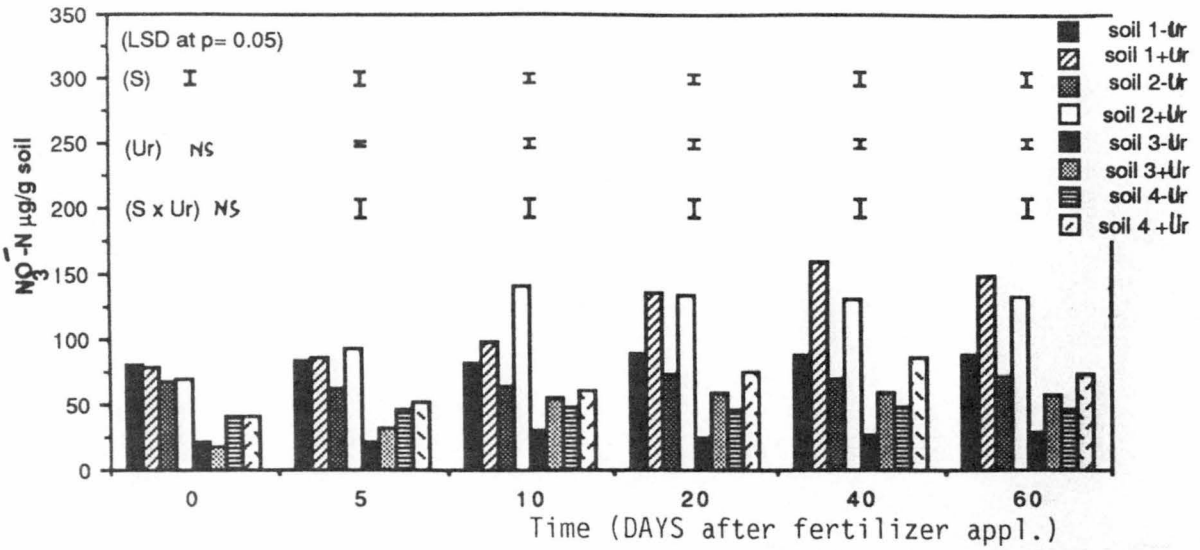
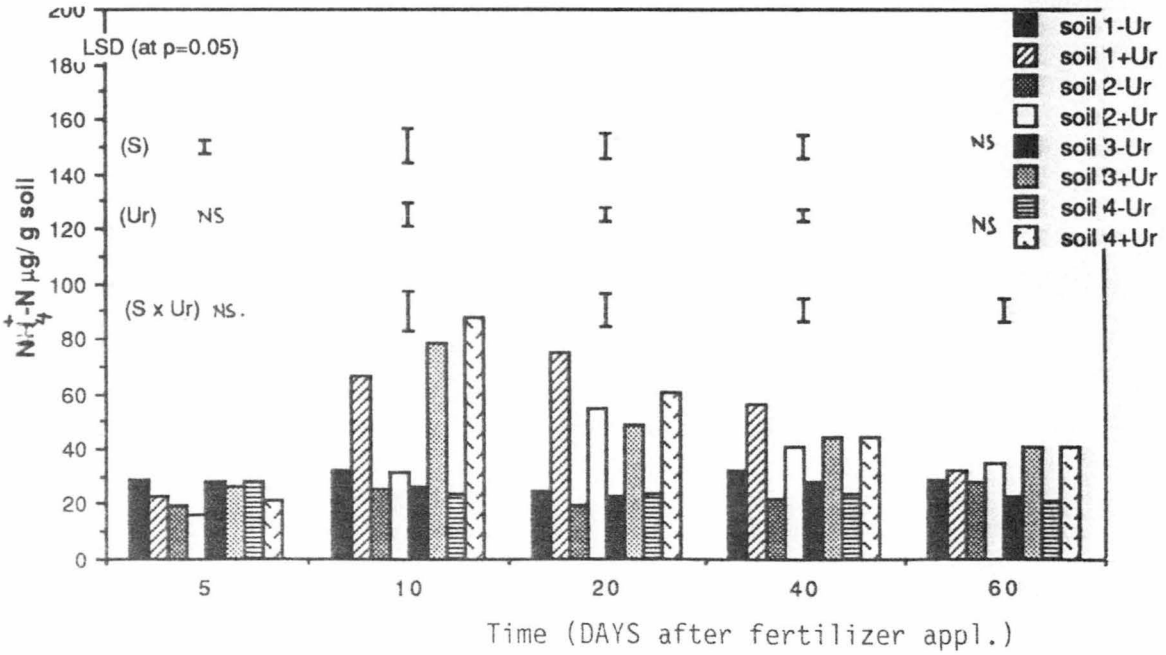


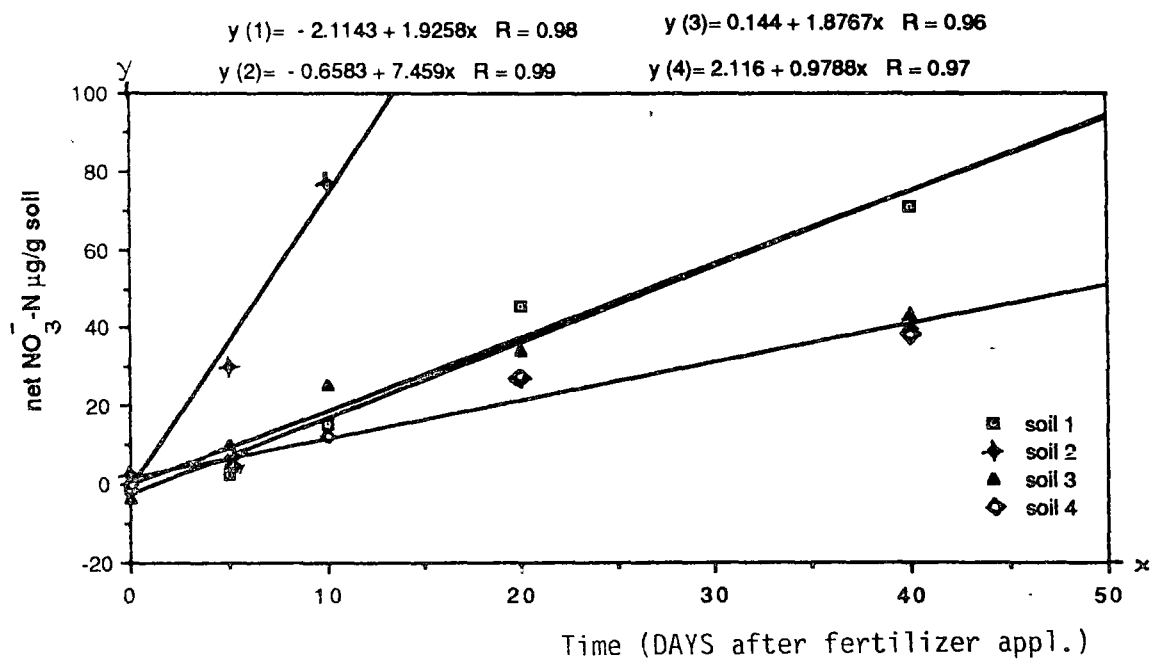
Fig. 4.2b Ammonium recovered from four soil types described in Table 4.1.



+Ur: with urea , -Ur: without urea.

Fig. 4.2c Net nitrification rates in four soil types described in Table 4.1.

(net NO_3^--N) = (NO_3^--N in urea added soil)- (NO_3^- -N in urea free soil)



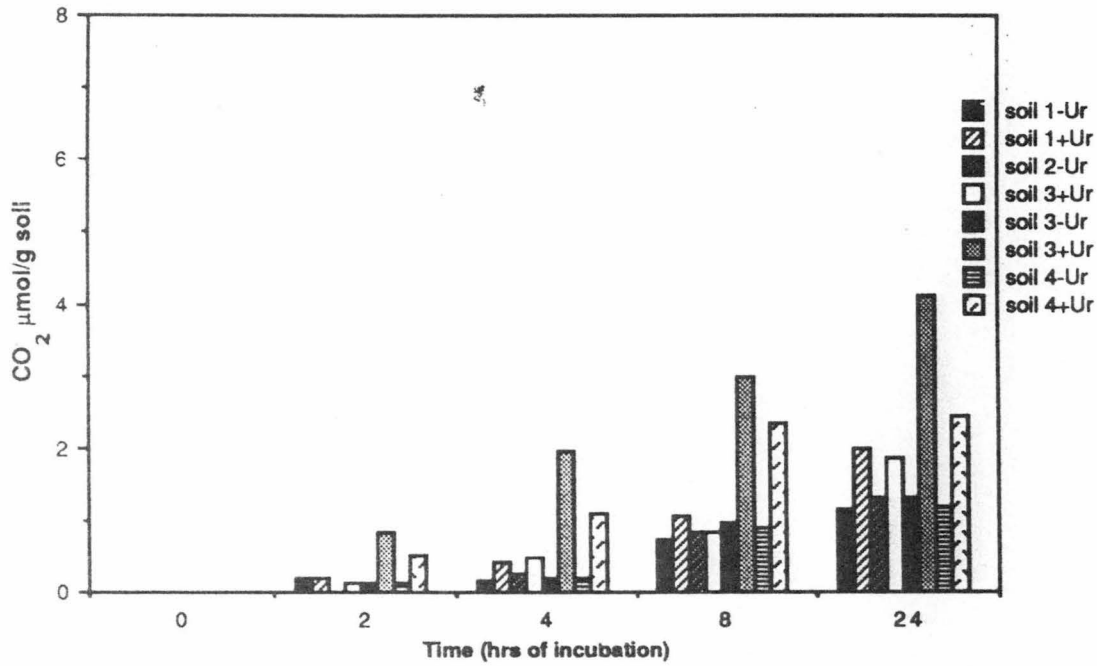
differences in nitrifying populations, and differences in ammonium losses (immobilization and/or ammonia volatilization) may be the more probable causes of the observed differences in nett nitrate production.

Although all soils were of almost similar reaction (pH) properties such as CEC, organic matter content, and C/N ratio were different. Organic matter content, depending on type and state of decomposition, could have influenced the extent of nitrification via competition by heterotrophs for ammonium-N. The high negative correlation between nitrification rates and per cent organic C at 20 DAF (r^2=-0.75) suggests that those soils with higher C/N ratio may have favoured the utilization of ammonium-N by heterotrophs rather than autotrophic nitrifiers. The sharper decline in ammonium-N levels compared to the slower increase in nitrate-N in soil types 3 and 4 may be due to immobilization of part of the ammonium-N (Fig. 4.2a and b).

Loss of nitrogen can occur via ammonia volatilization following increase in soil pH caused by urea hydrolysis. The relatively longer generation time of autotrophic nitrifiers (20-40 hrs. in situ for nitrifying organisms) in comparison with shorter times for most heterotrophs (Knowles et al., 1965; Schimdt, 1974) and the nature of the C assimilation process, with an incomplete tricarboxylic cycle which further decreases their already limited metabolic versatility (see section 2.3), make autotrophic nitrifiers less efficient in utilization of ammonium-N compared with heterotrophs (Quayle & Ferenci, 1978). Hendrickson et al., (1987), also found that pH increases associated with urea additions promoted the N immobilization process and and that this may have occurred in preference to nitrification.

Soil type 1 had a relatively steady nett nitrate-N production ending up with a significantly higher level of nitrate-N than soil types 3 and 4 by the end of the experiment. Soil type 2 on the other hand had the highest initial increase in level of nitrate-N production at 5 and 10 DAF(Fig. 4.2a). However, when nitrification rates were estimated (Fig. 4.2c), soil type 1 and 3 appeared to be similar with moderate rates when compared to nitrification rates of the other two soil types (nitrification rates estimated by using nett nitrate-N produced until peak levels were reached). Soil type 2 had the highest nitrification rate while soil type 4 showed the lowest rate, probably due to one or more of the above-cited reasons and/or because of heterotrophic nitrification which occurs at a much lower rate than the autotrophic process (Duggins, 1984; Section 2.1). It was also noted that although the nitrification rate of soil type 3 was similar to that of soil type 1, the amount of nitrate produced in soil type 3 reached a plateau after 20days and this amount was significantly less compared to that of soil type 1(fig 4.2a). On the other hand, since soil cation exchange capacity may affect the availability of N for nitrifiers (Sarathchandra, 1978) it is possible that in soil type 3, which had the highest CEC (Table 4.1), nitrifiers had access to less ammonium than

Fig. 4.3: CO_2 production in four soil types with (+Ur) and without(-Ur) urea.



Lsd (p=0.05)

Time	Soil	Urea	Urea x Soil
0	0	0.0	0.0
2	0	0.015	0.0
4	.035	0.025	0.045
8	.105	0.10	0.170
24	.125	.045	0.075

y 1(+ur) = -0.2369 + 0.2416 x R=0.95
y 2(+ur) = -0.2194 + 0.2214 x R=0.94
y 3(+ur) = -0.0916 + 0.5192 x R=1.00
y 4(+ur) = -0.056 + 0.3345 x R=0.98

in other soils. Therefore, soil types 1 and 2 were considered to be more suitable for further evaluation.

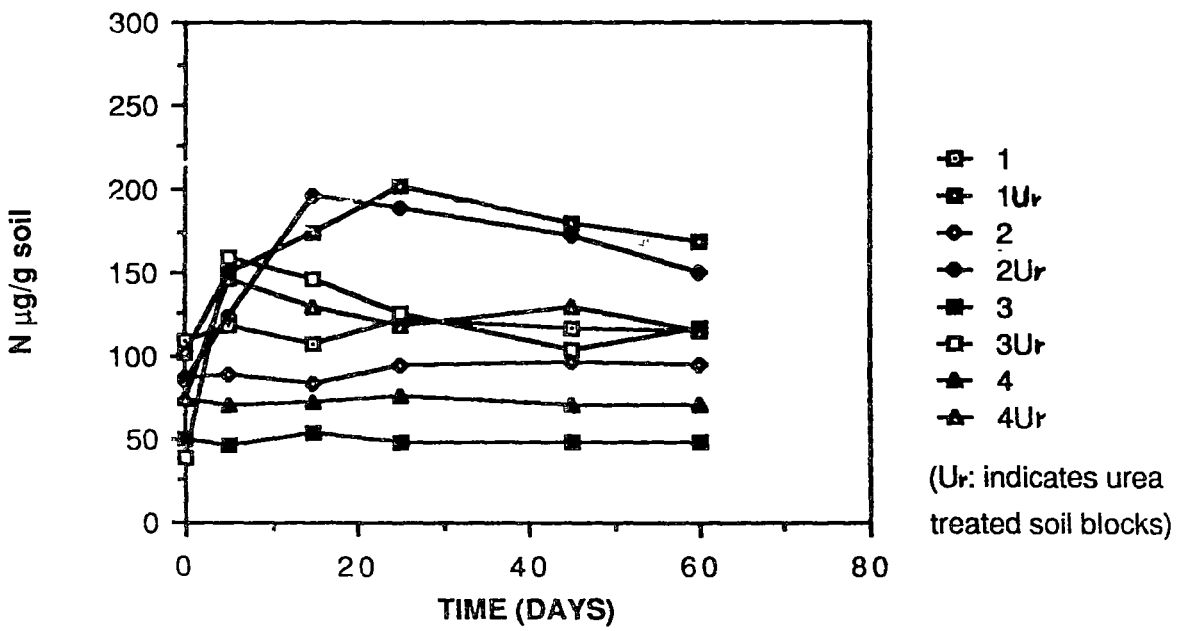
Soil respiration. Heterotrophic activity increased significantly within 48 hours of addition of urea (Figure. 4.3; Appendix 11). Levels of carbon dioxide produced after 2 hours of incubation were not significantly different, probably because of adaptation time. However, differences appeared after four hours of incubation.

Comparison of soil respiration after addition of urea with that of untreated soil revealed that all four soil types contained microbial populations that responded to urea, but to different degrees. The observed increases may be attributed to activation of urease-producing and ammonium-utilizing heterotrophic micro-organisms. Soil types 3 and 4, with their larger amounts of undecomposed organic plant residues and higher C/N ratio, probably supported a larger microbial population than that of soil types 1 and 2. This would mean that urea application may lead to greater nitrogen immobilization in soils 3 and 4 than in soils 1 and 2. Hendrickson et al., (1987) made a similar observation. Since, immobilization can remove a significant amount of ammonium from the available mineral-N pool, reducing the amount of ammonium available for nitrification, these results partly explain the much lower nitrification levels in soil types 3 and 4 than in soil types 1 and 2 (Fig. 4.2a).

The levels of total mineral N measured at each sampling time (Fig. 4.4) also supported the possibility of variability of immobilization processes in the four soil types. Five days after urea addition 84% and 86% of the initial amount of N (original N plus added urea-N) in soil types 1 and 2 respectively was present as inorganic-N , whereas in soil types 3 and 4 these values were only 65% and 64% respectively.

Denitrification activity and denitrification potential. Comparison of of denitrification potential was based on the assumption that the denitrifying population (form and size) directly influenced the rate of denitrification. In fact, depending on the type of active population, the extent of inhibition by acetylene could also vary (Keeney, 1986). Hence, depending on the activity of denitrifier organisms, soil specimens collected at intervals and incubated under optimum conditions could show varying rates of nitrous oxide production. That is, if the initial population or type of denitrifiers in the soils were different, then variable rates of "take-off" in nitrous oxide production could be expected. However, doubts have been raised recently about the suitability of the acetylene blockage technique for measuring denitrification (Rolston, 1986; Keeney, 1986) in the wake of

Fig. 4.4 Total mineral N (NH_4 +NO_3 and NO_2-N) in four (1,2,3 and 4) soils at different sampling times.



suggestions that inhibition of nitrous oxide reduction by acetylene may disappear with time (Mosier, 1980; Aulakh, 1984), that acetylene could even increase denitrification (Germon, 1980; Yeomans and Beauchamp, 1982), or that inhibition of nitrification may be effective only at low partial pressures of acetylene (Davidson et al., 1987). Short term incubation of nitrate-enriched media with sufficiently high concentrations of purified acetylene may overcome these problems to some extent and the advantages of the method have been widely accepted (Duxbury, 1986).

As this experiment was carried out to compare only the denitrification potential at each sampling time, rather than to study the effects on nitrate-N, no parallel estimates without nitrates were carried out. Denitrification potential in soils without added urea was not expected to show any significant change during the course of this experiment

Figure 4.5 (a-c) shows nitrous oxide production in incubated soil specimens collected from soil blocks at 5, 20 and 60 days after fertilization (DAF). Most specimens reached their maximum nitrous oxide production levels before 240 hours of incubation. The decrease in, or complete cessation, of nitrous oxide production after about 72 to 120 hours (depending on soil type) may be due to a decreasing inhibitory effect of acetylene on nitrous oxide reduction as has been reported in recent investigations (eg. Adkin and Knowles, 1986; Keeney, 1986; Davidson et al., 1987). Common reasons given for such an effect include the possible adaptation of the denitrifying population, loss of acetylene by microbial reduction and promotion of growth of some denitrifers that reduce nitrous oxide in the presence of acetylene. Since there is evidence that carbon dioxide production occurs even under anaerobic conditions, carbon dioxide toxicity could also be a factor influencing such decline in denitrification (M.A.Line, University of Tasmania, pers. Comm).

For these reasons, determination of denitrification potential via estimation of rate of nitrous oxide production was restricted to the initial period of incubation where a steady state was observed. Slopes calculated from linear regression equations of the fitted curves (Fig. 4.5) are given in the Table 4.2.

Rate of nitrous oxide production rose in all specimens at first, then declined progressively in all cases but at different rates. In specimens incubated at 20 DAF, lowest rates were realized with soil type 1 while the highest rate (denitrification potential) was seen with soil type 2. It is possible that waterlogged conditions that develop occasionally in the field from which soil type 2 was extracted may have promoted a higher denitrifying population. Soil types 3 and 4 had moderate rates and magnitude of nitrous oxide production. As the rate of denitrification is a function of the active denitrifying population (or different forms of denitrifiers),

Fig.4.5 : Nitrous oxide production in specimens of four soils sampled at 5,20 and 60 DAF and incubated anaerobically for 240 hours with added nitrate-N

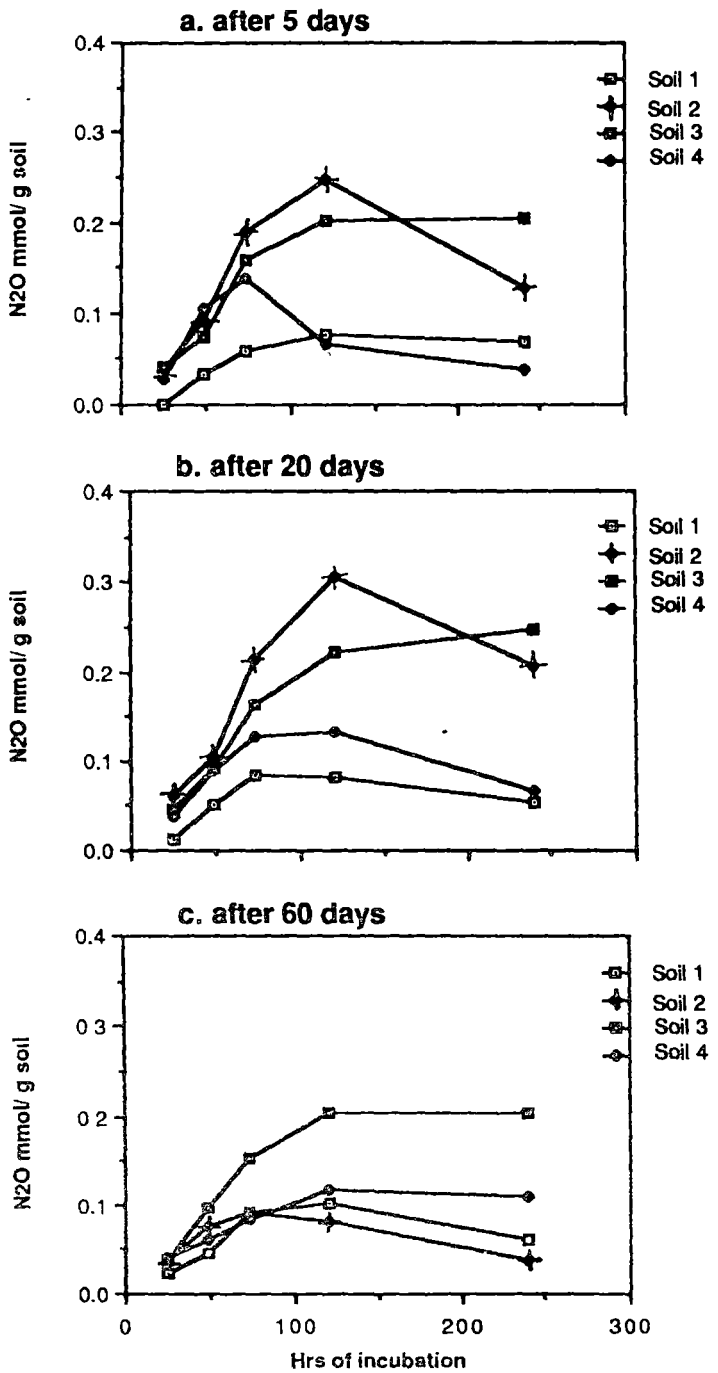


Table 4.2 Slopes of linear regression equations derived for relationships between nitrous oxide production and time of incubation for four soil types.

		Slope (rate in m mol/g/hr)			
DAF	soil type	1	2	3	4
5		0.77	2.2	1.7	2.2
20		1.5	2.7	1.9	1.9
60		0.85	1.1	1.7	1.7

(DAF: Days after fertilizer addition= times at which soil specimens were withdrawn from soil blocks treated with urea).

soil type 2 has the greatest potential denitrification (N-loss) if optimum conditions for denitrification occur. For this reason soil type 2 was eliminated in favour of soil type 1 for further studies.

It may be noted that nitrous oxide production occurred only after the creation of optimum conditions for denitrification. Thus no significant denitrification in undisturbed soil blocks used for this experiment would normally be expected. However suitable conditions for denitrification could develop in the field situation and if such were the case the impact of denitrification losses would be expected to be greatest in soil type 2, followed by soil types 3 and 4, with the least affect in soil type 1.

In summary the total mineral N recovered from each soil showed that losses from the mineral N pool were least in soil types 1 and 2 while soiltypes 3 and 4 had considerably higher immobilization losses. However, soil type 2 showed considerably higher potential denitrification when compared to soil type 1. Urease activity and soil respiration rate of soil type 1 were moderate indicating a satisfactory level of microbial activity. Therefore soil type 1 showing least potential loss of N by denitrification was selected for further studies on the transformation of ammonium-N to nitrate-N and to investigate the effects of lime and nitrapyrin on this process.

CHAPTER FIVE

5. The effect of urea and other common N fertilizers on growth and N nutrition of a barley cultivar (cv. Triumph).

5.1 Introduction

There is considerable variation in the efficiency of different N fertilizers measured in terms of crop response (Rennie and Rennie, 1973; Oluobi et al., 1986). Some studies have shown that urea compares poorly with other common N fertilizers such as ammonium nitrate, ammonium sulphate and calcium nitrate (Kucey, 1987; Campbell et al., 1986) whereas others found it to be no less effective than those fertilizers (Thorman et al., 1980; Walker et al., 1979; Terman et al., 1968). Possibly, such differences occur because different forms of added N undergo different kinds and intensity of N transformation processes. The extent of each process is known to depend on factors such as type of crop, cropping practice, climate and soil characteristics, all of which affect the growth of microorganisms involved in N transformation as well as, movement and stability of N in forms utilizable by plants and microbes .

The present experiment was designed to compare urea with other common N fertilizers in terms of "N use efficiency" (NUE) and the growth performance of a high yielding barley variety (cv. Triumph). Such information could be useful in designing experiments aimed at improving NUE of barley involving soil and fertilizer amendments (eg. liming and nitrapyrin respectively).

Although increased crop production has followed increased rates of fertilizer application under local conditions (Mendham and Russell, 1987; Abdul-Rahman, 1988), there has been no comparison of the effects of different N sources on cereal crop growth. Thus one of the objectives of this work was to determine whether and to what extent urea is a suitable alternative to other N sources for barley in local soils.

5.2. Materials and methods

Four commonly used nitrogenous fertilizers, namely urea (Ur), ammonium sulphate (AS), ammonium nitrate (AN), and calcium nitrate (CN), were used at two rates of application [low-(1)=50kg N/ha, and high-(2)=(120 kg N/ha] with a nil fertilizer treatment as control. The treatments were replicated twice in a randomized complete block design (3x4x2). The location and description of the site has been given in Chapter 3 and the procedure by which the site was selected has been described in Chapter 4

Main plots, 20m x 1.5m, were separated by 1m strips free of crop growth (Plate 2). On 6th June 1986, the individual nitrogen treatments were broadcast and

then incorporated by raking prior to sowing of Triumph barley at the rate of 120 kg/ha (approx, 20 x 10^5 seeds/ha) at 30-40mm depth. A 0-7-12 fertilizer mixture with P as super phosphate and K as muriate of potash was applied at the rate of 200 kg/ha at the time of sowing. About 30-40 mm of rain fell overnight.

Weedicide"One-shot" (mixture of MCPA and Dicamba) was sprayed a fortnight after emergence of the crop to control broad leaf weeds. "Mesurol", a bird repellent, was sprayed on four occasions during grain filling to reduce the threat of bird damage. However, total avoidance of bird damage could not be achieved and therefore the areas of obvious bird damage were avoided during harvesting.

Rainfall and temperature data for the growing season are given in Appendix 6. The good spread of rainfall during the season was sufficient for satisfactory crop growth. However, dry conditions associated with higher temperatures towards the end of season necessitated irrigation in October (20 and 28th) and then again in early and mid December (2, 8 and 15th) (sprinkler irrigation was equivalent to 10mm rainfall on each occasion).

Analysis of soil and plant specimens was carried out as described in the Chapter 3. Sub-plots of 0.25m^2 were harvested at 60(tillering), 80(early stem elongation), 120 (seed filling) and 180(close to maturity) days after sowing (DAS) to estimate top dry matter production and grain yield. Plant height and spikelet length were recorded at 180 DAS. A least square difference technique (Gomez and Gomez, 1984) was used for comparison of treatment means.

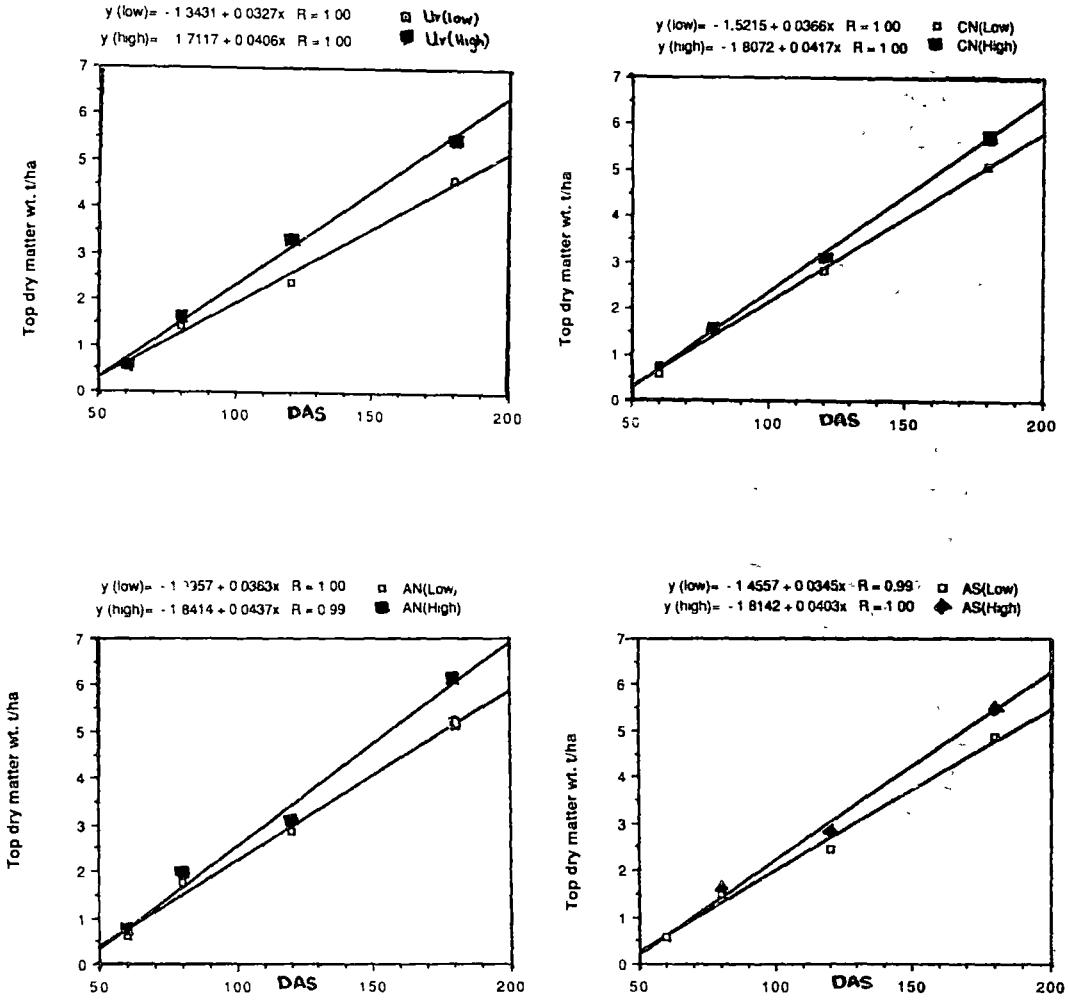
5.3. Results and Discussion

Plant growth and yield: Top dry matter production increased almost linearly with time. Each of the four forms of nitrogen fertilizer gave significant increases in top dry matter production at higher rates of N application (Figure 5.1) particularly at 60, 80 and 180 DAS. The mean dry matter weights in plots receiving equal rates of N but different N forms were not significantly different.

The increase in top dry matter production with increased rate of N (Table 5.1 and Figure 5.1) is in general agreement with dry matter production data obtained by Abdul-Rahman (pers. comm.) during 1985 using the same barley variety at a neighboring site also with N added as ammonium nitrate. In Australia many workers have demonstrated that nitrogen fertilization at levels ranging from 67 to 180 Kg N/ha increases forage yield of cereals and have proposed that this is an effective method of overcoming the problem of winter feed shortage faced by many farmers (Archer, 1969; Blunt and Fisher, 1976).

Fig. 5.1: Effect of N feritilizer source and application rate on dry matter production by Triumph barley.

(Ur= urea ; CN=$Ca(NO_3)_2$; AN=NH_4NO_3 ; AS= $(NH_4)_2SO_4$); low =50kg N/ha,



i. control (0 kg N/ha)

ii. 50 kg N/ha

iii. 120 kg N/ha

Plate 4. : The effect of three rates of urea application on plant height and canopy density.

Table 5.1: Dry matter production at different stages of growth and grain yield of Triumph barley

Fertilizer Type	Rate	Dry matter (t/ha) at (DAS) 60	80	120	180.	Yield t/ha	Harvest Index
control.(mean of 4x2)	0	0.55	1.12	2.33	4.72	4.91	-
Urea (Ur)	50	0.59	1.44	2.38	4.63	5.5	54.62
	120	0.60	1.62	3.3	5.52	5.85	52.23
$Ca(NO_3)_2$ (CN)	50	0.58	1.55	2.83	5.06	6.19	54.98
	120	0.75	1.53	3.1	5.76	6.89	54.5
NH_4NO_3 (AN)	50	0.62	1.76	2.85	5.14	5.79	52.97
	120	0.72	1.95	3.05	6.16	6.23	50.19
$(NH_4)_2SO_4$ (AS)	50	0.59	1.5	2.43	4.86	5.05	51.23
	120	0.58	1.6	2.77	5.54	5.88	51.51
Means table:							
low		0.595	1.557	2.624	4.922	5.87	53.45
high		0.661	1.670	3.055	5.745	6.10	52.1
Ur		0.594	1.525	2.838	5.075	5.73	53.42
CN		0.668	1.530	2.967	5.409	6.54	54.74
AN		0.668	1.855	2.950	5.650	6.01	51.58
AS		0.585	1.543	2.603	5.199	5.51	51.37
LSD (p=0.05)							
(type)		0.08	0.17	ns	0.36	ns	2.48
(rate)		0.06	0.12	0.23	0.25	0.56	ns
(rate x type)		ns	ns	ns	ns	ns	ns

The top dry matter yield at 180 DAS was considered as fairly representative of hay/straw production since the time of sampling was quite close to final harvesting (21 days before harvesting). Plant height (an important character as increased height could make plants susceptible to lodging) at the time of final harvesting was not affected by N source or rate of application (Table 5.2). Hence the significant differences in straw dry matter production due to treatments may be attributed to differences in no of tillers, thickness of stems or to differences in cabohydrate reserves that was not investigated in this experiment.

Grain yield: Yields varied from 4.9t/ha for the control to 6.9t/ha for the higher level of CN2 application (120kg N/ha). Yields obtained are moderate when compared with those reported in Europe where the Triumph variety was bred and also when compared to yields of other local trials (Mendham and Russell, 1987).

The "effective" spikelet length (i.e. the length of spikelet bearing fertile seeds) at harvest that was used as a measure of the number of seeds per tiller (Table 5.2) was influenced (p=0.05) by rate of N application and not by source of N. However, the grain yield and straw dry matter production showed a high correlation coefficient of r^2=0.73 indicating that that increased grain yields could be mainly due to increased number of fertile tillers per plant. Therefore it appears that for grazing and hay production there is scope for the use of different agro-technical soil and fertilizer amendments to improve N use efficiency of urea under local conditions within the range of the plant's capacity to use N.

Only rate of fertilizer application showed a significant influence (p=0.05) on yield while the effect of fertilizer type was less significant (p=0.1). At this probability level (p=0.1) the mean yields from plots receiving urea (Ur) and ammonium sulphate (AS) were significantly less than those obtained with calcium nitrate (CN) and ammonium nitrate (AN). It may be that a greater number of replications is necessary to improve the probability of detecting any effect of N source.

Figure 5.2 shows a Mitscherlich-type exponential relationship between grain yield and N application rate for all N sources except ammonium sulphate (AS). This result for AS may have been due to a higher proportion of N added in this form being unavailable to plants perhaps due to immobilization and/or ammonia losses

Some workers have suggested the ratio of grain yield to total biomass production (i.e. harvest index) as a better indicator of the relationship of vegetative growth and grain yield than the total top dry matter production alone (Riggs et al., 1981; White, 1987). Data given in Table 5.1 show the effect of treatment on the harvest index. The rate of N application did not have a significant effect on the harvest index (p=0.05). However only the CN treatment gave a significantly higher harvest index compared to the other three N sources (LSD=2.48, p=0.05). This may be because of the greater effective spikelet length in CN-treated plants. Abdul-

Rahman (un-published data) using the same barley variety obtained similar harvest index data in response to applied N in 1985.

Nitrogen content of plant parts: Nitrogen in straw and grain at maturity expressed in terms of N content per 100 kg of straw and grain respectively is shown in Table 5.3. Neither source of N nor rate of N application had a significant effect on straw N content. However the per cent grain N increased from 1.48 for Ur1-treated to 1.82 for AN2-treated plots and was significantly affected by N source (LSD= 0.073, p=0.05) and rate of application (LSD= 0.209, p=0.05). There was a positive interaction (LSD= 0.103 at p=0.05). Urea application resulted in the lowest mean increase in grain N content. This is in agreement with the results reported by Kucey (1987) who observed a generally lower N content of barley grain following urea application than following ammonium nitrate or anhydrous ammonia. Nevertheless the present results show that the per cent N in straw was least with AS1 (50 kg N/ha) and highest with CN2 (120 kg N/ha) although the differences were not significant (p=0.05).

Nitrogen use efficiency in terms of yield and per cent N in plant parts with respect to applied N is shown in Table 5.3. The method of calculation used in the present study was originally proposed by Bock (1984). Bock's equations were slightly modified in order to separate yield efficiency (YE) and N recovery efficiency (RE) by grain and straw.

Straw yield efficiency was affected by fertilizer type as well as by rate of application (p=0.05). The effect of fertilizer type on grain-yield efficiency was also significant.

In terms of recovery efficiency (RE) only total plant N (grain and straw N together) was significantly affected by different fertilizer forms. Again the lowest efficiency was observed in the case of the urea and ammonium sulphate treated plots.

Comparison of YE of grain and straw confirmed that N fertilizer in the form of ammonium sulphate and urea was not as efficient as ammonium nitrate and calcium nitrate under the conditions of this experiment. These results may need further evaluation regarding the effects of associated ions and pH changes subsequent to fertilizer application. The differences in YE and RE patterns within the same cultivar could be due to modified N absorption capacity of plants due to factors such as availability of different N forms including differences in their transport and storage in soil. Maynard et al., (1976) and Maynard and Barker (1979) reported that environmental factors, fertilizer management and crop production practices can modify the degree of N accumulation and transport. Nitrate toxicity in plants following high nitrate absorption has also been reported (Maynard and Barker, 1979) as well as ammonium accumulation in some plant tissues.

Table 5.2: Plant height and spikes length at harvesting.

Fertilizer Type	Rate kg N/ha	Plant Height mm	Effective Spikelet Length mm
Ur	50	667	73
	120	752	79
CN	50	713	75
	120	655	80
AN	50	661	76
	120	709	75
AS	50	660	73
	120	710	76
Means table			
low N		675	74
high N		707	77
Ur		709	76
CN		684	77
AN		685	76
AS		685	75
LSD table			
(type) p=.05		ns	ns
(rate) p=.05		ns	6
(type x rate)p=0.1		49	ns

(Ur= urea ; CN=$Ca(NO_3)_2$;AN = NH_4NO_3 ; AS=$(NH_4)_2SO_4$

Fig. 5.2: Effect of fertilizer N source and rate of N application on grain yield of Triumph barley

Ur= urea ; CN=$Ca(NO_3)_2$; AN=NH_4NO_3 ; AS= $(NH_4)_2SO_4$);
(low =50kg N/ha, high=120kg/ha)

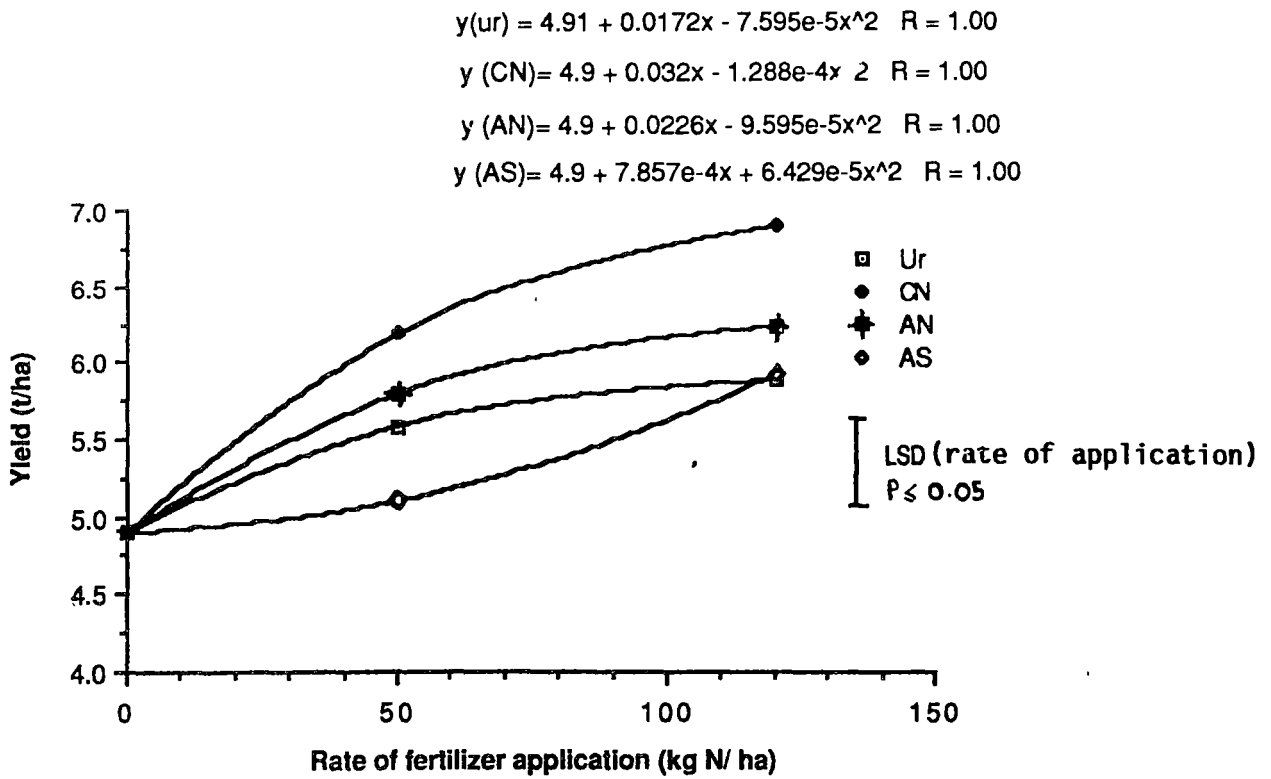


Table 5.3: Nitrogen content of grain and straw and N use efficiency of barley (cv. Triumph).

Fertilizer Form	Rate	% N con. in Grain	straw	Cumulative N use efficiency YE(grain)	RE(grain)	YE(straw)	RE(straw)
Cont	0	1·4	0·6				
Ur	50	1.48	0.7	13.37	31.8	9.82	7.77
	120	1.51	0.69	9.39	41.7	11.52	8.44
CN	50	1.56	0.70	25.48	41.78	18.45	13.61
	120	1.51	0.79	16.54	50.21	13.48	14.50
AN	50	1.63	0.63	17.68	44.29	20.08	7.64
	120	1.82	0.67	11.00	32.24	16.84	10.42
AS	50	1.66	0.65	4.36	40.43	14.37	5.71
	120	1.73	0.69	8.45	29.67	11.70	8.19
Means table:							
Low rate		1.583	0.67	15.22	39.58	15.68	8.68
High rate		1.64	0.71	11.34	38.45	13.39	10.39
Ur		1.50	0.69	11.38	36.75	10.67	8.11
CN		1.54	0.74	21.00	46.00	15.97	14.06
AN		1.72	0.65	14.34	38.26	18.46	9.03
AS		1.69	0.67	6.40	35.05	13.03	6.95
LSD (P=.05)							
(type)		0.073	ns	9.73	ns	3.41	ns
(rate)		0.209	ns	ns	19.49	ns	ns

All N use efficiencies were calculated on a cumulative basis using the following equations modified after Bock (1984)

$YE=(Y_{50 \text{ or } 120} - Y\text{control})/$ N rate of application

$RE=(N_{50 \text{ or } 120} - N\text{control})/$ N rate of application

YE= Yield efficiency; RE= Recovery efficiency

y= yield; N= N content of plant part (kg)

[Ur: Urea, CN: $Ca(NO_3)_2$, AN: NH_4NO_3, AS: $(NH_4)_2SO_4$]

However the present results cannot be unqualifiedly interpreted to infer a plant "preference" for a particular N form (see p. 9 of Section 2.1.12, Chapter 2), or to indicate the effects of ammonium and nitrate forms on plant growth. It may be that the variations observed in plant growth under the experimental conditions may have been the result of the fertilizer forms and rates at which they were applied but effects of subsequent changes in the soil pH and associated changes in N availability following addition of fertilizer should be considered (Tinker, 1978; Goh and Haynes, 1986). Although no significant variation in bulk soil pH was apparent, it is possible that significant pH changes may have occurred at the soil/fertilizer/root microsite interface (Haynes, 1986).

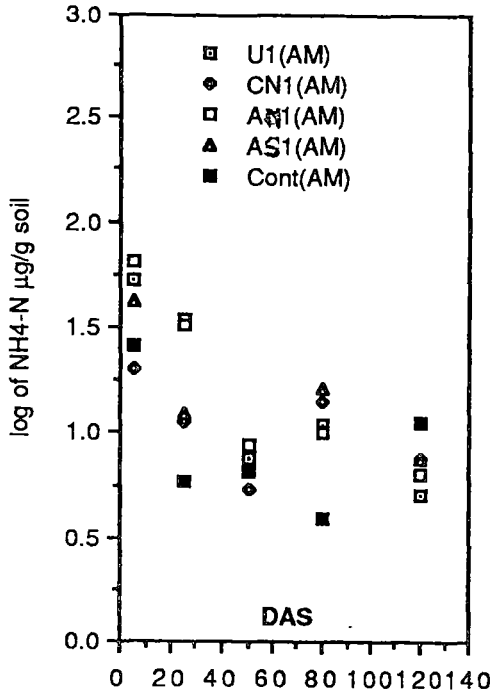
Soil mineral N The variations in soil ammonium and nitrate levels during the growing season are shown in Fig 5.3 (a,b,c and d).

Ammonium levels had changed significantly at 5 DAS in relation to fertilizer type and rate of application. The relatively lower increases in ammonium levels associated with application of nitrate fertilizers suggest that nett mineralization of soil organic N increased, probably due to increased activity of heterotrophic microorganisms resulting from increased aeration during sowing and to the addition of fertilizer nutrients (N, P and K). The levels of ammonium declined rapidly and the treatment effect on ammonium level was not significant thereafter. Higher ammonium levels in urea-treated soils at 5 DAS indicated rapid urea hydrolysis even though low temperature conditions prevailed (Appendix 6). The decline in ammonium concentration by 45 DAS could be due to loss of ammonia by nitrification and/or plant uptake, immobilization, fixation, or perhaps volatilization. However, the low soil pH may not have been conducive to ammonia volatilization (Freney et al., 1986) and incorporation of fertilizer-N into soil by raking immediately after application should have reduced such losses. The data indicate that loss of ammonia by immobilization in this soil, even under higher temperature conditions, may not be substantial. Moreover increased nitrate levels by the time of later samplings indicated active nitrification which, together with plant uptake, must have been the main cause of N loss.

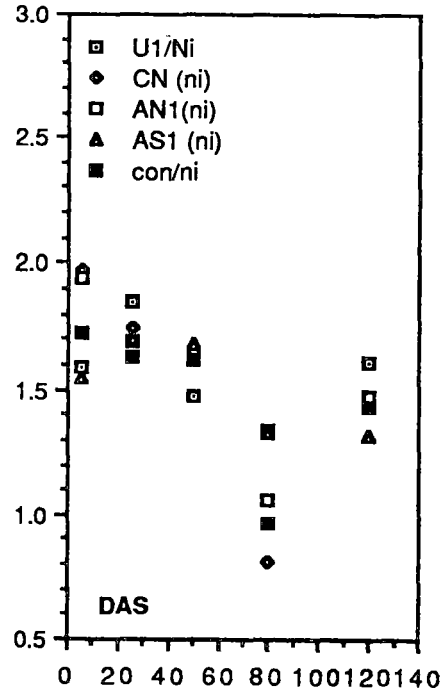
Nitrate-N, on the other hand, was significantly affected throughout the growing season (from DAS 5 to 150) due to the form of fertilizer N. The effect of application rates showed a similar trend except for the samples collected at 25 DAS. Highest concentrations of nitrate-N were realized at 5 DAS but by 25 DAS the levels started declining, probably due to plant uptake or to leaching losses and/or denitrification. At approximately two and half months after sowing (80 DAS) nitrate levels were close to those of control plots.

Fig. 5.3 Soil Mineral N (a & b) at low rate of application: and (c & d) at high rate of application.

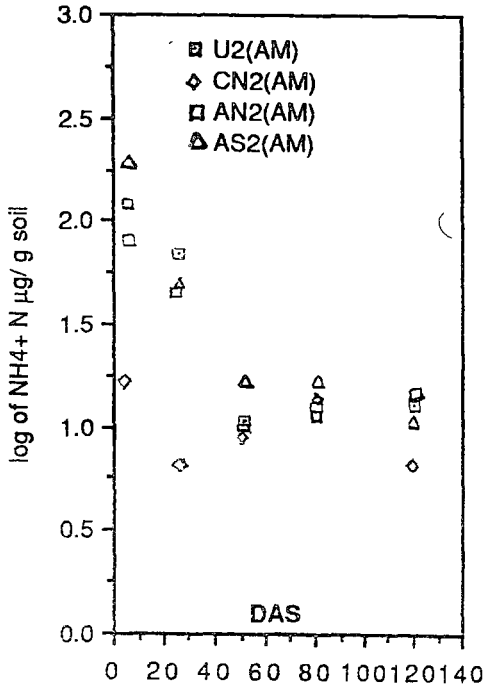
a. Ammonium-N (AM)



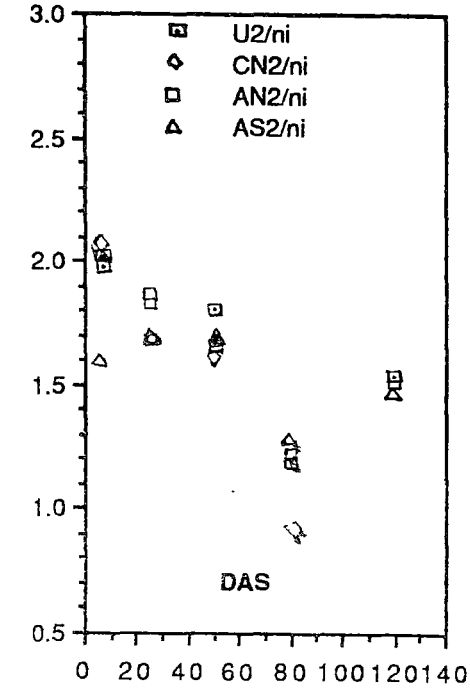
b nitrate-N (ni)



c. Ammonium -N



d. nitrate -N



Ur:Urea, CN: $Ca(NO_3)_3$, AN : NH_4NO_4, AS: $(NH_4)_2SO_4$

The total mineral N contents in soils treated with urea and ammonium sulphate were not significantly different to those of calcium nitrate and ammonium nitrate treated soils indicating that the observed differences in NUE depending on the form of fertilizer-N have been influenced by factors other than mineral-N availability as determined by the analytical techniques used. Again, these results do not necessarily imply that the ammonium form is less favoured than nitrate forms for plant uptake as the experiment did not cover the role of associated ions and changes that may have occurred (such as pH changes, osmotic potentials) at the soil/fertilizer (micro-environment) interface.

Relatively low NUE and crop yield observed in response to urea application could have been due to one or more of several reasons. Since soil pH is fairly low, loss of N due to ammonia volatilization following rapid hydrolysis of urea was expected to be small. However, nitrification rapidly converted ammonium formed in the soil to nitrate (Figure 5.3). It may also be that a considerable amount of the ammonium form could have been rapidly immobilized by microbes or fixed on the exchange complex during the initial growth stages. Furthermore, although the impermeable clay subsoil may have minimized vertical leaching, the 2% slope of the site may have allowed substantial lateral leaching and in loss of nitrate-N.

Nevertheless, the observations support the planned use of urea in subsequent experiments. The N use efficiency of the crop has been shown to be higher than the comparative N supply efficiency of urea applied to this soil.

CHAPTER SIX

6. The effect of lime and nitrapyrin (soil and fertilizer amendments respectively) on urea N transformation in the selected acid soil.

6.1. Introduction

Liming is recommended for ameliorating acid soils with problems such as aluminium and manganese toxicities and deficiencies of calcium and magnesium. This practice can also affect soil biological activities including N transformations whereby both mineralization and nitrification processes in soil are enhanced (Nyborg & Hoyt, 1978). Ammoniacal fertilizers applied to such soils may be rapidly converted to NO_3^- N with the possibility of increased N losses by leaching and/or denitrification. One of the ways of reducing such losses is by the use of a nitrification inhibitor (NI) such as nitrapyrin. Reports on the effectiveness of nitrapyrin in limed soils vary considerably (Laskowsky and Bidlack, 1977; Hendrickson and Keeney, 1979; Sims and MacKown, 1987).

The experiment described in Chapter 5, showed that urea application to an acid soil resulted in relatively low nitrogen use efficiency by barley when compared to other N fertilizers. The effectiveness of urea might be improved by liming to provide a better soil pH environment for N transformation and/or by amending the fertilizer with nitrapyrin to prevent nitrification, thus reducing the possibility of N losses due to denitrification and leaching. There are records of improved performance of barley following liming under local conditions (Mendham and Russell, 1987).

Hence the present experiment was undertaken to evaluate only effects of lime and nitrapyrin on nitrogen transformation processes in an acid soil following urea application and was carried out in the absence of plants.

6.2 Materials and Methods

Experimental: Soil was air dried and gently crushed to pass a 2 mm sieve. Lime treatments (Lo,no lime; Li,2.3g/kg soil; Lii, 6.9g/kg soil, oven dry basis) were applied by spreading the required quantity of soil on a polythene sheet and mixing thoroughly with finely powdered 'Limil'® Soil and soil + lime mixtures were packed into plastic pots to a bulk density of 1.3, watered to field capacity (F.C.) and left to settle for four weeks. During this period moisture content was maintained at field capacity by regular weighing and addition of distilled water as necessary. Just prior to each watering the surface of the soil in the pots was raked to a depth of 3-4 cm. Watering was stopped after three weeks and the soil in the pots was allowed to dry for one more week after which urease activity and ammonium and nitrate levels were determined immediately in soil specimens withdrawn using a short PVC tube. Urea

was then applied in 20ml of solution at a rate of 50mg N/kg soil (equivalent to approximately 100kg N/ha) with and without nitrification inhibitor. NIi (inhibitor added) treatments received nitrapyrin ("N-serve EC": Xylene based) at a rate of 0.3mg(a.i.)/kg soil (approx. 0.56 kg(a.i)/ha). The nitrapyrin was mixed in 20ml of urea solution just prior to application. Quickfit glassware was used as far as possible during solution preparation to minimize loss of nitrapyrin (Bremner, 1978; Hendrickson and Keeney, 1978). The control (NIo) pots received 20ml of urea solution mixed with and the same volume of xylene as that added with the nitrapyrin in the NIi treatment. Solutions were added to each pot after removing the top 2cm. of soil which was replaced immediately to minimize possible loss of ammonia and also to ensure a good mix of soil and added chemicals. Thereafter water was added to each pot in amounts calculated to maintain an average water content around 70-75% F.C. to minimize losses due to denitrification. Deionized water was used for water replacement throughout the experiment. Pots were laid out in a split plot design with lime as the main treatment and NI as the subplot treatment. While maintaining the experimental design the positions of main and subplot pots were changed regularly to minimize the any position effects. The whole experiment was replicated three times. Soil specimens were withdrawn 5, 15, 25, 45, and 60 days after urea application, using a PVC tube. After specimen withdrawal the soil in each pot was re-mixed. A period of 60 days was considered adequate to cover the normal period of crop growth.

To determine the effects of NI on denitrification potential separate 10g soil specimens were treated with 10 ml of O.05M KNO_3 in 50ml McCartney bottles with suba seals. Specimens representing soil at two levels of soil pH (Lo and Li) and one of nitrapyrin (NIi) were set up to provide triplicate determinations. Soil inside the bottles was submerged and air was replaced with gaseous nitrogen (CIG-lab grade) to provide an optimum environment for denitrification. The procedure adopted to determine nitrous oxide production by gas chromatography has been described in Chapter 3.

Analytical: Total N was determined by Kjeldhal digestion followed by steam distillation with 40% NaOH (Bremner, 1965). Forms of mineral-N (NH_4^+,NO_2^-& NO_3^-) were measured by a modified Kjeldhal method (Bremner, 1982). The buffer method of Tabatabai & Bremner (1972), was used to determine soil urease activity. Steam distillations were carried out using a Kjeltec distillation unit.

The analysis of variance was performed on data collected for each sampling time . Nitrification rate was calculated at each sampling time to estimate the size of the nitrifying population and percent inhibition was calculated to reflect change in effectiveness of nitrapyrin over time.

6.3. Results and Discussion

Soil pH: At the end of four weeks of incubation, prior to the addition of urea, the non-limed soil pH was slightly lower (5.2) than the pH of the soil before incubation (pH 5.3). Following the addition of urea soil pH increased initially to a level above that of the field soil before declining again. No marked effect on soil pH due to nitrapyrin was evident. These data together with those showing the effect of liming on soil pH are given in Table 6.1.

Table 6.1: Effect of lime and NI on soil pH

Treatments	0 day	5day	25day	45day
NIoLo	5.2	5.6	5.3	5.3
NIoLi	7.2	7.3	6.9	6.8
NIoLii	8.4	8.3	8.4	8.3
NIiLo	-	5.5	5.3	5.3
NIiLi	-	7.5	6.9	6.8
NIiLii	-	8.3	8.4	8.5

Day 0-the day before the addition of urea.
(pH was measured in 1:5 soil:water suspension using a glass electrode)
NIo=No inhibitor; NIi=Inhibitor added; Lo=No Lime; Li=Lime added at 2.3g kg-1(2.5 t/ha); Lii -Lime added at 6.9g kg-1(7.5 t/ha)

Urease activity: Liming had a significant effect on urease activity (Fig. 6.1). The control, non-limed soil without nitrification inhibitor (NIoLo), showed significantly lower activity than limed soil at 5, 15 and 25 days after addition of urea. Increasing the rate of liming from 2.5t/ha(Li, pH 6.8) to 7.5t/ha (Lii, pH 8.3) did not result in further significant increase in urease activity (Lsd =6.057, p=0.05). However, it should be noted that the increase in urease activity from Li to Lii with or without nitrapyrin was consistent over time. This could mean that the near neutral pH of the Li treatment was more favourable for urease producing miroorganisms than the higher pH of the Lii treated soil. Accordingly, a high positive correlation (r^2.=0.74-0.91, p=0.05) was observed between urease activity and soil pH at different sampling times. A similar relationship between pH and urease activity was reported by Zantua & Bremner (1977). However there was no significant effect (P=0.05) of nitrapyrin or lime+nitrification inhibitor on urease activity, in spite of the fact that there was lower urease activity in non-limed soil plus NI when compared to limed soil plus NI for which no reasonable explanation can be given. The absence of a significant effect of NI on urease activity has been widely reported (eg.,Bremner & Douglas, 1971).

Fig 6.1: Effect of lime (Lo, Li and Lii) and nitrapyrin (NIo and NIi) on urease activity.

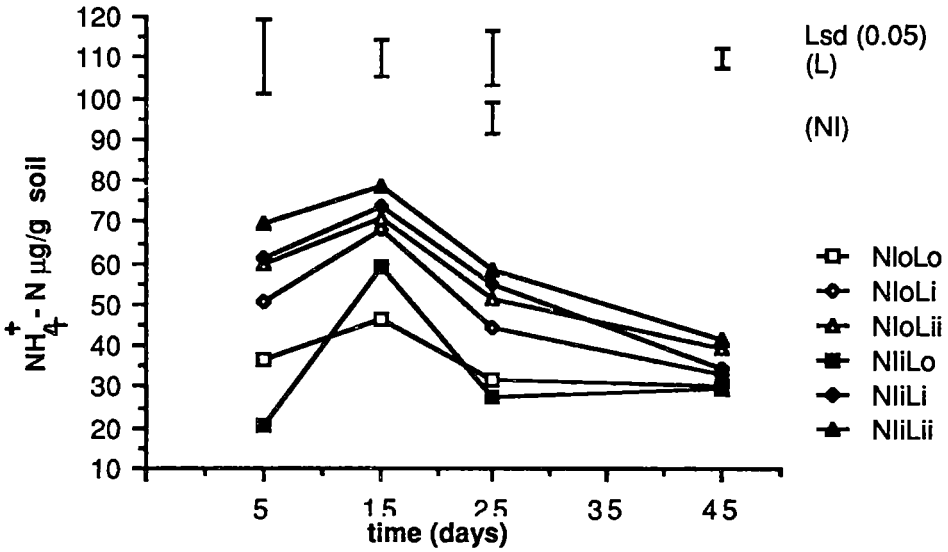
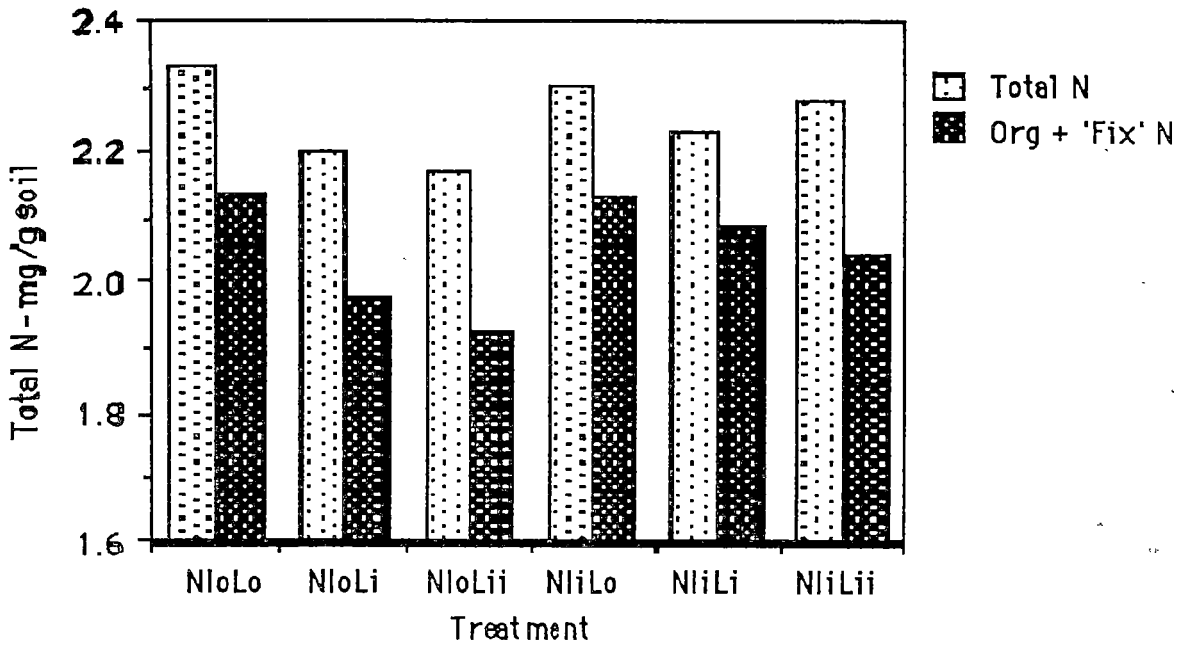


Fig.6.2: Total soil N 60 days after urea application to variably limed (Lo, Li & Lii) with (NIi) and without (NIo) addition of nitrapyrin.



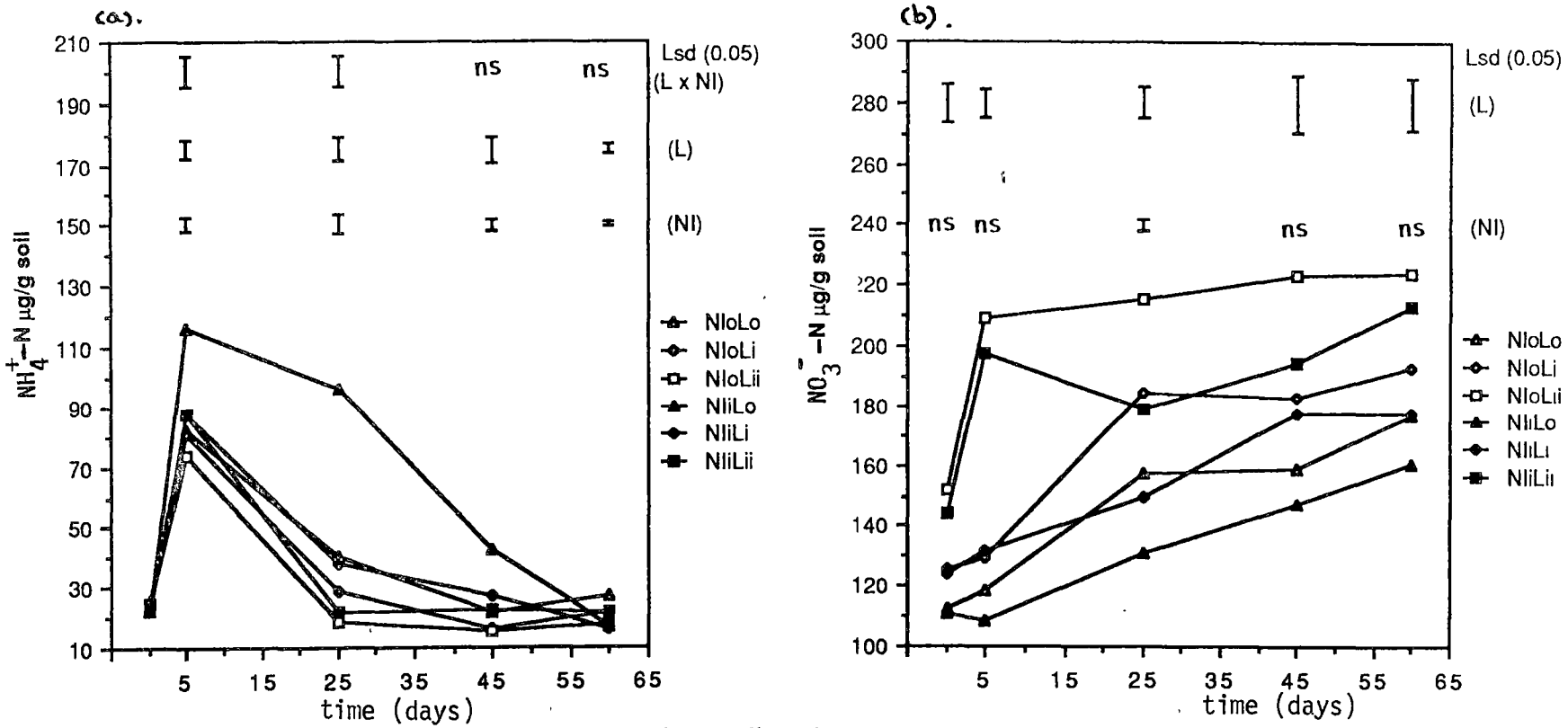
In all treatments urease activity increased up to 15 days, presumably due to stimulation of microorganisms capable of producing exocellular ureases in the presence of urea (Mulvaney and Bremner, 1981). Subsequent decline in urease activity may be explained as due to decreased substrate concentration (urea). The enzyme activity then appeared to stabilize at a level close to the original level of the field soil. The present results agree with those of Zantua & Bremner (1977) who attributed the pattern of urease activity to the formation of complexes of urease with different soil constituents such as clay and organic matter, preventing further degradation of the enzyme. They suggested that "...different soils may have different minimum levels of urease activity determined by the soil constituent's capacity to protect and stabilize urease against microbial degradation and other inactivation processes." More recent results of O'Toole & Morgan (1985) support this suggestion.

Considerable variability in pH optima for soil urease activity has been reported (Tabatabai & Bremner, 1977; Kumar & Wagenet , 1984). Most studies indicate neutral a to slightly alkaline soil pH to be optimal for urease activity. Increased urease activity at higher pH shown by the present results suggests that the optimum for urease activity in the soil used may be in the upper pH range. However, these results conflict with the observations of Moe (1967) and Peltser (1972) who reported a decrease in urease activity following liming. Kumar and Wagenet (1984) also reported an 8% reduction in urease activity following addition of calcium carbonate.

Soil N content: Neither total nitrogen nor organic N + N fixed (N-fixed refers to nitrogen "fixed" on the exchange complex) levels were significantly affected by treatments. This was most likely due to the inadequacy of the Kjeldhal technique to detect a small change in organic N content rather than to the absence of any effect on the organic-N fraction. Hence, only the trends of total Kjeldhal N and organic N+ N-fixed have been discussed (Figure 6.2).

Liming resulted in a reproducible declining trend in total N and in organic N+ N-fixed although this was not statistically significant (Fig. 6.2). Since no significant change in nett 'N-fixed' would have occurred, the decrease may be attributed to a possible increase in mineralization of organic N due to liming as reported by Nyborg and Hoyt (1978). The total Kjeldhal-N recovered was always less (especially in Li and Lii treated soils) than the total of "added + native-N " for the original soil, indicating N loss from the system due to denitrification or NH_3 volatilization.

Fig.6.3: Effect of lime (L) and nitrapyrin (NI) on soil NH_4^+-and NO_3^--N.



Analysis of variance was performed on data for each sampling time . Lsd(s) ($p \leq 0.05$) shown as vertical bars are used to compare the effects of the tre· ments each point represent the mean of three determinations.

Table 6.2: Nitrous oxide production (umol/100g soil) in the presence (NIi) and absence of nitrapyrin (NIo) in limed (Li) and non limed soil (Lo) treated with nitrate-N.

		N_2O produced (x umol/10 g soil)		
Treatment	days(1)	2	7	10
NIoLo		12.55	26.72	20.62
NI1Lo		-	8.69	12.9
NIoLi		14.504	24.61	32.56
NI1Li		-	18.69	24.72
mean table				
Lo		6.28	17.71	16.76
Li		7.25	21.65	28.64
NIo		13.53	25.67	26.59
NIi		-	13.69	18.81
Lsd (p=0.05)				
lime		ns	ns	5.79
NI		3.17	3.42	2.56
LxNI		ns	1.89	ns

1) Flasks were incubated under anaerobic conditions at 25°C with assays at the days specified.

Application of urea gave an immediate significant increase in ammonium-N in all the pots (Figure 6.3a). This was followed by a rapid decrease except in the unlimed soil receiving nitrification inhibitor (NIiLo) indicating rapid hydrolysis of urea followed by nitrification and/or NH_3 volatilization in the limed soil. Previous workers have attributed the decline in ammonium-N following liming to increased nitrification and ammonia volatilization due to increased soil pH (Nyborg and Hoyt, 1978; Adams and Martin, 1984).

The higher rate of liming (Lii) did not produce significantly greater ammonium levels than those associated with the lower rate (Li). On the other hand, addition of NI into limed soil resulted in markedly higher ammonium-N levels than those in limed soil containing no NI, suggesting that nitrification was a major cause of the decline in ammonium-N. A significant positive interaction (p=0.05) of NI and lime on the level of ammonium-N was observed initially and up to 15 days after fertilizer addition (15 DAF). However, at 40 and 60 DAF, there was no detectable interaction of NI and lime although the effects due to lime and to NI were individually significant. Application of NI to strongly acid unlimed soil (pH 5.3) resulted in greater retention of ammonium-N when compared with the effect of its application to limed soil (pH

6.3 and 7.2). A similar effect of NI in relation to soil pH was reported by Sims and Mackown, (1987).

The separate incubation experiment carried out to investigate whether the denitrification process was significant under the present experimental conditions and also to determine whether denitrification potential was affected by liming and/or nitrapyrin showed that nitrapyrin significantly inhibited the nitrous oxide production (Table 6.2). The inhibitory effect was more pronounced when soil pH was low and the amount of nitrous oxide produced after two days of incubation was not detectable. The interaction between lime and NI had a significant influence on denitrification when tested following 7 days incubation but the effect disappeared after further incubation (Table 6.2). Meanwhile the production of nitrous oxide declined with further incubation (probably for the same reasons given in Chapter 4, p.76).

Table 6.3: Changes in the effect of lime and nitrapyrin with time on nitrification rate[1] following addition of urea.

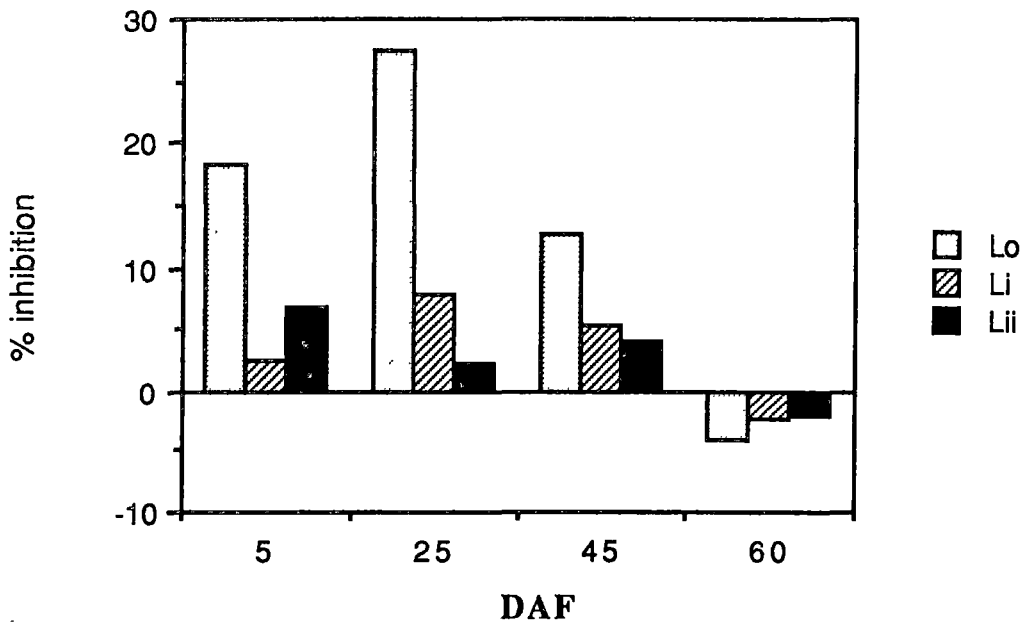
Treatment	5	25	45	60
	days after addition of urea			
NIoLo	59.05	79.67	87.69	86.77
NIoLi	60.62	82.63	91.81	90.04
NIoLii	73.25	92.02	93.58	92.53
NIiLo	48.26	57.77	76.58	90.23
NIiLi	59.11	79.8	86.8	92.10
NIiLii	68.22	89.94	89.76	94.49

(1) Calculated by using the equation (modified after Sahrawat, 1980): (%)

$$\text{Nitrification rate} = \frac{(NO_3)\text{-N}}{(NH_4+NO_3)\text{-N}} \times 100$$

The delay in denitrification due to nitrapyrin may not be attributed to a secondary effect of nitrification inhibition since adequate nitrate-N was present. Although the direct inhibition of denitrification by nitrapyrin has not been widely reported, its dual role in inhibiting nitrification and denitrification has received some attention recently (Mills et al., 1976, 1978; McElhannon & Mills, 1981). Further investigations need to be carried out since some authors find it difficult to accept these findings in view of the diversity of denitrifiers (Keeney, 1986; Section 2.3 of this thesis).

Fig 6.4: Change in effectiveness of nitrapyrin under different lime treatments (Lo, Li & Lii) expressed as a % inhibition.



The effectiveness shown in the Figure 6.4 is given in terms of % inhibition at different times. This is calculated using the equation proposed by Saharawat, (1980). ie.

$$\% \text{ inhibition} = \frac{\text{Nr in control(NIo)} - \text{Nr in inhibitor treated sample (NIi)}}{\text{Nr in control (NIo)}} \times 100$$

Nr-Nitrification rate

Since anaerobic conditions did not develop in the main pot experiment due to regular mixing and watering to 70-75% F.C. no substantial denitrification would have occurred. On the other hand there was no leaching under the controlled watering regime. Hence any N loss that may have occurred could have been mainly due to NH_3 volatilization.

Nitrate-N levels were also monitored (Figure 6.3) to estimate the contribution of nitrification to the decline in ammonium-N levels with increased pH. The levels of nitrate-N and rate of nitrification (Table 6.3) were significantly increased by liming with or without NI. Although the application of NI consistently reduced nitrate-N production at all levels of lime application this effect was significant only at 25 DAF. There was no significant interaction between lime and NI in relation to nitrate-N levels. However the increases in nitrate-N did not fully account for the disappearance of ammonium-N, particularly in lime-treated soils, further supporting the possibility that loss of gaseous ammonia contributed to lower levels of ammonium-N. Similar observations have been reported by many authors (eg., Laskowski and Bidlack, 1977; Nyborg and Hoyt, 1978).

The increases in nitrification rates that occurred in response to increased lime application in the presence of inhibitor were lower compared to those in the respective lime treatments with no nitrification inhibitor. This effect persisted up to 45 DAF, being most marked at 25 DAF. The effect of NI on nitrification was not significant after 45 DAF probably due to insufficient concentration of NI for effective inhibition.

The nitrification rate was used to estimate the per cent inhibition at each sampling time. The percent inhibition estimated using the equation proposed by Sahrawat (1980) for each lime treatment is shown in Figure 6.4.

Liming appeared to reduce the effectiveness of nitrapyrin up to 45 DAF but by 60th DAF more nitrate-N (relative to the respective control treatment) had formed in Lii treated soil than in Li soil. Lo soil had the lowest nitrate-N relative to the control and the lowest percent inhibition. These results are in general agreement with earlier findings by Goring (1962), Hendrickson et al., (1978), Laskowski and Bidlack (1977) and Sims and MacKown (1987) but are contrary to the observations of Hendrickson and Keeney (1979b) who found that increased pH following liming could lead to increased effectiveness of nitrapyrin. Hendrickson and Keeney (1979a) earlier reported that hydrolysis of nitrapyrin was not affected by pH, thus eliminating the possibility of dissociation of nitrapyrin with changing pH . The greater effectiveness of nitrapyrin at higher soil pH was attributed to the presence of a greater number of nitrifiers (higher nitrification rate) in such an environment (Appendix 4). However short term effectiveness of nitrapyrin may disappear more quickly at higher pH than at lower pH, as suggested by the present results, due to a faster recovery of nitrifiers at higher pH . Therefore it may not be appropriate to consider a greater short

term inhibition as more effective if such effectiveness does not persist long enough to provide a significant benefit for crops .

However Hendrickson and Keeney's results point to an important factor in the use of nitrification inhibitors in the field, namely that there could be different strains of nitrifiers active at different soil pH levels, and that the degree of nitrification inhibition may change depending on the dominant strain in the system. It is also of interest to note that at higher soil pH the initial decline in nitrification rate may be greater because of the larger nitrifying population but this phenomenon may soon disappear because of faster recovery of nitrifiers at higher soil pH (Appendix 4).

Further evidence of the significance of nitrifier strain differences on the degree of inhibition was reported by Belser and Schmidt (1986) who pointed out that a change in population from more susceptible to less susceptible strains will result in the need for a greater amount of NI for the same degree of inhibition. Powell and Prosser (1987) also observed that bacterial strain differences may be a major cause of differences in susceptibility to nitrapyrin.

In conclusion the present experiment shows that:

a. urease activity in the soil increased with liming but was not affected significantly by the addition of NI, the soil also showed a characteristic stable level of urease activity.

b. increased liming may have produced an environment more conducive to nitrifiers thus leading to a rapid build-up of nitrate-N assisted by the absence of substantial denitrification due to the aerobic condition of the soils; it was also observed that nitrapyrin was less effective in inhibiting nitrification in limed soil;

c. the observed inhibition of denitrification by nitrapyrin merits further investigation since in environments such as water-logged, acid rice growing soils denitrification is the significant mechanism of nitrogen loss and therefore such an inhibition could be very useful.

CHAPTER SEVEN

7 Effect of nitrapyrin and liming on early growth of barley.

7.1. Introduction

Nitrapyrin (NI) can effectively inhibit the nitrification process and thereby reduce loss of nitrate-N from soil plant systems. However its capability to improve plant growth or yield is widely disputed. Many studies have shown no significant advantage of using nitrification inhibitors (Appendix 1; York and Tucker, 1985). Some workers have reported that NI and/or its by-products may even be phyto-toxic and retard plant growth (Jones, 1973; Rajendra Prasad et al., 1980). Lynd et al., (1967) reported that a concentration of 1ppm N-serve in an acid sand (pH 5.7)caused leaf curling, stem twisting, and formation of a restricted club-like root system in *Robinia*. Nitrapyrin also inhibited the growth of alfalfa at all stages up to 11 weeks with effects increasing over a range of 1-20ppm of applied nitrapyrin (McKell and Whalley, 1964). Hauck (1975) cited reports of plants recovering from early stunting attributable to added "N-serve" but with no apparent reduction in final grain yield.

Redmann et al., (1964) found that nitrapyrin (NI) added to soil degraded rapidly to 6-chloropicolinic acid (6-CPA) and then to 6-hydroxypicolonic acid, both supposedly harmless to plants and microbes. Although several reports indicate either very low or no phyto-toxic effects due to the main degradation products of NI (Goring, 1962b; Mullison and Norris, 1976) Geronimo et al., (1973a,b) found direct phyto-toxic symptoms in cotton, wheat and maize seedlings following nitrapyrin application.

Reportedly, at recommended levels of application nitrapryin has no direct effect on soil microorganisms other than ammonium oxidizers. Nevertheless, in an earlier experiment described in Chapter 6, it was shown that some denitrifying bacteria may also be affected following nitrapyrin application in accordance with earlier findings of Mills and McElhannon, (1984), and McElhannon et al. (1984). On the other hand it is known that an increased level of one form of mineral N relative to other(s) and also the pH changes that result from nitrification, may affect the susceptibility of plants to pathogens. For example the incidence of Fusarium and Rhizoctonia root rot may increase if more ammonium-N is taken up relative to nitrate-N while plants taking up excess nitrate-N are more susceptible to attack by Verticillium, Cercosporella and Ophiolobus (Hauck, 1975; Papendick and Cook, 1974). However the exact mechanism(s) are not yet well understood.

If heterotrophic microflora are influenced by nitrapyrin, (or products of its hydrolysis) or by changes in pH and ammonium to nitrate ratio, the effects should be reflected in soil respiration (ie. net carbon dioxide production). On the other hand if

autotrophic nitrification is inhibited the accumulation of carbon dioxide should increase since autotrophic nitrifiers have an obligate dependence on carbon dioxide as the major source of carbon (Bock, 1978, Matin, 1978). It is estimated that in the process of oxidizing 35 molecules of ammonium, nitrifiers fix one carbon atom (Wood, 1986).

In view of the relatively short half-life of NI (4-22 days at 2°C; according to Laskowski, 1972 and Redemann et al., 1964) the toxic effects on growth of plant and general soil microflora sould become apparent during the early stages of plant growth or soon after nitrapyrin application.

A glasshouse experiment was set up to determine the effect of NI applied to soils limed to different equilibrium levels of soil pH, on (i) early growth, (ii) N-uptake and (iii) the appearance of toxic symptoms in the barley cultivar used (cv. Triumph). A separate parallel experiment was carried out to test the effect of NI and soil pH on soil respiration at 10°C and 25°C.

7.2 Materials and methods

Soil preparation: Soil collected from the site described in Chapter 2 was air dried and passed through a 2mm round-holed sieve. Mixing of appropriate amounts of lime (Li=2.3g/kg soil and Lii=6.9g/kg soil oven dry basis) was carried out approximately six months prior to the start of this experiment to allow sufficient time for the stabilization of both pH and soil microflora population. Soil packed into pots was kept in a glasshouse (mean temperature 15°C) with regular watering to maintain moisture conditions without drainage outflow.

Experiment 1. Plant growth response: Barley seedlings were raised in washed sand trays. Two days after emergence four washed seedlings were transplanted into each pot. Upon establishment (3 days after transplanting) the number of seedlings was thinned to two plants per pot. Five days after thinning, 10ml of urea solution(4mg/ml) mixed with specified rates of nitrapyrin [NIo, no nitrapyrin; NIi, 0.56mg (a.i)/100mg of urea-N; and NIii, 2mg (a.i.)/100mg of urea-N] was added followed by 40ml of water. The soil surface was scratched so that the solutions infiltrated easily.

The length of first two leaves was measured six days after application of NI/urea treatments (9/6/87). Plant tops were harvested 40 days after treatment application and total plant leaf area, weight of top dry matter, number of tillers/plant, and total N content of harvested material were determined. During the growth period the general appearance of plants was examined for any symptoms of toxic effects such as mosaics, scorching, stunted growth, or leaf curling.

Experiment 2. Soil respiration: This was estimated at three soil pH levels (5.3, 7.1 and 8.3). 10g of less than 2 mm soil was added to each of eighteen 125 ml sampling bottles. To each was added 1ml ammonium sulphate solution containing 2mg/ml ammonium-N. Nitrapyrin(NI) was added to give concentrations of 20ug and 200ug NI per mg of added N in each set of nine bottles. The moisture content of the soil was brought to 70% of field capacity with glass-distilled water. One ml of 0.5% nutrient broth (Oxoid™) solution was then added as asupplementary energy source for heterotrophic activity. Air in the bottles was replaced with moist carbon dioxide free air by passing air first through a KOH trap and then through water. The bottles were sealed with suba plugs. Two sets of treatments (18 x 2) were prepared and incubated at 10°C and 25 °C. A sample of 1 ml of air was withdrawn after 2, 5, 8, 24, 72 and 120 hrs of incubation for estimation of carbon dioxide production by thermal conductivity gas chromatography.

Statistical analysis: Experiment 1 was laid out according to a split-plot design with three soil pH values as main plot treatments and three rates of application of nitrapyrin as sub-plot treatments. Measurements of growth response were based on three replications while each replication consisted of measurements of two plants. Experiment 2 was conducted at two different temperatures as two separate experiments. Each experiment was laid out in a split-plot design with three replications. The significance of the difference between treatment means were compared using LSD values. Rates of carbon dioxide production were estimated by simple linear regression.

7.3 Results and discussion

Plant growth was measured in terms of total top dry matter, leaf area per plant and number of tillers per plant at the end of experiment. None of the growth parameters measured varied significantly (p=0.05) with soil pH or nitrapyrin, singly or in combination (Table 7.1). However, the total N content of plants was significantly affected by soil pH. Nitrapyrin had no significant effect on total N content of plant dry matter nor was there any significant interaction effect of nitrapyrin and pH.

. The higher lime treatments (Lii) appeared to have had some effect on leaf area per plant. Although an effect similar to that observed for leaf area per plant was evident in plant top dry matter production, the differences were again not statistically significant (p=0.05). Number of tillers was not influenced by either treatment. Total N content (mg/g of plant dry matter) showed a slight but significant decline at higher soil pH (Lii). Similar observations have been made by Sim and Mackown (1987) using tobacco as the test plant.

The high level of soil mineral N recovered at the end of the experiment (40 DAT) suggests that N supply was adequate for plant requirements throughout.

These result given in Table 7.1 indicate that liming may have influenced the total "N-off take" by plants but neither nitrapyrin nor the liming with nitrapyrin had a significant effect on total N content (p=0.05). The general growth of the barley cultivar Triumph does not appear to have been affected to any significant extent by any of the treatments.

Table 7.1: Leaf area per plant, mean number of tillers per plant, top dry matter production and total plant nitrogen at 40 days after treatments (DAT).

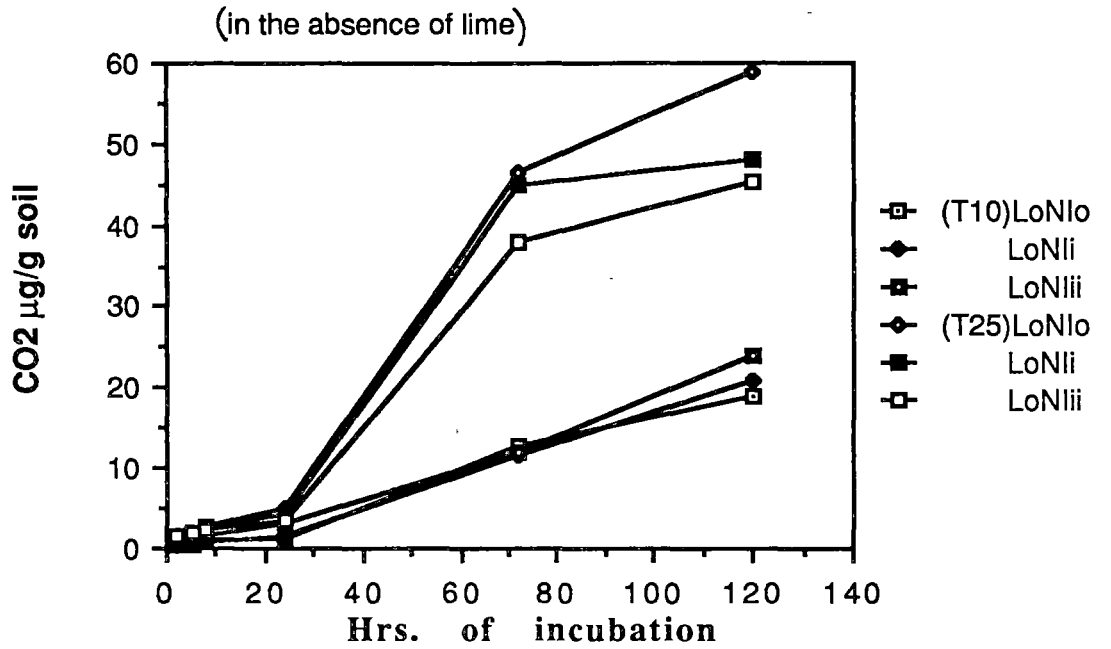
Treatments	LA/plant mm^2.	Top dry matter wt. g/plant	Mean no.of tillers/plant	Total plant N mg/g
LoNIo	2416	0.67	2.7	5.9
LoNIi	2617	0.74	3.0	6.7
LoNIii	2438	0.54	2.5	5.9
LiNIo	2384	0.58	2.2	6.0
LiNIi	2572	0.67	2.3	5.5
LiNIii	2443	0.71	2.8	5.4
LiiNIo	2665	0.74	2.8	4.8
LiiNIi	2959	0.69	3.0	4.9
LiiNIii	2801	0.72	2.8	5.1
Lsd (p=0.05)				
L	285	NS	NS	0.39

Lsd at p=0.05 for (NI and LxNI) were not significant for any of the measurements.

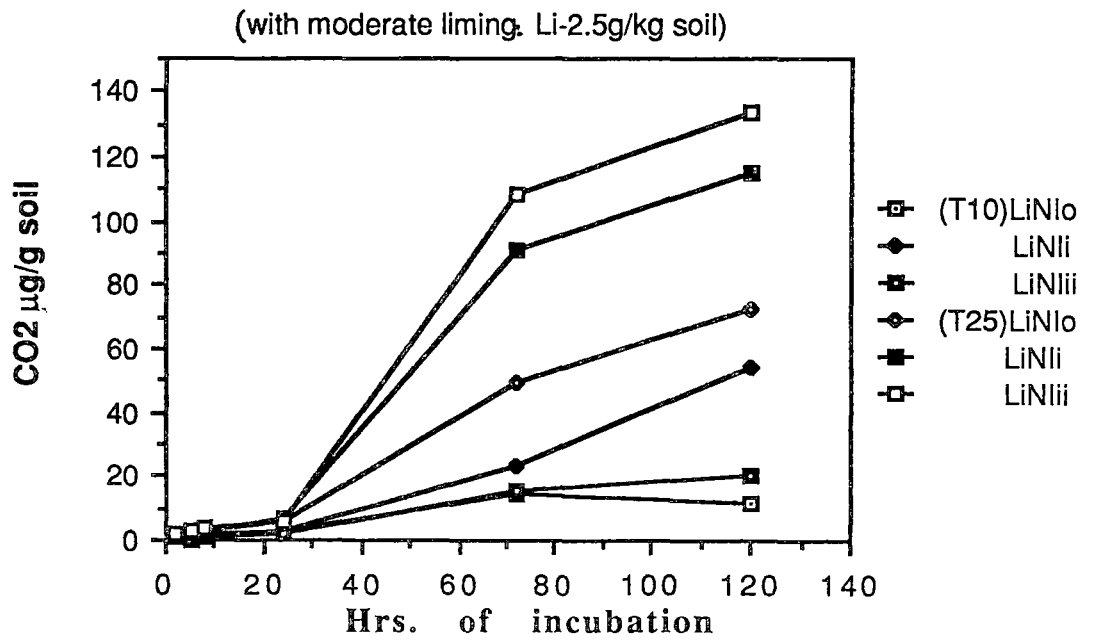
Slight wrinkling of initial leaves of plants was observed in some pots treated with the higher concentration of nitrapyrin (NIii) but no visible symptoms of toxicity reported by other workers (such as scorched leaf margins and/or tips, leaf curling, stunted growth, chlorosis) were noted. Length of leaf blades was measured regularly and mean leaf length was calculated in an attempt to quantify any reduction in photosynthetic area. Measurements made 6, 15, 25, and 35 days after treatment (DAT) are given in Table 7.2 and show no significant differences due to treatments employed.

Fig. 7.1: Effect of nitrapyrin (NI) and soil pH on carbon dioxide production at 10° and 25°C

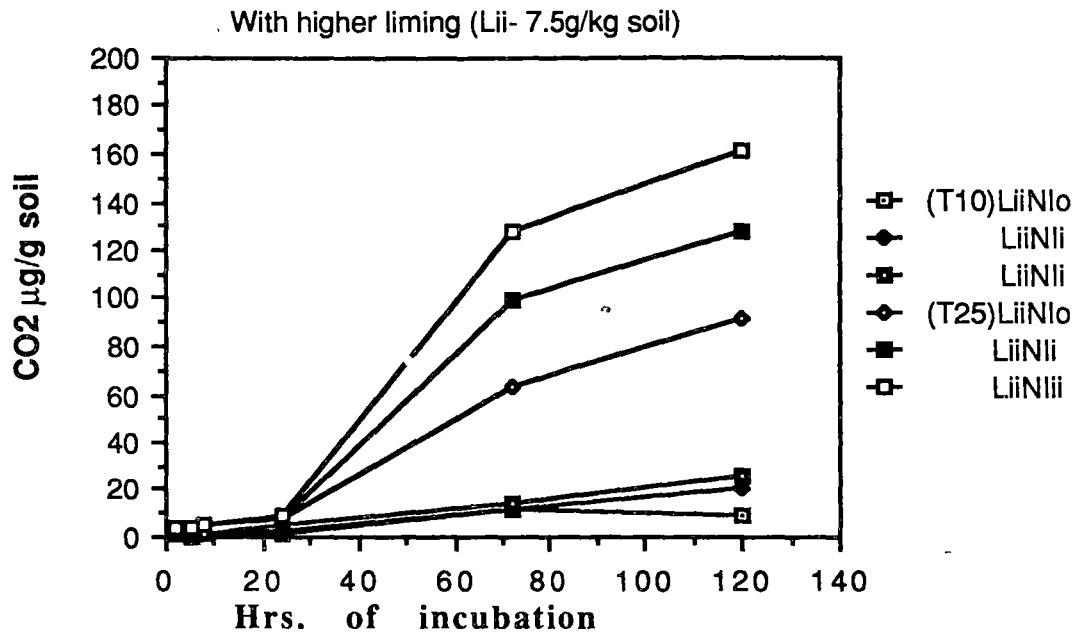
a. at pH 5.6 (Lo)



b. at pH 6.2 (Li)



c. at pH 7.3 (Lii)



T10 & T25 refer to 10 and 25°C respectively.

See Appendix 14 for data and LSD (p=0.05) values.

Table 7.2: Mean change in blade length of the first two leaves during the first 35 days after treatments (DAT)

	Length of blade /leaf (mm)			
DAT	6	15	25	35
Treatment				
LoNIo	30(7)	113(8)	328(27)	395(2)
LoNIi	26(10)	103(15)	325(44)	409(15)
LoNIii	31(8)	100(18)	286(36)	381(3)
LiNIo	30(12)	102(14)	292(32)	393(2)
LiNIi	30(8)	113(23)	298(57)	405(34)
LiNIii	38(5)	111(15)	283(54)	392(71)
LiiNIo	26(7)	99(10)	343(37)	410(24)
LiiNIi	23(12)	100(16)	298(42)	412(58)
LiiNIii	25(10)	102(13)	312(47)	419(36)

Lsd (p=0.05) was not significant at any stage of sampling
Figures within parenthesis are standard errors

Soil respiration: Soil respiration changed markedly in response to to soil pH and application of nitrapyrin. Figure 7.1 (a,b,c) shows that the pattern of carbon dioxide production at 10°Cwas essentially similar to that at 25°C although the amounts produced were significantly different. Hence the discussion of carbon dioxide production has been restricted to results obtained at 25°C(Table 7.3) and Lsds for comparison of treatment means at this temperature are given in Appendix 13.

Table 7.3: Rate* of carbon dioxide production at 25°C, μmol/g/hr

Treatment	Lo	Li	Lii
NIo(control)	0.87	0.9	1.17
NIi	0.84	1.76	1.89
NIii	0.72	2.13	2.48

*Calculated from simple linear regression of data obtained for 25 and 72 hours.

Although there were significant differences (p=0.05) in the amount of carbon dioxide produced due to treatments, the rates of production were quite low. The lag period, which lasted for about 24 hours, may have been due to soil handling and treatment stress on microorganisms (M.A. Line, pers. commun.). Therefore, the calculation of rate of carbon dioxide production was based on the results obtained between 24 and 72 hours. After 72 hours of incubation the rate of evolution of carbon dioxide began to decline. This decline after 72 hourss. could be a result of toxicity associated with the then high level of carbon dioxide in the closed

environment.

Production of carbon dioxide was affected both by pH and NI. The significance of the result for 120 hours, needs to be considered cautiously since the high level of carbon dioxide in the head space of the incubation bottles could have had different effects on organisms with variable pH optima.

Although the application of nitrapyrin at the rate of 20ug/mg of added N to limed soil considerably increased the rate of carbon dioxide production, its addition to unlimed soil had no significant effect (p=0.05). Rates of carbon dioxide production for the two liming rates did not significantly differ at the low rate of nitrapyrin (ie LiNIi and LiiNIi). The increase in rate of NI from 20 to 200ug/mg of N added further enhanced the CO_2 production at both rates of liming.

Mullison and Norris (1976) reported that nitrapyrin may not affect soil bacteria at low concentrations but that it may increase the number of colonies obtained from soil extracts at concentrations of 400 and 1000ppm suggesting that at higher rates nitrapyrin could favour the growth of some soil microflora. On the other hand Laskowski et al.,(1975) reported that nitrapyrin was inactive on soil microorganisms other than Nitrosomonas at 100ppm and that it had no effect on carbon dioxide production at 95ppm although at 950ppm the amount of carbon dioxide produced was significantly lower. Since the concentrations used in the present investigation (4 ppm and 40 ppm) were far below the levels used by Laskowski et al., (1975) it may be suggested that the effects observed in the present experiment may be attributable to a simultaneous stimulation of heterotrophic flora and inhibition of autotrophic nitrifiers that utilize carbon dioxide.

Liming is known to enhance both mineralization immobilization turnover (MIT) and nitrification processes (Adams and Martin, 1984). Jenkinson (1984) stated that when a readily available carbon source is present both mineralization and immobilization are likely to be favoured over nitrification processes and heterotrophs capable of immobilization may compete successfully with nitrifiers for ammonium as an N-source. Therefore factors that cause partial or complete cessation of nitrification could encourage immobilization. The increased rate of carbon dioxide production with application of lime when compared to that of the control (no lime, no nitrapyrin) may have been due to the activity of heterotrophic bacteria exhibiting optimum activity at higher pH. The activity of heterotrophic bacteria has been shown to result in much greater carbon dioxide production vis-a-vis its consumption by autotrophs (Wood, 1986). This result may also suggest that some heterotrophic microorganisms in soil are capable of using nitrapyrin or one of its hydrolysis products (6-CPA or 6-HPA) in their metabolic pathways. The absence of a significant residue level of any of these major hydrolysis products in soil has been attributed by Mullison and Norris (1976) to their photolysis and microbiological decomposition.

The lack of a significant increase in carbon dioxide production following addition of nitrapyrin to unlimed soil (Lo treatments) may be due to an unfavorable pH for growth of heterotrophic microbes and utilization of available C sources (nutrient broth or nitrapyrin). It appears the stimulatory effect of NI on carbon dioxide production becomes significant only at higher soil pH probably because of better environmental conditions for heterotrophic activity.

It is also possible that at different pH the soil materials used were supporting different microbial populations (Belser and Schmidt, 1986) with different pH optima. Such different microbial populations may react differently towards NI as pointed out by Hendrickson and Keeney (1979) when they examined the response of nitrifiers to nitrapyrin at different pH levels.

Thus it would be interesting to investigate further whether there are specific soil-borne microorganisms capable of utilizing nitrapyrin or its hydrolysis products as their C and N source and to determine the possible mechanism of such use. (i.e. whether nitrapyrin or its hydrolysed products are biodegradable).

CHAPTER EIGHT

8. Re-distribution of added urea, ammonium-N and nitrapyrin by leaching in artificially packed soil columns, and persistence of nitrapyrin in soil.

8.1 Introduction

The use of nitrification inhibitors may provide an effective means of reducing losses of soil N via nitrate leaching and denitrification (Malhi and Nyborg, 1988b; Powell, 1986). Although such chemicals are already used commercially in some countries in order to improve crop performance, the reported performance of common nitrification inhibitors in many field trials has been highly variable (Appendix 1, Sahrawat et al., 1987). Hence the factors that influence the effectiveness of these chemicals continue to be the subject of considerable research interest.

The factors determining inhibitor performance can be broadly classified as environmental (soil, climatic, plant, and microbial) and as factors associated with the chemical itself (see Section 2.3). According to Hauck (1975) this latter group of factors (eg. "product" characteristics such as mobility, specificity and persistence) are as important as environmental factors in determining the effectiveness of inhibitory compounds. Surprisingly, only a few workers have dealt with "product"-related characteristics of nitrification inhibitors.

Bock (1981) carried out a series of experiments on the mobility of another nitrification inhibitor dicyandiamide, in an attempt to determine the extent to which this compound separated from applied ammonium ions or urea during mass flow. No similar investigations appear to have been undertaken on nitrapyrin except for related studies by Briggs (1975), Landua, (1976) and Rodgers and Ashworth (1982) (Section 2.3). In view of the lack of research data on "product"-related characteristics of nitrification inhibitors and the observed variability in their effectiveness when tested in the field and in glasshouse experiments (Goring, 1962; Slangen and Kerkhoff, 1984) further studies on their behavioral characteristics under different environmental conditions are warranted.

The present experiment was designed to investigate the relative mobility and vertical distribution of urea, ammonium, nitrate and nitrapyrin (NI) in a soil column in response to successive leachings. Also, the persistence of nitrapyrin was studied when subjected to regimes of simulated spring (10°C and summer (25°C) average temperatures, with a constant air flow over the soil surface. A knowledge of such characteristics should be relevant for a satisfactory explanation of variations in results from field and glasshouse experiments.

8.2 Material and Methods

Initially, undisturbed soil columns were to be used because of their closer similarity to field soil conditions. However preliminary investigations with undisturbed soil columns (600 mm in length and 120 mm in diameter) extracted with a motor driven core sampler into PVC tubes showed that this system was unsatisfactory for the planned leaching experiment for reasons described later in this chapter (see Section 8.3).

Thus it was decided to use artificially packed soil columns. Three types of column containers were tested viz., glass tubing of internal diameter 45mm with an outlet of 8mm in the bottom end; longitudinally split pvc tubing of 50mm internal diameter; and a column comprised of segments separated by brass washers as described by Bodman and Colman, (1943) but modified to suit the present experiment were tested. The Bodman and Colman column was selected because of its advantages for the present experiment which are discussed below (P. 132).

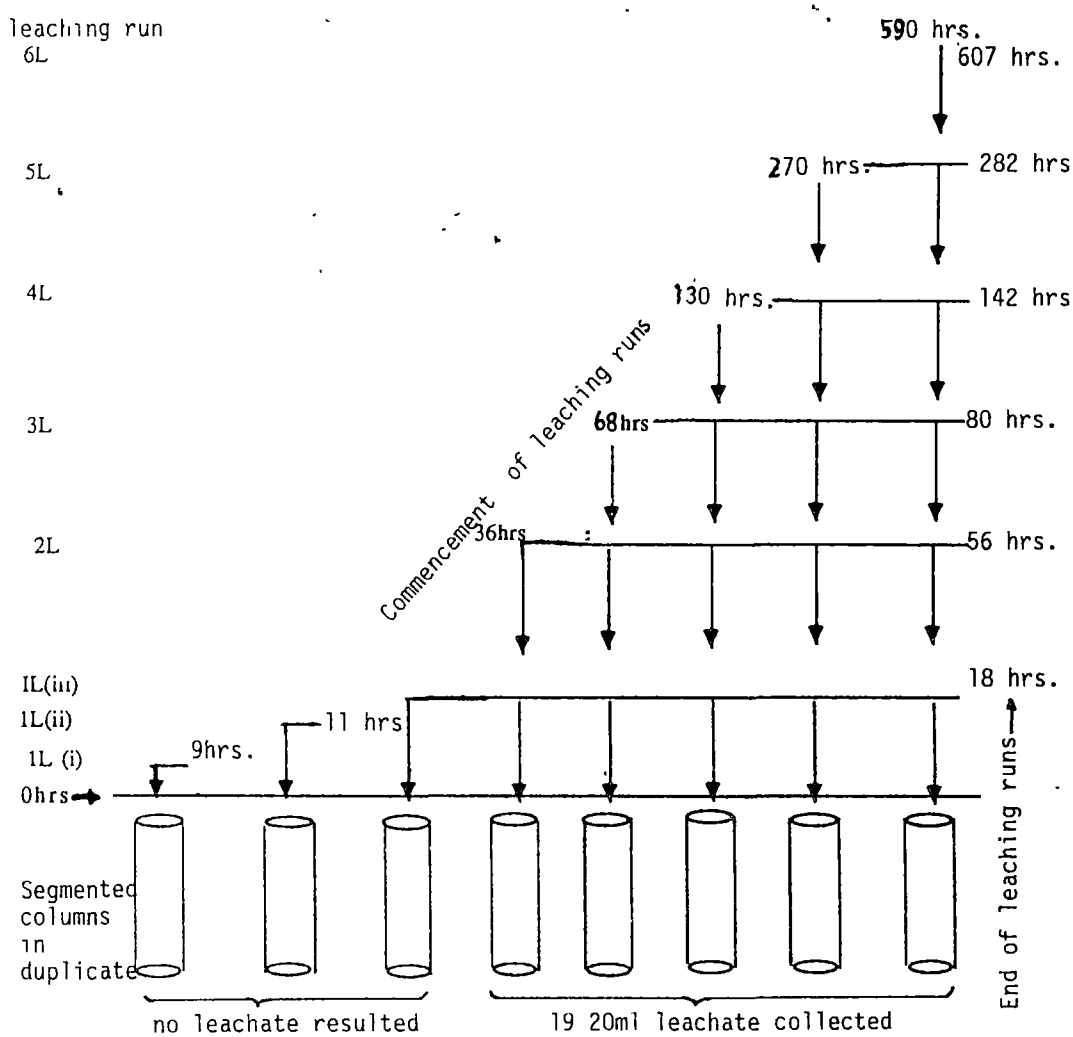
Soil columns: Preparation of the soil columns was carried out according to the procedure proposed by Bodman and Colman (1943). The modifications to the column included a 50 ml beaker that was inserted in the lower part of the column (Plate 5), while, instead of the brass segments used by Bodman and Colman, the columns used for the present work were constructed using 6.5mm thick square perspex plates with a central circular hole 40 mm (i.d.) (Plate 6). All cut surfaces were polished for good visibility of the soil column. When forming the column the segments were assembled on two diagonally opposed rods and adjacent segments were separated by two 1mm thick brass washers. Two layers of fibre-glass gauze fixed to the bottom segment and a little glasswool prevented soil from falling through but allowed rapid passage of water. When filled with soil the total effective length of soil column was 163mm. When sampling the column, copper plates were slid through alternate 1mm gaps thus separating paired segments to give a sample thickness of 14mm (2x 6.5mm + 1mm). As each segment accommodated about 7-8g of soil (OD basis) the weight of soil in fully packed columns averaged about 132g giving an average bulk density of 0.80g/ml.

The soil used in this experiment was the same as that described in Chapter 3 except that it had been limed to pH 6.5 after passing through a 2mm seive. The limed soil was stored in a plastic container for six months prior to packing into columns.

Figure 8.1 Schematic diagram showing the experimental procedure followed during the leaching experiment

The diagram shows leaching of air dry soil columns only

Initially wet soil columns were subjected to 18hrs (1L) and 36 hrs(2L) leaching runs only.



Air-dry soil columns: Eight sets of duplicate columns (16 columns in all) were filled with air-dried soil. 2ml of a solution containing 0.325mg urea/ml (150ug urea N/ml), ammonium sulphate (NH_4^+-N 0.35mg/ml) and 100ul of NI (0.24mg a.i.) was added to the top of each column. Immediately after addition of the solution the surfaces were covered to a depth of 5mm with air-dry soil to minimize volatilization losses of ammonia and nitrapyrin. With this addition the total length of soil column was increased to 168mm.

Initially saturated soil columns : To study the movement of NI and fertilizer-N under saturated water flow conditions, two additional sets (2x2) of similarly prepared soil columns were used. The level of water was raised gradually until free water stood above the soil surface at the top of the column. These columns are termed "wet" columns below.

Infiltration and Leaching Runs: Time zero for all infiltration and leaching runs was that at which water was first applied at the top of the column. Sampling times and the duration of each leaching run are shown in Figure 8.1. Although a similar number of leaching runs was initiated with both air-dry and wet soil columns the last three sets of the latter columns had to be discarded following accidental disruption of the regulated water supply during the third leaching. Hence, only two samplings of the wet columns under leaching conditions were carried out i.e. following a first leaching immediately after fertilizer addition (1L) and a second leaching 3 days after fertilizer application (2L).

Leaching : Leaching was carried out by releasing water onto the upper surface of the soil column protected with a moist filter paper and maintaining a head of less than 1mm depth of water on the surface. A marriotte tube arrangement was used with the addition of a regulatory valve on each inlet tube to control water flow (Fig. 8.2). A syringe needle was installed 1mm above the surface and connected to a constant flow pump operated at 5ml/hr to suck out any excess water (Plate 6). Distilled water from a common reservoir was released at a rate of 1.2-2.5ml/hr. At the beginning of each leaching run, it was necessary to supplement water supply manually to establish a water head (<1mm) as rapidly as possible to encourage uniform infiltration.

The term "leaching run" used below describes the passage of water through the columns. Supply of water to all twenty of columns (8 x 2 of air-dry columns and 2 x 2 of wet columns) was started almost simultaneously. During the first leaching run (which may be called "pre-leaching" since controlled application of water resulted only in wetting of the complete length of the columns with no measurable outflow) three sets of air-dry soil columns were sampled at three different stages. This condition was achieved experimentally by interrupting infiltration initially in six air-dry soil columns (three sets of duplicates) when the wetting front had reached depths of 9-10cm (8hrs), 13-14cm (13hrs) and 16-17cm (18hrs). These three sampling

stages have been referred to as stage 1, 2 and 3 of the first leaching run in the text and in text figures as 1L(i), 1L(ii) and 1L(iii). This part of the experimental work served to demonstrate uniform advance of wetting front and also confirmed that uniformity of column packing could be achieved with considerable precision. The final procedure was adopted only after repeated trials involving at least seven separate column packings for testing of water control system, infiltration characteristics and sectioning to determine moisture relationships.

For each leaching run (runs 2 to 6 for initially air-dry soil columns and during both runs of wet soil columns) enough water was supplied to produce an outflow volume of about 19-20 ml which was earlier measured as equal to one pore volume for the columns (mean of eight sets of air-dry columns). The determination of the distance travelled was possible only during the first leaching run when the advancing wetting front was clearly visible. At the end of 18 hrs water supply to the columns was terminated and the columns were kept covered until the next leaching. One set of columns was sampled at the end of each run. Although water continued to re-distribute downwards within the columns after stopping the water supply, no leachate emerged from the columns at the end of first run.

At the end of 18 hours of infiltration, water supply was cut off and twelve columns (ten initially air dry and two wet soil columns) were wrapped overall in aluminium foil and stored at 10°C in a closed cool room until 36 hours had elapsed from time zero. Infiltration was then resumed and continued until approximately one pore volume of out flow (19- 20 ml) had been collected. At this point infiltration was again interrupted and the columns were stored as before until 68 hours had elapsed from time zero. This sequence of operations was repeated with leaching runs commencing at 36, 68, 130, 270, and finally 590 hours (Figure 8.1). One set of two columns was sampled after each of these leaching runs and the soil segments were analyzed as described. The leachates were analyzed separately so that after leaching run 2, ten leachates were available for analysis, eight after leaching run 3 and so on until there were two final leachate samples after leaching run 6.

The actual leaching runs were carried out over periods of 36-50 hours (2L) 68-80 hours (3L), 130-148 hours (4L), 270-282 (5L) and 590-607 hours (6L) (Time periods given are rounded up to the nearest hour).

Plate 5: Demonstration set-up showing the various stages of the present experiment.

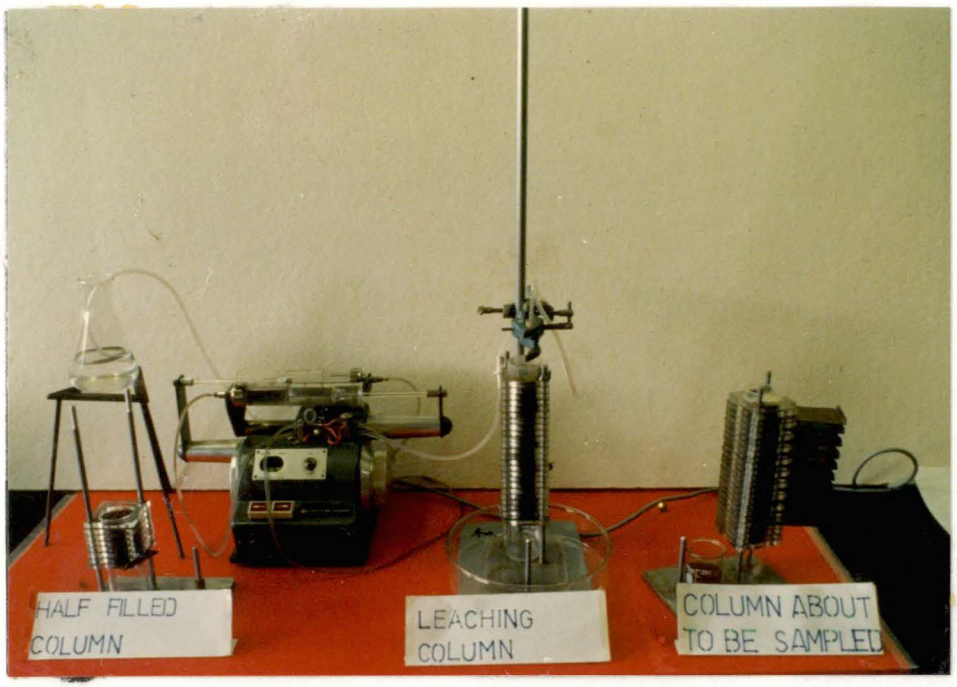


Plate 6 : Upper surface of a leaching column with protecting filter paper in position

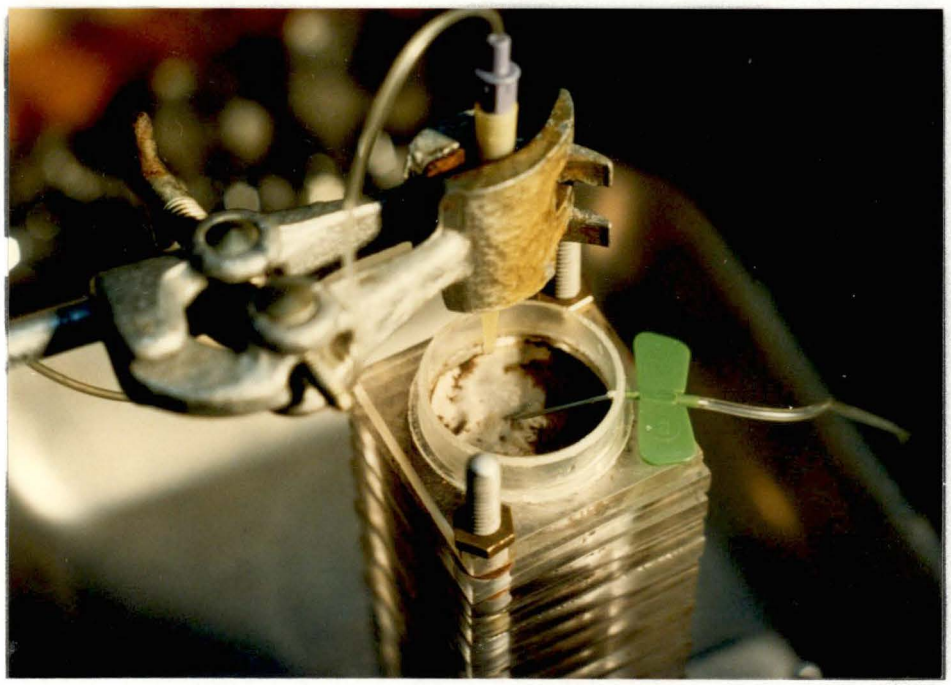
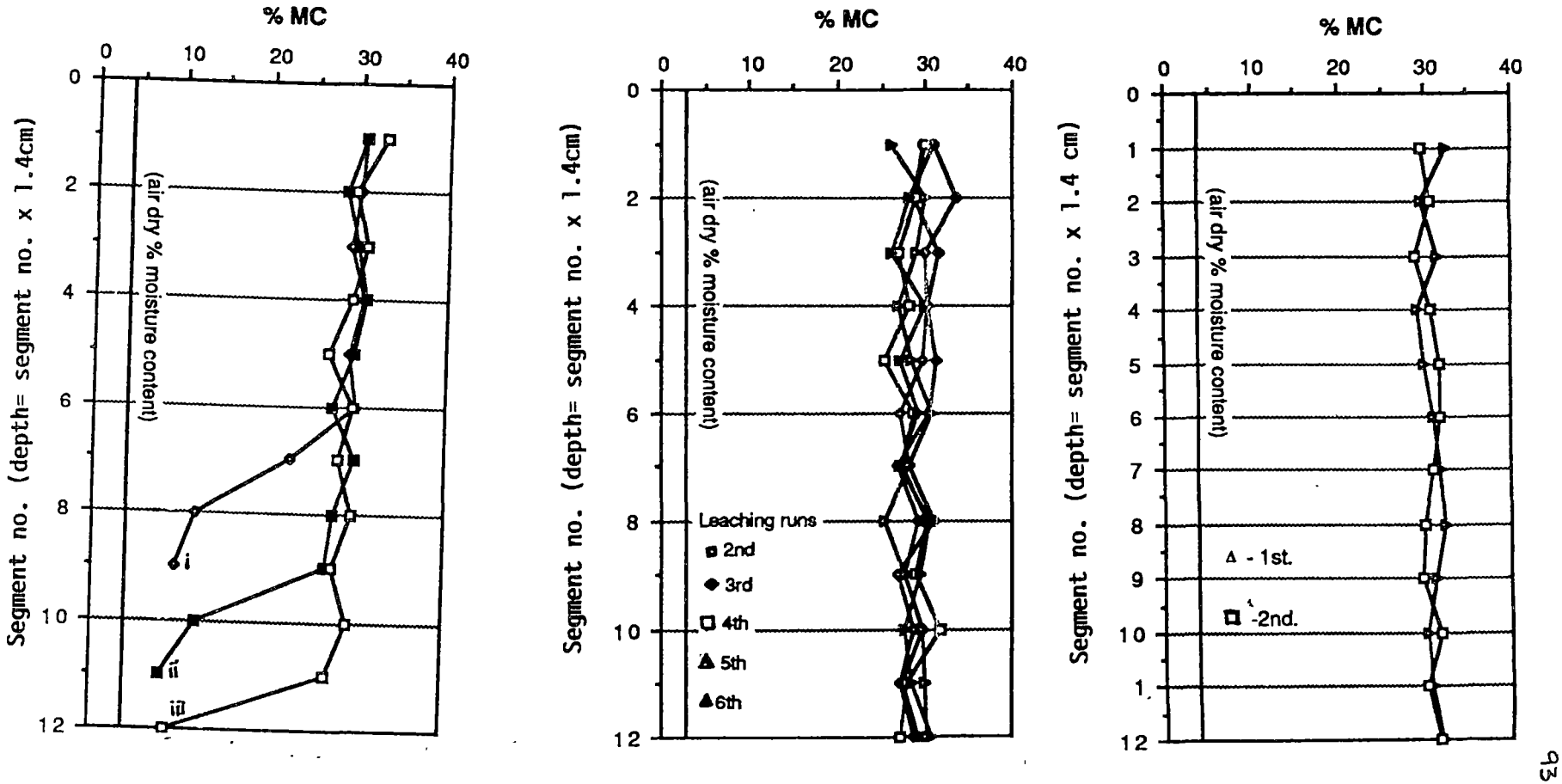


Fig. 8.2: Moisture distribution in initially air-dry (a & b) and initially wet soil columns

a. after i,ii,and iii stages of first leaching run

b. after 2,3,4,5 and 6 leaching runs

c. after 1 and 2 leaching runs



Actual depth=Y axis (segment no.) X 1.4cm (height of one segment)
air dry %mc = 4.6 (w/w O.D basis)
%MC: per cent moisture content, Number shown on Y axis correspond to segment numbers.

Sampling: At the end of each leaching run sectioning of soil columns was carried out using thin metal slides. Plate 5 shows a column about to be sectioned with copper slides positioned between column segments. The soil separates (about 15g soil, O.D.) were transferred immediately into aluminium weighing boxes with tight-fitting lids and weighed. After mixing, the soil was sub-sampled for analysis of urea, ammonium and nitrate plus nitrite (about 5g of sample OD basis), nitrapyrin (1-3g of sample O.D.) and the remaining soil was used to determine moisture content.

Persistence of nitrapyrin: Presence of the chemical in the active form in soil is important for effective nitrification inhibition. Hence the persistence of nitrapyrin in the soil used was determined by a method similar to that used by McCall and Swann (1978). Air-dried soil (25g O.D.) in each of sixty 600 ml tall bottles (210mm tall and 70mm i.d.) was moistened to about 75% of field capacity and mixed thoroughly before addition of 50µl of nitrapyrin solution. Half the number of bottles maintained at each temperature (10° and 25°Cwas subjected to a constant air flow of 120 ml/min. Air flow was directed through a water bath that was used to adjust the flow rate as well as to moisten the air.

Analytical procedures: For the analysis of urea, ammonium and nitrate (including nitrite) extracts were prepared by shaking 5g (O.D> basis) of soil with 25ml of KCl-phenylmercuric acetate (PMA) solution for two hours followed by filtering (whatman 42). The PMA solution was prepared by dissolving 1500g of KCl in 9 liters of water and adding 1 liter of 50ppm PMA solution. An aliquot of the filtered extract was used to analyze urea by the modified diacetyl monoxime method (Douglas and Bremner, 1970). A further aliquot was used to determine ammonium and nitrate plus nitrite N by the steam distillation method described by Keeney and Nelson, (1982).

Nitrapyrin was determined by extracting 1-2g (OD basis) of soil with 1ml of re-distilled Analar™ hexane containing hexadecane (120ug/ml). Hexadecane was used as an internal standard. An aliquot of the extractant solution (about 1-2ul) mixed with soil was kept overnight at 2°C before determination of nitrapyrin by MS/GC (mass spectrometry coupled with gas chromatography). The concentration of nitrapyrin in the extract was estimated using a standard curve prepared from a series of known NI/hexadecane concentrations (Appendix 16a).

A Hewlett-Packard model 5890 gas chromatograph coupled with a model 5970 mass selective detector equipped with a 25m (0.32um-i.d. x 0.52um o.d.) HP 5 column with 5% phenyl methyl silicone (cross-linked) was used for the analysis. The oven temperature was 200°C isothermal, and the injector temperature was 250°C.Head pressure was 75.845 N/m^2. Helium was used as the carrier gas at a flow rate of 1.7ml/min (measured at room temp with oven temp at 200 °C). An application volume of 1-2ul was split in a 10:1 ratio for GC/MS measurements.

This method consistently gave very high reproducibility and was sensitive

enough to detect trace amounts (0.001ug) of nitrapyrin. A typical mass-spectrogram of nitrapyrin and the standard curve used in the assay is given in Appendix 16b.

8.3 Results and discussion

Experimental procedures : The decision to use artificially packed soil columns instead of undisturbed soil columns was based on observations during preliminary experimentation. The use of intact soil columns extracted into PVC tubes resulted in seepage of water along the PVC tube wall/soil interface which could not be prevented satisfactorily without altering the soil structure. When tightly-fitting soil cores were extracted a considerable degree of compaction of soil around the tube wall was unavoidable, thus reducing the entry of water into such areas. Furthermore, the permeability along the column varied because of the duplex nature of the soil profile, with water moving rapidly through the sandy loam A horizon, but very slowly through the clay B2 horizon thus causing water stagnation within the column. However it was noted that some workers reportedly achieved a condition of field capacity in undisturbed duplex soil columns and appeared to have overcome the problem of stagnating water as no reference was made to such difficulties (Davey and Simpson, 1987). The permeability of undisturbed columns was affected also by large stones within the columns that were discovered only at the time of sectioning. Cutting these columns into thin sections was difficult and time consuming. Use of longitudinally-split PVC tubes helped to overcome the problem of delay in sectioning but posed a further complication as the horizontal placement of undisturbed columns for sectioning resulted in change of direction of water movement and was also conducive to contamination of neighboring sections. These two problems do not appear to have been addressed by Bock et al. (1981) in his study using unsplit PVC tubes containing undisturbed soil columns.

In view of the difficulties in realizing a reasonably uniform water flow and the problems associated with sampling of undisturbed soil columns three different types of artificially packed soil columns as described earlier were tested. In addition to the benefits highlighted by the original users, further advantages of the column proposed by Bodman and Colman (1943) were that:

1. it was possible to avoid almost completely any contamination of neighboring samples at the time of sampling;
2. sectioning was easy and almost instantaneous so that no change in flow direction resulted during the sampling;
3. it was possible to maintain uniformly aerobic conditions throughout the column as free lateral movement of air could occur through the gaps between the sections;
4. the development of positive air pressure in front of the wetting front associated with flow in closed or tapered columns as described by Sander and Paralang (1984)

was avoided;
5. the moving wetting front was clearly visible through the polished perspex sections of the column so that uniformity of water flow could be confirmed; and
6. each segment was packed with the same amount of soil so that uniform bulk density was achieved throughout the column.

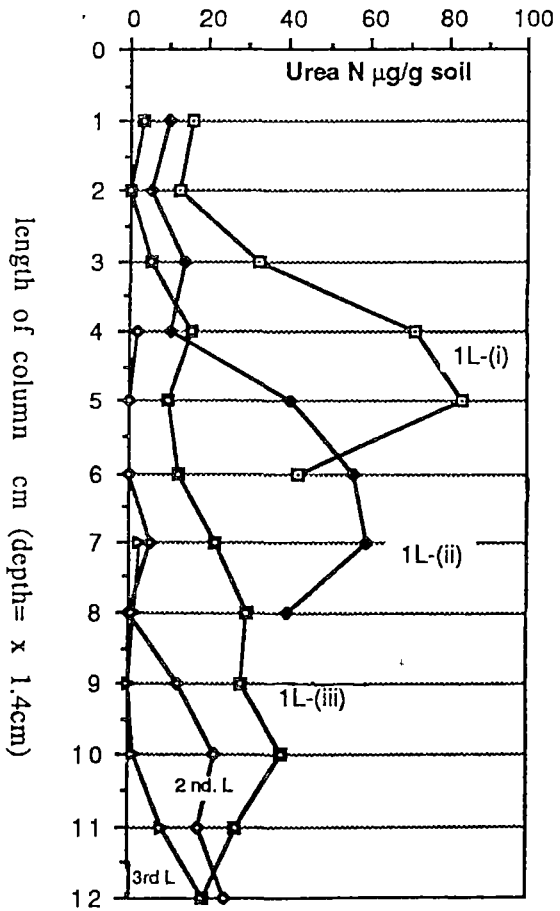
Relative mobility and distribution of moisture, urea, ammonium N, nitrate N, and nitrapyrin during infiltration:
In initially air dry soil columns.

Movement of water and urea: The distribution of moisture during the first leaching run of the air-dry soil columns is shown in Figure 8.2a. The moisture content in initially air-dry soil columns subjected to further leaching (runs 2 to 6) are shown in Figure 8.2b. The observed moisture distribution pattern through the column closely resembled that reported by Bodman and Colman (1943) who also used air-dried sandy loam soil in their investigation of moisture distribution during downward movement of water. The distribution of moisture in initially wet soil columns (Figure 8.2c) was not much different to that observed after leaching runs 2 to 6 into initially air-dry soil columns. The infiltration rate for initially air-dry soil columns during the first leaching run averaged 1.1 ml per hour. It was not possible to measure directly the infiltration rate for initially wet soil columns as the wetting front in these columns was not visible and also because leachate began emerging from the bottom of the columns soon after the start of the run. However the detection of traces of urea in the leachate collected from initially wet columns after the first leaching run suggested that the infiltration rate for wet soil was higher than the infiltration rate for initially air-dry soil. As stated earlier no leachate emerged at the end of the first leaching run in initially air-dry soil.(See Figure 8.2 a-c).

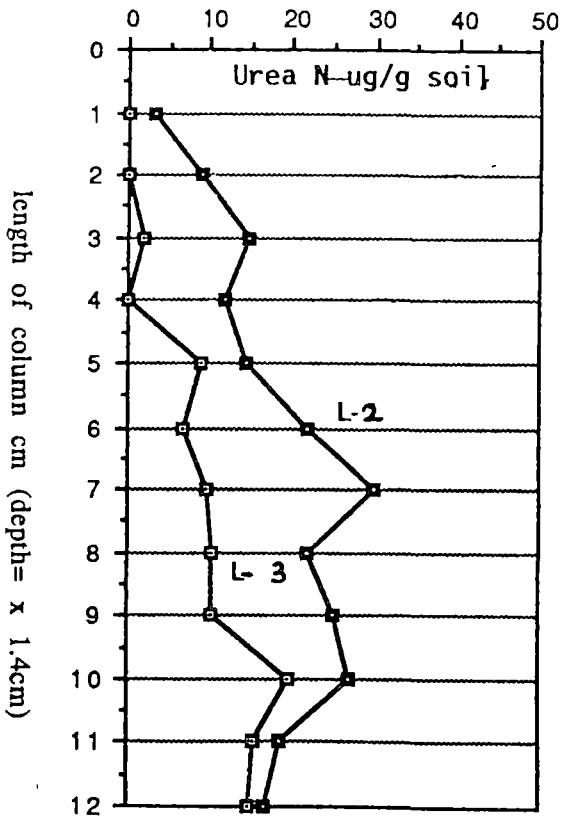
The movement of urea in initially air-dry soil columns took place quite rapidly with the urea peak appearing a few mm behind the wetting front (Figure 8.3a and b). As shown in the Figure 8.3a continuation of leaching from stage i to stage iii resulted in a marked shift of the urea peak down the soil column. Although no leachate emerged at the end of the first leaching run, detectable levels of urea were found in leachates collected after the second and third leaching runs. A similar movement and distribution pattern of urea was observed by Mahendra Singh et al. (1985) when they leached a sandy loam soil, while Broadbent et al., (1958) and Wagenet, et al. (1977) reported rapid movement of urea in other types of soil. Rapid translocation of urea during leaching may be attributed to the organic matter (Chin and Kroontje, 1962), and/or low clay content of soils (Balwinder Singh and Bajwa, 1985) as well as uniformity of the soil columns in the present study.

Fig. 8.3 : Distribution of urea–N in soil columns subjected to leaching.

a. after first(1L), second (2L) and third (3L) leaching runs of initially air–dry soil columns

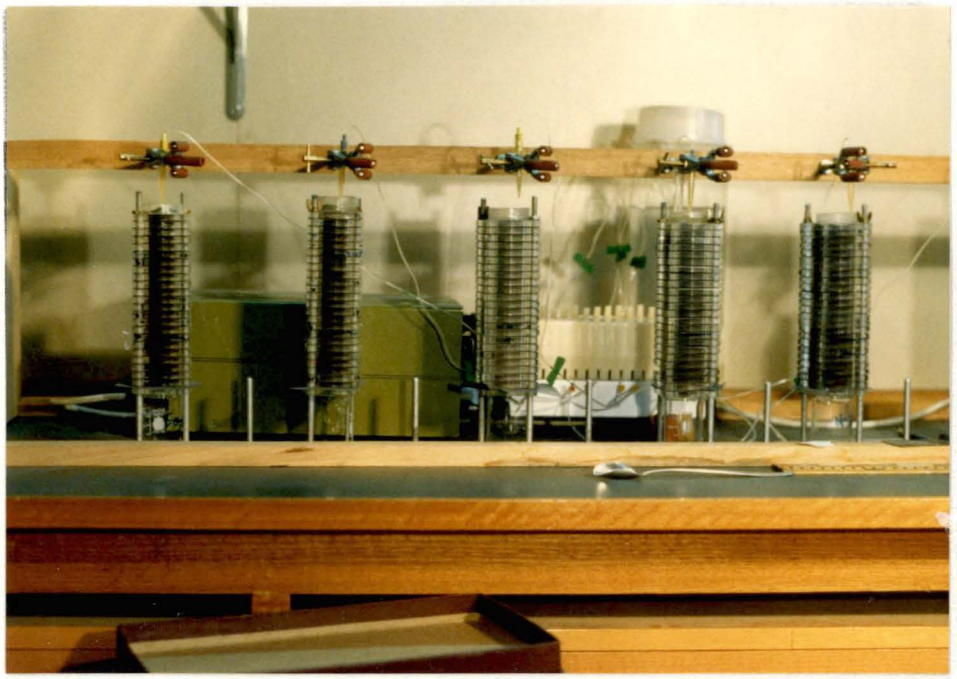


b. after second (L–2) and third (L–3)leaching runs of initially wet soil columns



(i), (ii) and (iii) represent one to three stages of sampling during the first leaching run respectively.

Actual depth of sampling= Y axis (segment no.) x 1.4cm (height of one segment)



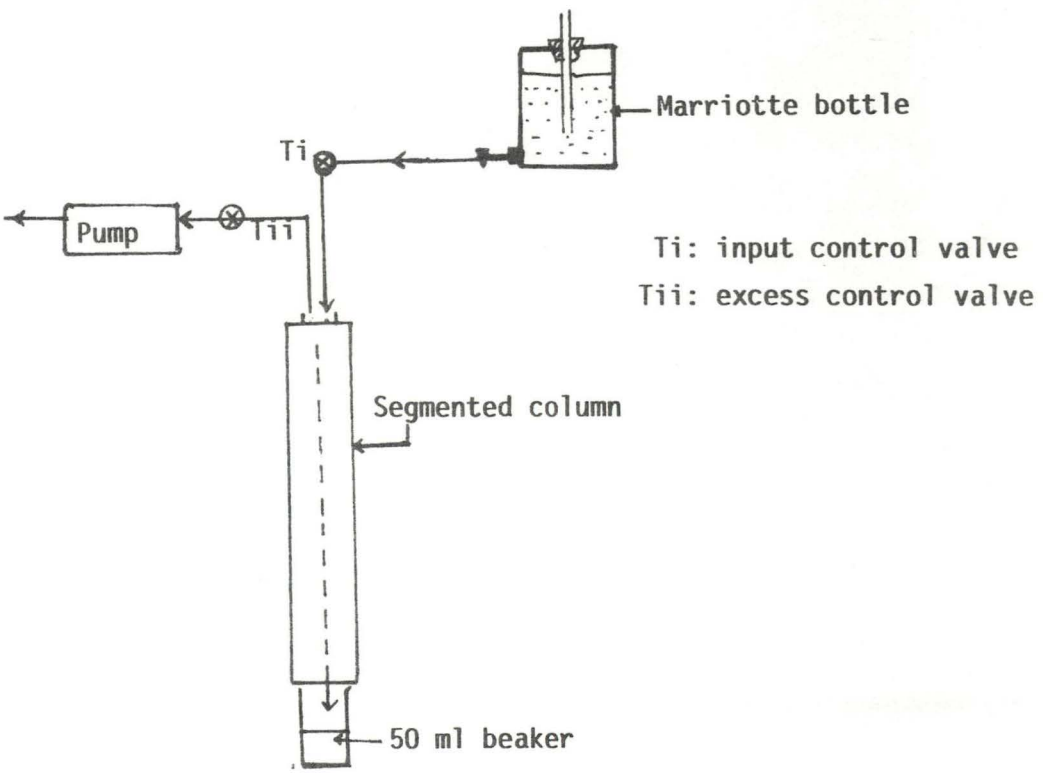


Diagram 1: Schematic diagram of leaching column shown in picture.

Recovery of urea-N declined rapidly from 85.9% of the total urea-N added at 8hr to 78.7% at 13 hr and then to 70.3% at 18 hr after the start of first leaching run (zero time). The recovery of urea-N after the 2nd and 3rd leachings was much lower 32.3 and 15.4 per cent of the total urea-N added respectively (Figure 8.4a and Appendix 14). Urea-N appeared in the soil and leachates collected for the second and third leaching but no urea was detected in either soil or leachates thereafter. Even at the end of the second and third leachings some samples from the upper half of the columns gave only a trace of pink colour in the diacetyl monoxime test, and could not be measured satisfactorily (Figures 8.3a and 8.4c).

The estimated rate of urea hydrolysis increased with time from 1.75 ug urea-N/hr at the 5 hr sampling to 4.34 ug urea-N/hr at 13 hrs and then increased further to 5.06 ug urea-N/hr at 18 hrs. However the rate of urea hydrolysis estimated at the end of the 2nd and 3rd leachings showed a marked decline (Figure 8.4b). The rate was calculated both on an incremental and cumulative basis using the equations shown in Appendix 14. The initial increase in urease hydrolysis was caused probably because of unused exocellular urease present in the lower part of the column coming into contact with advancing urea molecules. This increase may also be attributed partly to the increased activity of urease-producing microorganisms stimulated by the supply of urea. The decline in the rate of urea hydrolysis that followed with subsequent leaching probably resulted because, by the time of the 2nd and 3rd leachings, a significant volume of the columns had much less urea due to its earlier rapid leaching and hydrolysis. No detectable urea-N was detected in any of the later columns or leachates.

The disappearance of urea seemed to be relatively rapid in this experiment when compared to experiments described previously which gave peak urease activity between 5 and 15 days after urea addition (see Chapters 4 and 6 above). This may have been due to the greater soil/urea ratio and also to the fact that there were no leaching losses of urea in the earlier pot and incubation experiments.

Initially wet soil columns were analyzed at the end of the first and second leaching runs (i.e. 18 hrs and 72 hrs respectively after the beginning of the experiment). Since the moving wetting front in initially wet soil could not be observed, an initial three stage sampling as for initially air-dry soil was not carried out. However, it was noted that leachate started appearing from initially wet columns soon after the introduction of water to the top of the columns. It seems that this infiltrating water displaced the moisture in the wet soil. A volume of about 19 to 20 ml of leachate was collected for each run. The amount of urea-N recovered and its pattern of distribution did not differ much from that of the initially air-dry soil columns except for the small quantity of urea recovered from the leachate of the first leaching run of initially wet columns. Mahendra Singh et al. (1985) also noted the

similarity in pattern of distribution of urea in air-dry and wet soil columns. However, the present study showed that the rate of movement of urea through initially wet soil columns was slightly higher than that in initially air-dry soil columns (Figure 8.4c) whereas Mahendra Singh et al., (1985) found movement of urea to be similar irrespective of soil moisture status .

Some workers have reported increased urea hydrolysis at higher moisture contents (Kumar and Wagenet, 1984; Rachinsky and Peltser, 1965). However the results obtained from the present experiment did not show a significant difference in residual urea between initially air-dry and wet soils. The total recovered from the initially wet columns was slightly higher than that from the corresponding initially air-dry soil columns. The present observations agree with the findings of Overrein, (1963) and Zantua and Bremner (1977b) who reported that there was no effect of moisture on urea hydrolysis. On the other hand Dalal (1975) reported that urea hydrolysis decreased with increased soil moisture.

Urea-N recovered from initially wet soil columns was spread over a greater column length than for initially air-dry soil. The distribution pattern shown in Figure 8.4c compares the percent urea-N recovered from each section (at 14 mm depth intervals) in relation to total urea added.

Distribution of ammonium and nitrate N: Ammonium-N recovered at a given sampling time was the net result of native ammonium in the soil plus added ammonium, plus ammonium formed due to urea hydrolysis and by mineralization, less losses of ammonium (nitrification, leaching, ammonia volatilization and immobilization of ammonium). Ammonia volatilization was not measured but was assumed to be minimal due to low temperature and wrapping of the columns with Al foil to reduce air circulation during the experiment. Mineralization and immobilization turnover was also assumed to be minimal because of the low temperature and also because of no additional C source. In a separate experiment it was found that the activity of heterotrophic soil microorganisms did not change when N was added unless a C supplement was also incorporated (M.A. Line, pers. commun.). On the other hand urea hydrolysis and nitrification may be expected to have contributed to the net ammonium recovered at each sampling. Hence the distribution patterns of ammonium ions during a leaching run can be influenced by these factors in addition to the mass flow effect.

As shown in Figure 8.5 (a and b) and the data given in Appendix 17a the introduction of ammonium into columns from the surface resulted in a sharp increase in ammonium concentration in the upper segments of the soil columns. The ammonium-N levels at different depths after each leaching run can be presented as a percentage of the mean total ammonium-N recovered for each set of columns.

Figure 8.4a.: Urea–N recovered from columns after leaching runs 1 – 3 (initially air–dry soil; total urea –N added = 300 ug per column)

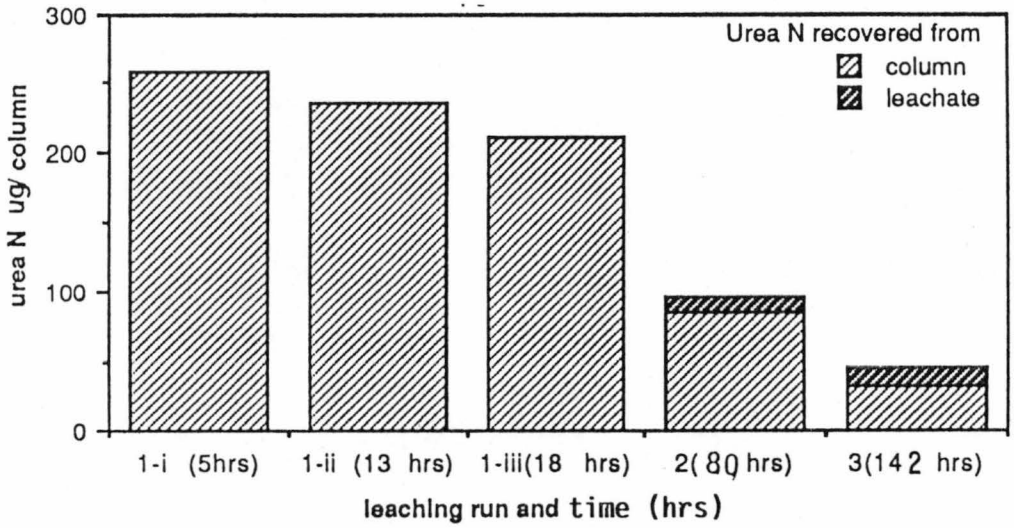
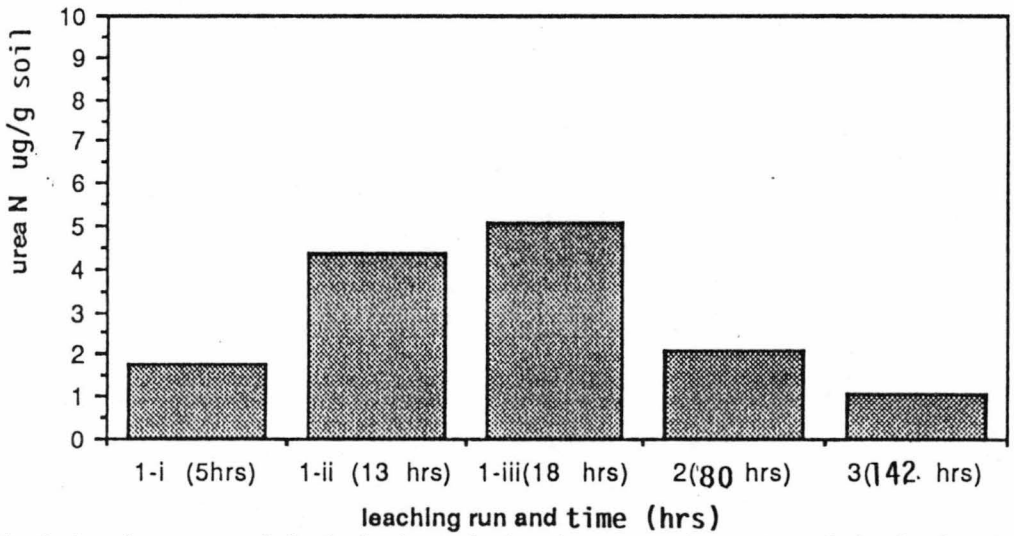
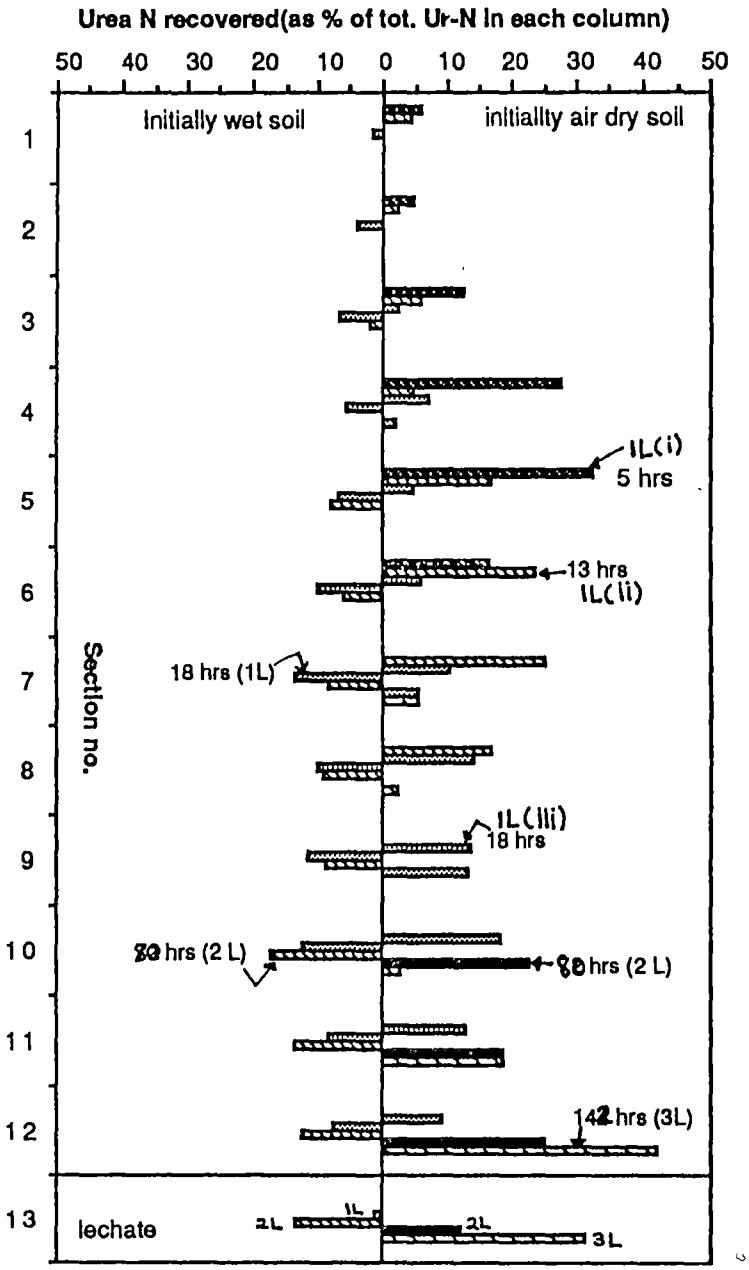


Figure 8.4b. Rate of urea hydrolysis calculated on incremental time basis.*



(* Only the rate of hydrolysis calculated on an incremental basis is shown here . The rate of urea hydrolysis are given in Appendix 14).

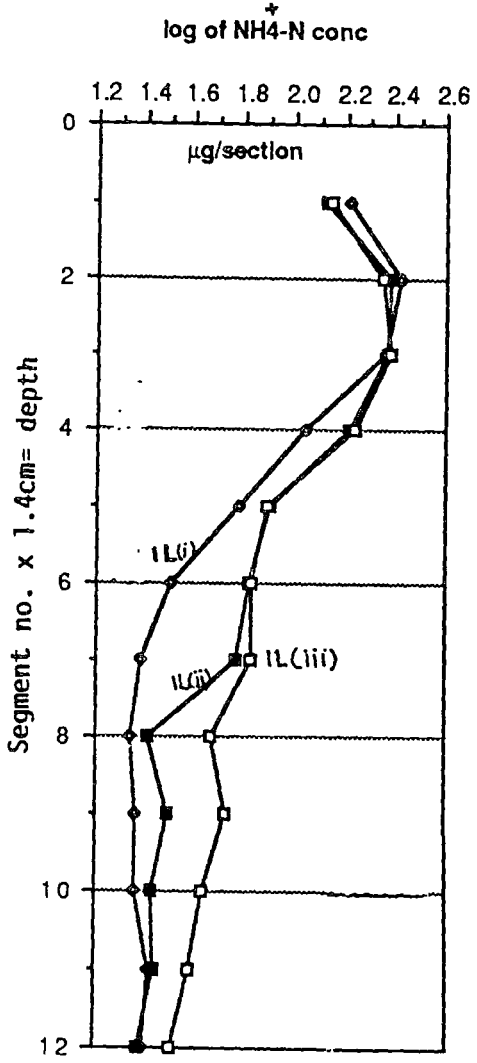
Figure 8.4c. : Percent of urea in initially air –dry soil columns and in initially wet soil columns after leaching.



Actual depth of sampling = Y axis (segment no.) x 1.4cm (ht. of one segment)

Figure 8.5(a , b): Amounts of ammonium-N recovered in initially air-dry soil columns

a. at the end of first three stage of first leaching run



b. at the end of 2nd to 6th leaching runs

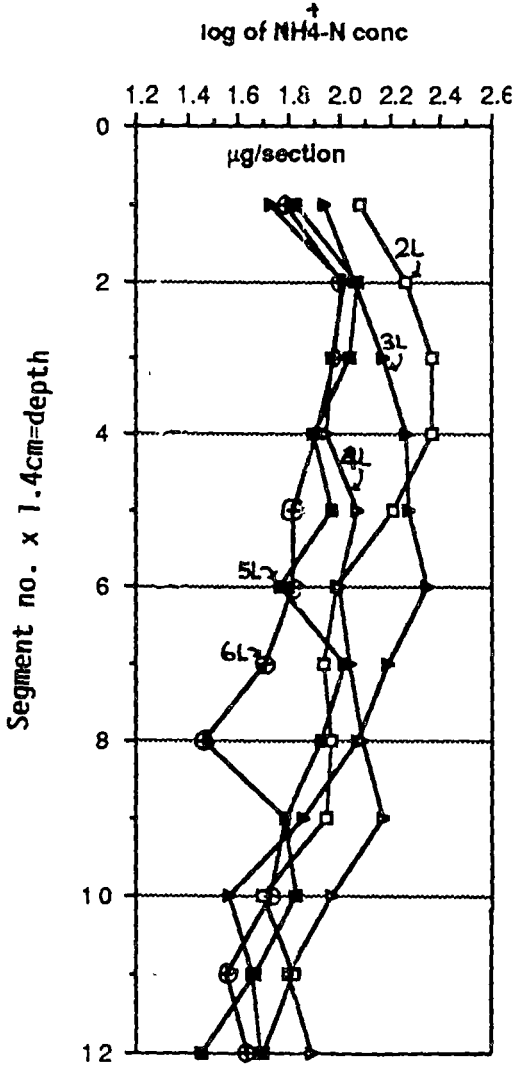
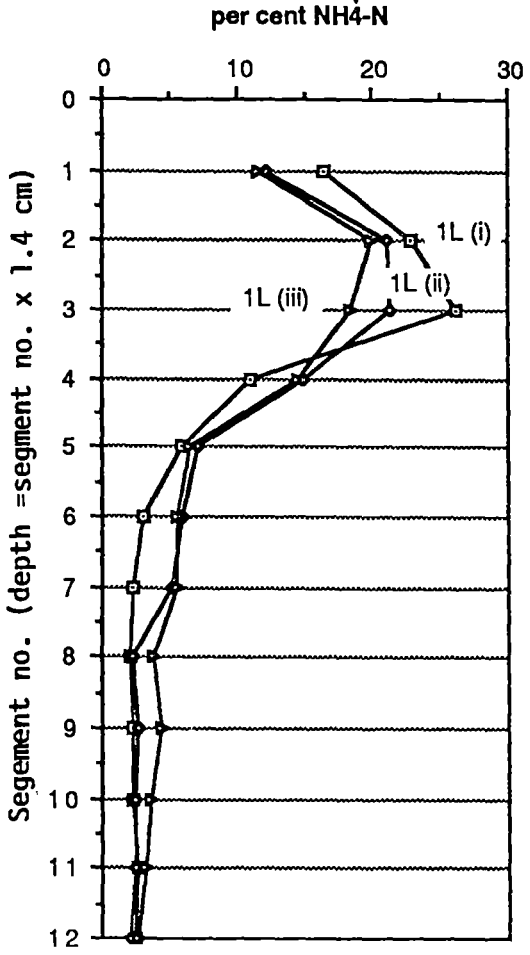
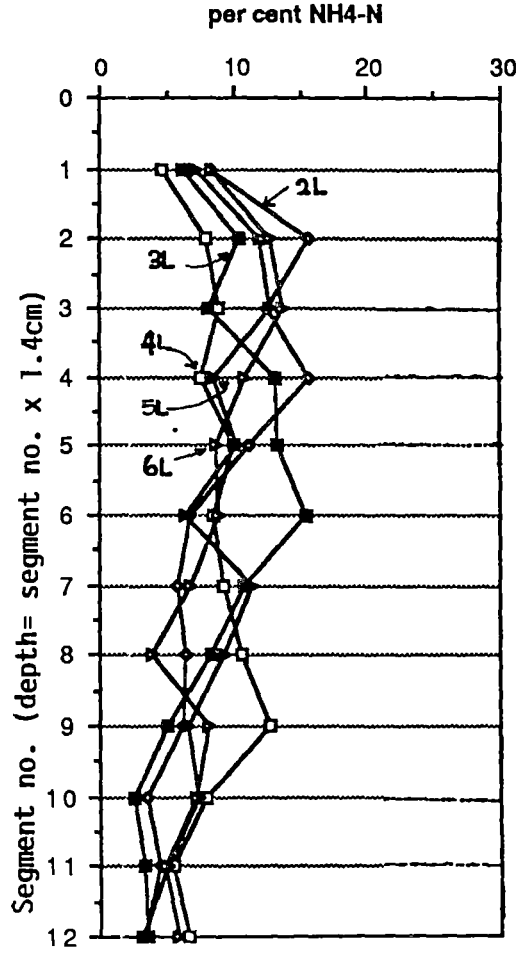


Figure 8.5 (c-d) Per cent distribution of ammonium-N

c. after three stages (i,ii,iii) of first leaching run (1L)



d. after 2nd to 6th. leaching runs.



This facilitates comparison of distribution patterns of ammonium-N without complications that may arise from differences in the balance of ammonium gains and losses between leaching runs (See Fig 8.5c and d).

The concentration in the lower half did not change during the first and second stages of the first leaching run simply because water did not reach lower depths. Therefore the data for depths greater than 10cm for 1L(i) and 14cm for 1L(ii) represent the native ammonium concentrations. However the ammonium-N concentrations increased appreciably in the lower part of the columns after the end of first [1L(iii)] and second leachings (2L).

Subsequent leaching runs resulted in progressively shorter but broader peaks of ammonium-N in the upper part of the soil columns that continued to diminish until the end of the experiment (Figure 8.5b). However the concentration of ammonium-N in the upper half of the columns was always higher when compared to the original levels of soil ammonium-N. The slow and regular decline in ammonium-N in the upper part of the columns was in contrast to the rapid and irregular fluctuations of ammonium-N in the lower parts of the columns (Figure 8.4b). The ammonium levels in the lower part of the columns continued to increase until the end of the second or third leaching but declined rapidly thereafter. Ammonium-N in the lower parts of the columns seemed to be more "active" than that in the upper layers. These changes were reflected to a lesser degree in the levels of ammonium-N recovered from the corresponding leachates (Appendix 17a). However the leachates did not contain particularly high amounts of ammonium-N.

If ammonia volatilization had a significant influence on levels of ammonium-N more of this form of N should have been lost from the upper parts of the columns where the concentration was higher. Presumably the initial increase in ammonium-N in the lower part of the columns occurred due to both translocation of urea and its subsequent hydrolysis, and to slow translocation of ammonium ions, while the later decline may be attributed mainly to the nitrification process. In order to assess the contribution of nitrification to the observed fluctuation of ammonium-N levels, the nitrate-N levels in columns and leachates were also assayed.

The original nitrate-N concentration in the soil was quite low (5-10 μg/ g soil). Figures 8.6a and b show the pattern of change in nitrate-N in the columns. Although the pattern of nitrate-N distribution changed markedly at each of the three stages of the first leaching run the total amount recovered (Appendix 17b) did not vary significantly. This indicates that the nitrate peaks that were located just behind the wetting fronts (Figure 8.6a) were caused by the movement of native nitrate-N with mass flow rather than the nett effect of nitrate production. The levels of nitrate-N beyond the wetting front at stages (i) and (ii) of the first leaching run represent the native soil nitrate-N. At the end of leaching run-2 (2L) the nitrate-N concentrations

showed an almost linear increase with depth while the 20ml of leachate collected contained 24.5% of the total nitrate-N recovered. Subsequent leachings resulted in a gradual decline in the nitrate-N recovered from the columns, but the concentration of nitrate-N in the leachate fluctuated widely as shown in the Figure 8.6c and Appendix 17b. The nitrate-N content of the leachate was the lowest after the third leaching run (3L) and increased again until after the fifth leaching run (5L). The amount of nitrate-N in the leachate again declined after the sixth leaching run (6L). It may be noted that, unlike ammonium-N, the changes in nitrate-N concentration in the columns had little relation to the changes in nitrate-N in the leachates. (Figure 8.6a,b, and c)

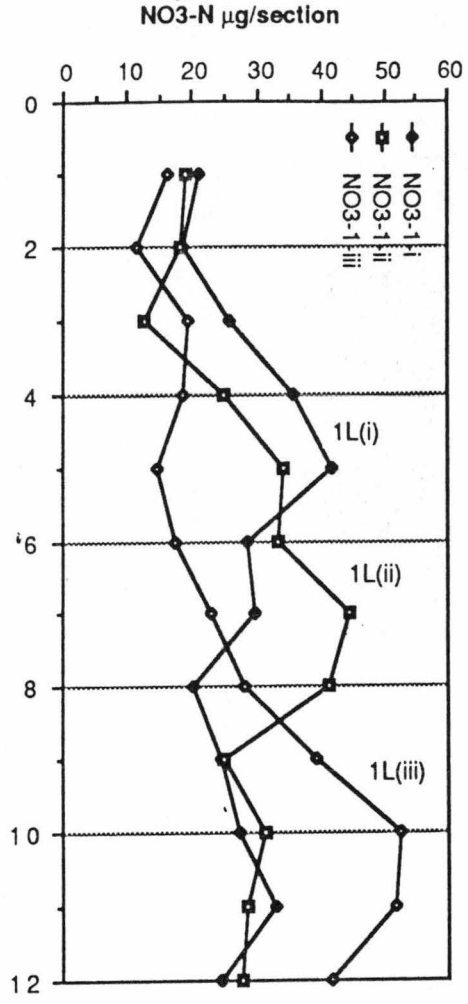
Leaching of columns appeared to have removed a total of 386(+37)ug nitrate-N per column. This amount was greater than that measured in the soil columns after the first leaching run which had no leachate (about 340(+22)ug per column). Since another 168(+31)ug of nitrate-N (mean of duplicate) was recovered from the soil in the columns subjected to the sixth leaching it would appear that a significant amount of nitrate-N was produced in the columns during the period of this experiment.

The distribution patterns of ammonium-N after leaching of initially wet soil columns were similar to those observed in the case of columns of initially air-dry soil. The higher ammonium-N concentration found in the upper half of the initially wet soil columns after first and second leaching runs indicated restricted movement . In contrast, nitrate-N seemed to have moved rapidly and appeared even in the leachate collected after the first leaching run. Although leachates were rich in nitrate-N, no distinct nitrate-N peaks could be found within the initially wet columns sampled after leaching. Presumably nitrification had occurred during the periods between successive leaching runs and the nitrate-N produced was subsequently leached from the columns (See Figure 8.6b)

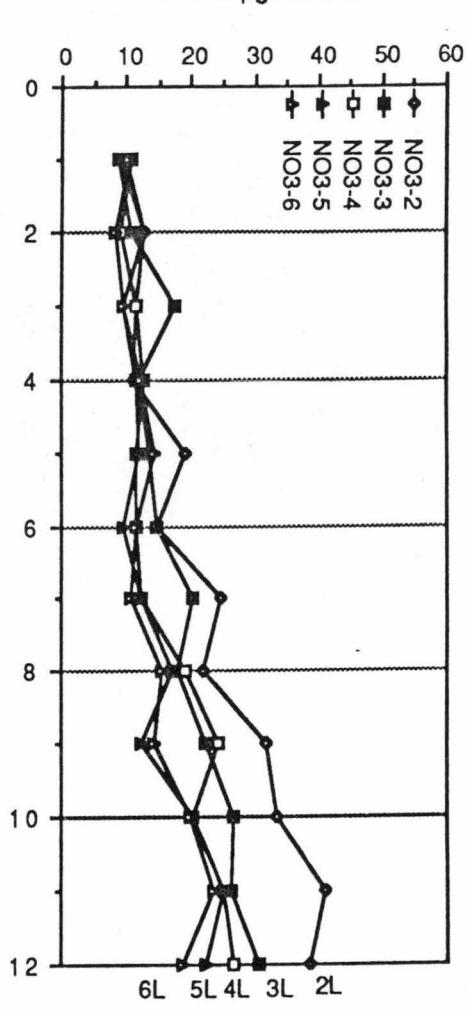
Although the total amount of nitrate-N recovered at any given sampling time was much less than the ammonium-N recovered it was still greater than the mean total native nitrate-N levels, thus indicating positive net nitrification in all the soil columns. Since nitrification inhibitor was added to the soil with ammonium even the small increase in nitrate production that occurred was somewhat unexpected. Moreover, in view of the low temperature that prevailed during this experiment volatilization and/or hydrolysis losses of nitrification inhibitor would have been negligible, thus giving a better likelihood of inhibition. As the rate of nitrification increases with temperature (Mahendrappa et al.,1966; Myres,1974) there is a high possibility that such rapid increase in nitrification could significantly affect N loss by leaching.

Figure 8.6(a & b) : Distribution of nitrate N in (air-dried soil) columns

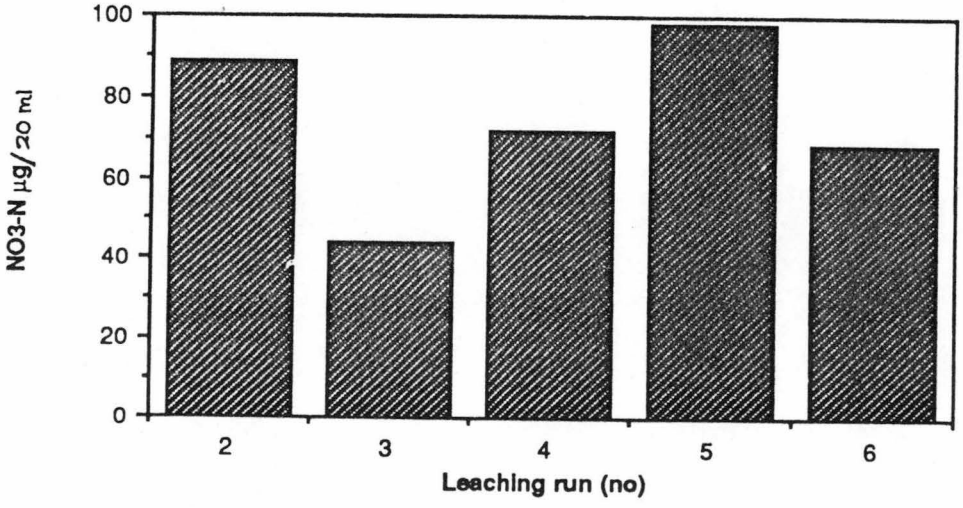
a. afer three stages of first leaching run



b. after 2nd to 6th leaching runs



c. Nitrate-N in leachates (initially air-dry soil columns)



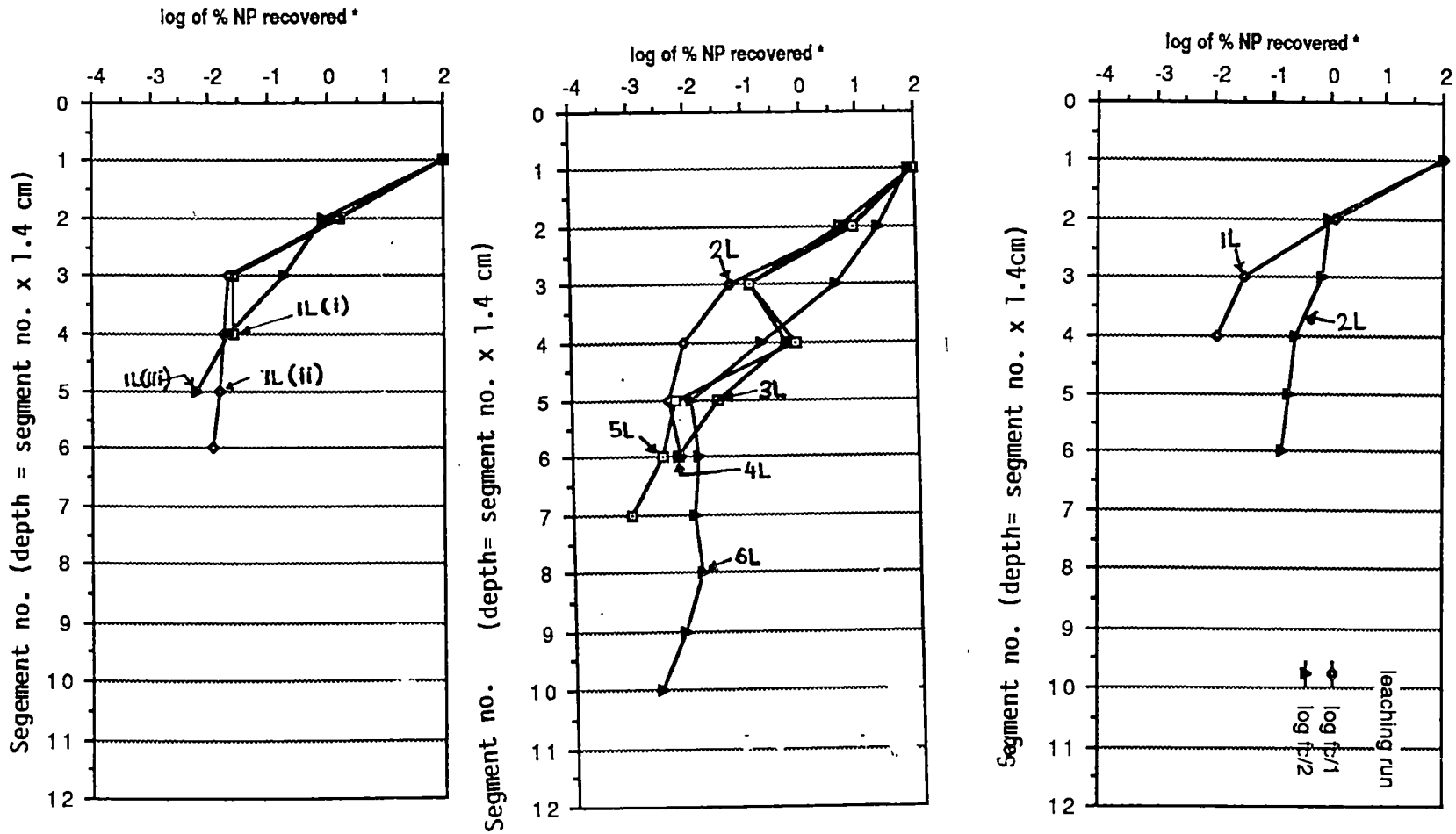
As there was no distinct distribution pattern of nitrate-N in the columns except during the first leaching (probably due to rapid translocation of this ion) it was not possible to discover whether nitrification occurred throughout the columns or whether it showed spatial heterogeneity. However, for reasons given above, and assuming that ammonium in the columns declined mainly due to nitrification rather than due to immobilization or ammonia loss, the pattern of decline in ammonium concentration may be used as an indirect indication of the distribution pattern of nitrate-N produced in the columns. Further, the observation that ammonium-N in the upper parts of the columns declined relatively slowly when compared to the rapid changes (especially rapid decreases) in the lower part of the columns, suggests that there could have been an additional factor or factors involved in the decline of ammonium-N in the lower part of the columns. As physical losses of ammonium-N (by leaching and volatilization) should obviously be greater from sites of higher concentration (i.e. upper part of column) the main reason for greater fluctuation of ammonium-N levels observed in the lower part of columns may be attributed mainly to nitrification. Therefore the distribution pattern of nitrification inhibitor was determined in order to facilitate explanation of the indicated spatial heterogeneity of nitrification within the columns.

Movement of nitrapyrin: Movement of nitrapyrin (NI) in initially air-dried as well as in initially wet soil was very slow during the entire period of the experiment. As nitrapyrin could be lost by volatilization and hydrolysis, and also to facilitate its detection, the amount added was four times that used in previous experiments (Chapter 6 and 7). More than 90% of nitrapyrin recovered after each leaching run was found in the first 3cm of the columns (Figure 8.7a and b). Because of this limited movement of nitrapyrin, the urea peak advanced ahead of the NP peak soon after the start of the first leaching (compare Fig.8.2a and Fig. 8.7a). On the other hand, because of the slower rate of movement of ammonium ions, the zone of peak concentration of NI overlapped that of ammonium-N for a longer period i.e., during the first three leaching runs (Figure 8.3a and 8.7a) after which ammonium-N moved ahead of NI (Figure 8.3b and Figure 8.7b).

Even with successive leaching little nitrapyrin was transported towards the middle of the columns (Figure 8.7b) and the amount recovered from depths greater than 4cm did not exceed 1% of the total NI recovered after any leaching run. The amount of NI recovered from each column (shown in absolute terms in Appendix 16) indicates that the concentration of NI at lower depths in the columns was much lower than the minimum concentration of 5-10ug nitrapyrin/g soil necessary to effectively inhibit nitrification in mineral soils (Rodgers and Ashworth., 1984; Sahrawat, 1987).

Figure 8.7 (a-c): Distribution of nitrapyrin in soil columns,(a & b)= initially air dry soil, c= initially wet soil

a. after three stages of first leaching b. after 2nd to 6th leaching c. after 1st and 2nd leachings



In the initially wet soil columns the pattern of distribution was slightly different. Traces of NI moved relatively faster when compared to NI movement in the corresponding leaching runs of initially air-dry soil columns. Nonetheless, as for air-dry soil, the bulk of the NI (more than 90% of the total recovered at any given time) was found in the upper 1-3 cm of the column with traces only extending to the middle of the columns by the end of the second leaching run (Figure 8.7c).

In laboratory studies, Goring (1962) found that nitrapyrin broadcast on a pre-fertilized soil was more effective in controlling nitrification if followed by 25mm of water (rain) than a broadcast mixture of nitrapyrin and fertilizer without immediate leaching. His conclusions were based on nitrate production rates of the confined total soil mass. However if the conditions exist for spatial (physical) separation of ammonium-N from nitrapyrin, as in the present experiment and in most field situations, the extent of inhibition may be reduced. Excess watering or leaching may result in a significant shift of the ammonium-N away from areas with effective nitrapyrin concentrations. Further, the extent of translocation of nitrapyrin and ammonium-N can be influenced by soil characteristics such as cation exchange capacity and the amount of organic matter present (Sahrawat et al., 1987). Since nitrapyrin moved into wet soil much faster than into dry soil (Figure 8.7c) application of a fertilizer/nitrapyrin mixture between waterings could prove a useful technique for irrigated soil.

However in field situations where preferential water channelling or fingering could occur, the situation may be different to that observed here. Briggs (1975) observed a fairly slow rate of movement of nitrapyrin in field experiments. He reported that NI moved only 7.5cm into a soil in field condition (he used intact soil columns buried in the field) after receiving 140mm of rainfall over a period of four weeks.

Despite the low temperature and confined conditions maintained during the present experiment the total amount of nitrapyrin recovered after each leaching declined steadily. Goring (1962b) and Laskowsky et al., (1978) found that loss of nitrapyrin could occur due to volatilization and also due to hydrolysis to 6 chloro picolonic acid. Hence a knowledge of nitrapyrin persistence in soils to which it is to be applied would be of considerable relevance in interpretation of experimental results and for its use in the field.

Persistence of nitrapyrin : The effect of the air flow on the persistence of NI was marked at both temperatures (Appendix 15). Concentration of NI declined logarithmically over the period of experimentation at both temperatures. The rate of decline was much slower at low temperature than at high temperature. At both temperatures air flow caused an accelerated loss of nitrapyrin (Figure 8.8). As explained by previous workers (Goring 1962a, Laskowsky et al., 1977) this decline

in NI concentration could have occurred due to natural breakdown into 6-chloro picolonic acid (or another major derivative as proposed by Kallilo et al., 1980) or by direct volatilization losses. The air-flow effect was much greater than that of temperature, hence that the loss of NI could be attributed mainly to the volatilization.

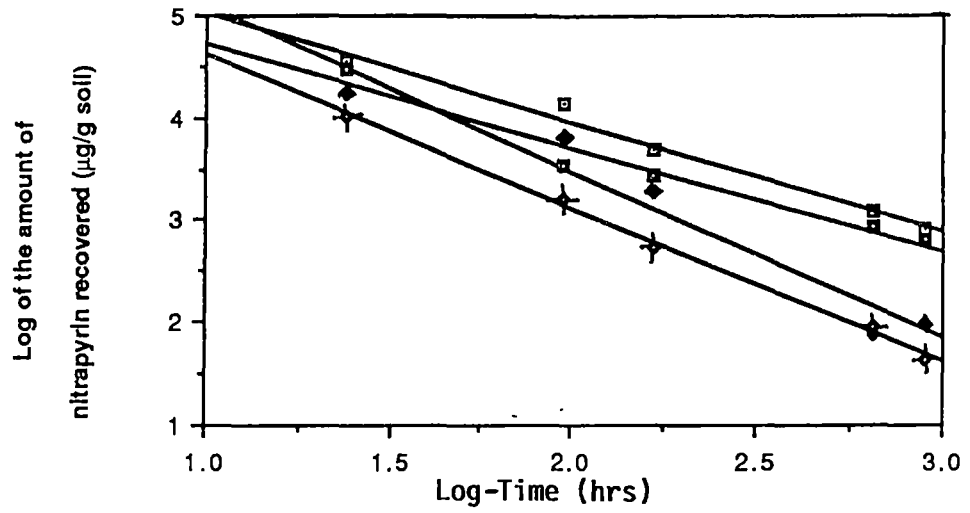
Soil drying during the experiment would be expected to have caused greater NI losses at 25°C than at 10°C This effect may be seen in the different gradients of the curves in Figure 8.8. The results also suggest that the effect of air flow is enhanced at the higher temperature and that these two factors in combination result in greater loss than the sum of the individual losses due to air flow and temperature alone. McCall and Swann (1977), however, showed that although evaporation of nitrapyrin increased with increasing temperature, losses of NI were greater at higher temperatures from wet than from dry-soil.

As shown in Figure 8.8 the rates of disappearance of NI varied with both temperature and air flow. However, since the moisture contents at the two temperatures were different, it appears that the persistence of NI could have been influenced by the confounding effect of soil moisture with temperature and air flow.

In conclusion it seems that under the conditions of the main experiment, nitrapyrin has a very low mobility both in air-dry and wet soil. If a nitrogen source as mobile as urea is used as a fertilizer the effectiveness of nitrapyrin may be greatly reduced due to a small proportion of the applied urea that would remain with the bulk of the nitrapyrin in a leaching situation. However, since the ammonium ion has a much lower mobility than urea,it will remain in closer proximity to NI for a longer time thus allowing greater inhibition of nitrification. The indications are that under wet conditions applied nitrapyrin will move slightly faster in soil than when leaching follows its addition to initially dry soil. However, since NI persistence may be reduced under wet conditions as suggested by McCall and Swann (1977) the effectiveness of inhibitor applied to initially wet soil may still be reduced.

This slowness in movement of NI probably allowed nitrification to occur in the lower part of the soil columns, while inhibiting it in the upper parts of the column as indicated by low rate of disappearance of ammonium of from from the upper part of the columns throughout the experiment. This could mean that complete incorporation and mixing of NI into soil might greatly improve its effectiveness. Such a technique would also help to reduce volatilization losses due to wind which could be substantial for surface-applied NI. Application when temperatures are low would further help to prolong NI persistence.

Figure 8.8: Effect of air flow and temperature on persistence of NP added to soil



log/ FoT10 y = 6.1013 - 1.0683x R = 0.99
log/F1T10 y = 6.7308 - 1.6253x R = 0.97
log FoT25 y = 5.7482 - 1.0168x R = 0.98
log/Fi T25 y = 6.1145 - 1.498x R = 1.00

F_o - NO Air Flow T_{10} - AT 10°C
F_1 - With Air Flow T_{25} - AT 25°C

CHAPTER NINE

9 Effect of urea and nitrification inhibitor with and without liming on soil nitrogen transformation and on growth and grain yield of barley (cv. Triumph)

9.1 Introduction.

The experiments discussed earlier in this thesis indicated that application of a nitrification inhibitor (nitrapyrin) could affect N transformation processes in the soil, effectively inhibiting the nitrification process (Chapter 6). Addition of nitrapyrin to the test soil did not significantly affect the early growth stages of barley in the glasshouse whereas lime amendment resulted in significantly increased growth of young plants (chapter 7). However, results of experiments carried out in the controlled environment of a glasshouse are of limited agronomic application

In view of reported growth responses of grain crops such as barley and wheat to liming under local conditions, the practice of liming can be expected to gain greater acceptance in management of widely distributed acidic, sandy surface soils. A further consideration is the increasing use of nitrogenous fertilizers such as urea that are contributing to soil acidification. It is known that lime-mediated soil pH increases may bring about changes in biochemical activities involved in N transformations in soil (Adams and Martin, 1984; section 2.2 of this thesis). Hence, it is essential to study field crop performance in relation to such biochemical changes in order to achieve optimum crop production. The relationship of N transformation in soil to N availability has added significance with respect to barley since N content is a vital factor determining quality for different purposes (high N content is needed for feed whereas malting quality grain production requires a specific level of N). New or introduced cultivars should be evaluated in terms of different agronomic practices that affect soil N availability especially since in the case of malting barley even though increased yields are desirable,it is absolutely necessary to maintain grain N concentration below 17.5 g kg^{-1}.

As pointed out in the general introduction (Chapter 1) the commercial use of a fertilizer amendment such as nitrapyrin must be justified in terms of its efficiency in comparison to that of various other field fertilizer management practices such as time of application, method of application and number of times the fertilizer is applied. Therefore comparative research on N management practices is well justified.

The present experiment was designed to show the effects of liming and urea application on performance of barley grown in the field. The efficiency of nitrapyrin as a fertilizer amendment was compared to single and split fertilizer applications at different soil pH values (obtained by variable liming) in terms of growth performance

(straw and grain production) and N availability.

9.2 Materials and Methods

The site used in this experiment was situated adjacent to the experimental site described in chapter 3 (Plate 1, page 57). The experiment was conducted in 1987-1988. The site had a long history (about 30 years) of pasture and had been cropped to wheat and oats respectively in the two previous years (1985,1986).

Duplicate main plots (26 x 6.5 m) were treated with lime on 6th February 1987. The treatments were applied according to a three way split plot design (Lo, nil lime; Li, 2.5 mT/ha; and Lii -7.5 mt/ha). Immediately after application lime was raked in and after two days the plots were disc harrowed for a better mix. Plots were then left until June and then chisel ploughed before sowing at the rate of 120 kg seed/ha. Nine sub-plot treatments were applied within the lime treatments (Table 9.1). The size of a sub-plot was 8 x 1.5m. A 1m and 0.5m wide buffer was maintained between main plots and sub-plots respectively. 1.5m wide buffer strips were established around main plots. Rainfall received during the period between February and May is shown in Appendix 6.

Table 9.1 Treatment symbols and description.

Main plot treatments

Lo -Control -no lime application, (pH 5.2)

Li -Soil limed at the rate of 2.5mt/ha (pH 5.8)

Lii-Soil limed at the rate of 7.5mt/ha (pH 6.6)

Sub plot treatments

T0 -No added nitrogen no nitrapyrin (NIo)(control).

T1 -50 kg of N/ha at sowing, no nitrapyrin (NIo).

T2 -50 kg of N/ha with 0.56 kg (a.i)/ha of NI at sowing.

T3 -50 kg of N/ha with 2 kg (a.i.)/ha of NI at sowing.

T4 -100 kg of N/ha at sowing, no Nitrapyrin (NIo).

T5 -100 kg of N/ha with 0.56 kg (a.i)/ha of NI at sowing.

T6 -100 kg of N/ha with 2 (a.i) kg (a.i)/ha of NI at sowing.

T7 -50 kg of N/ha with no NI (NIo), at stem elongation.

T8 -100 kg of N/ha, split 50kg at sowing and 50kg at stem elongation, no NI.

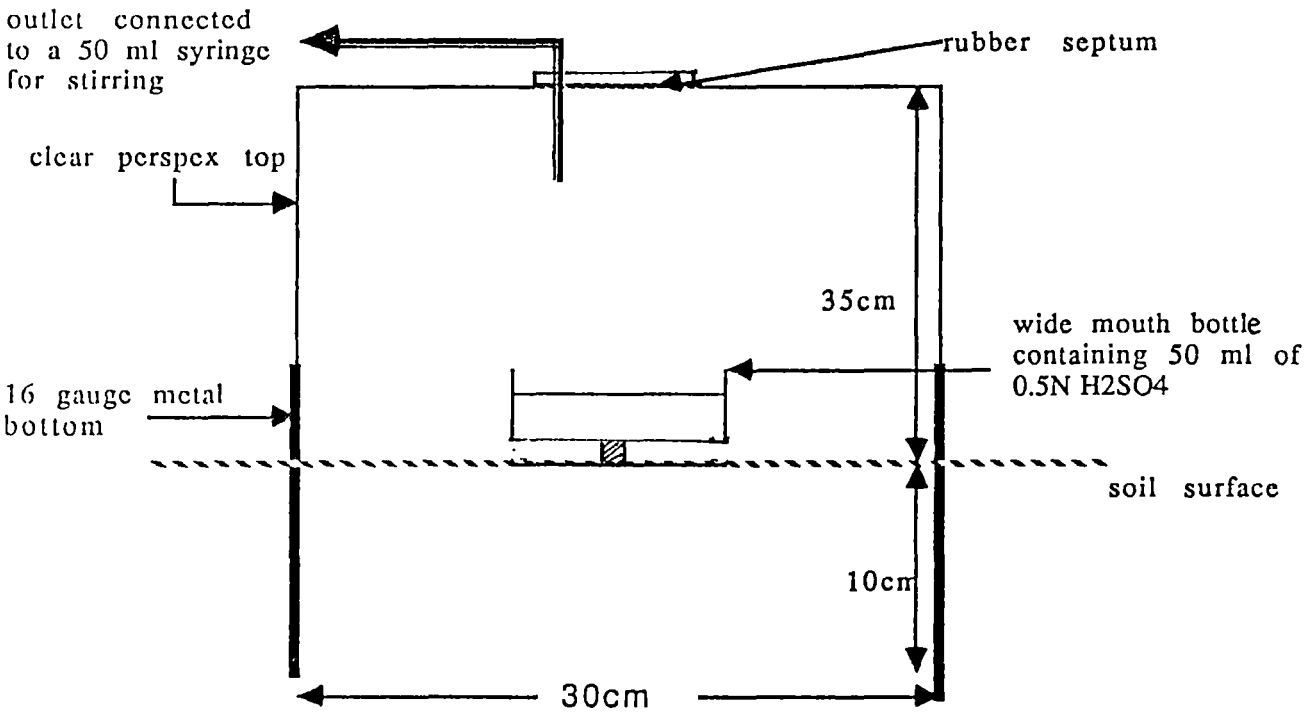


Diagram 2: Schematic drawing of a chamber used for measuring ammonia volatilization.

The appropriate concentrations of urea and nitrapyrin were blended by pouring nitrapyrin solution over the solid urea in a glass jar and mixing thoroughly with a glass rod. Immediately after broadcasting, the fertilizer/NI mixture was carefully incorporated into the soil by hand raking of individual plots. This procedure was carried out in the field just prior to sowing.

Plant and soil specimens were collected and analyzed as described in Chapter 3. Progressive crop performance was measured in terms of top dry matter production, leaf area index (LAI:estimated using ten randomly selected individual plants at various growth stages), grain and straw yield and harvest index (HI) (Chapter 5). Light interception data were obtained at 120 days after sowing (DAS)

Ammonia volatilization was measured using the type of chamber shown in Diagram 2. The chambers were installed in triplicate on three differently limed sites ie. Lo, Li and Lii (without NI) immediately after sowing. Chambers were dismantled on three occasions at two day intervals (i.e., at 2, 4 and 6 DAS) and ammonium-N in 20 ml of the 0.5N sulphuric acid absorbent solution was determined by steam distillation (Bremner, 1965) following treatment with sufficient fused MgO to ensure complete release of ammonia. The locations of the chambers were changed after each dismantling for specimen collection since prolonged confinement of one site will cause the site to differ considerably from the outside unconfined area (eg. differences in wind effect, humidity, and partial pressure of ammonia).

9.3 Results and Discussion.

Crop performances

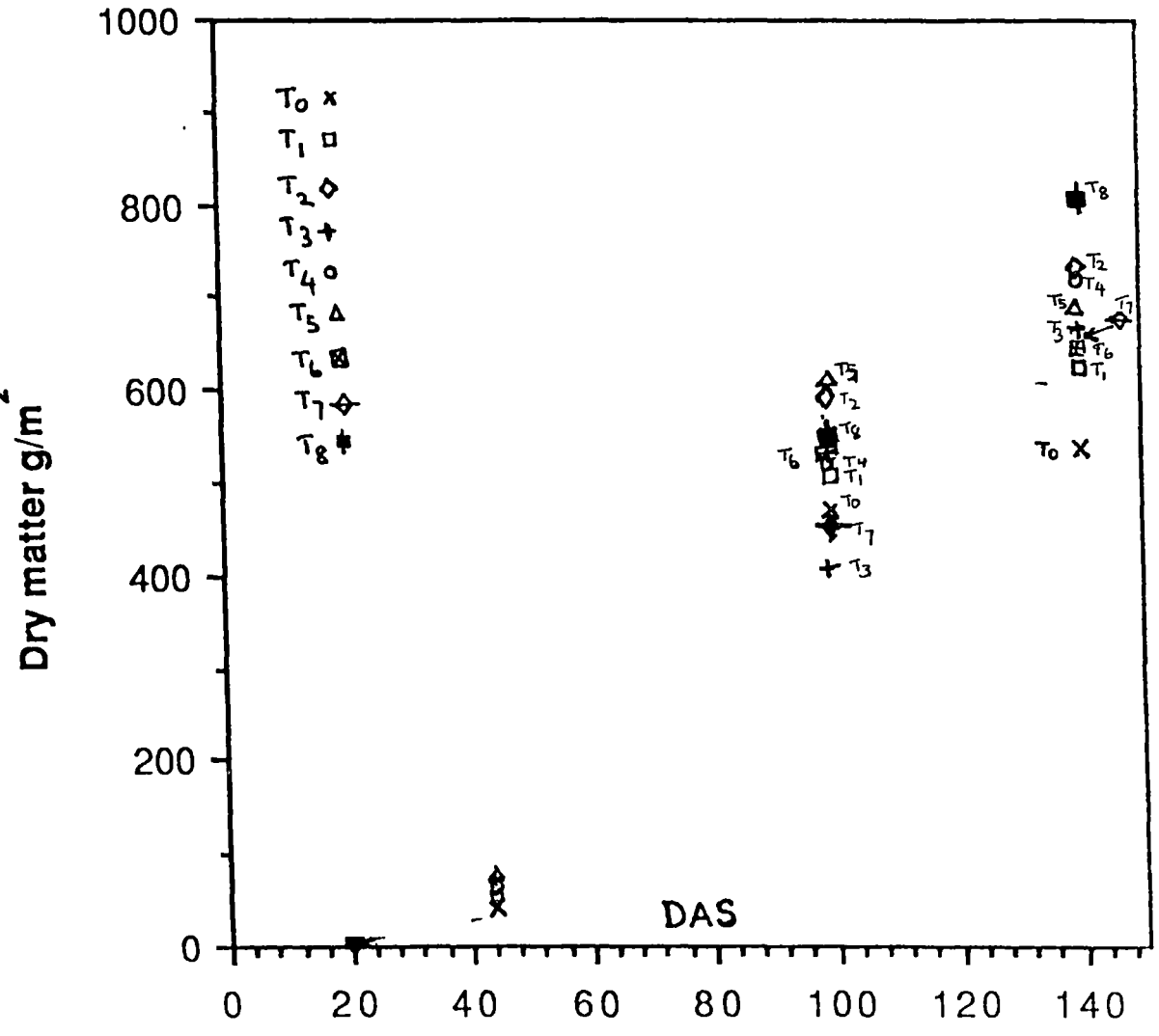
Dry matter production and the leaf area index: Data have been plotted in Figure 9.1 (a, b, c) and means and analysis of variance are given in Appendix 17. The regressions used to determine the rate of top dry matter production are given in Appendix 18

Slight indications of treatment effects at 20 DAS became more apparent at 45DAS and were further accentuated at 100 and 140 DAS. (Figure 9.1). On the other hand, although the responses to lime were significant (p=0.05), the results with respect to N/NI treatments (T0-T8) were rather variable and no clear trends could be established in early stages of growth. However, with the season progressing, the effect of N began to appear.

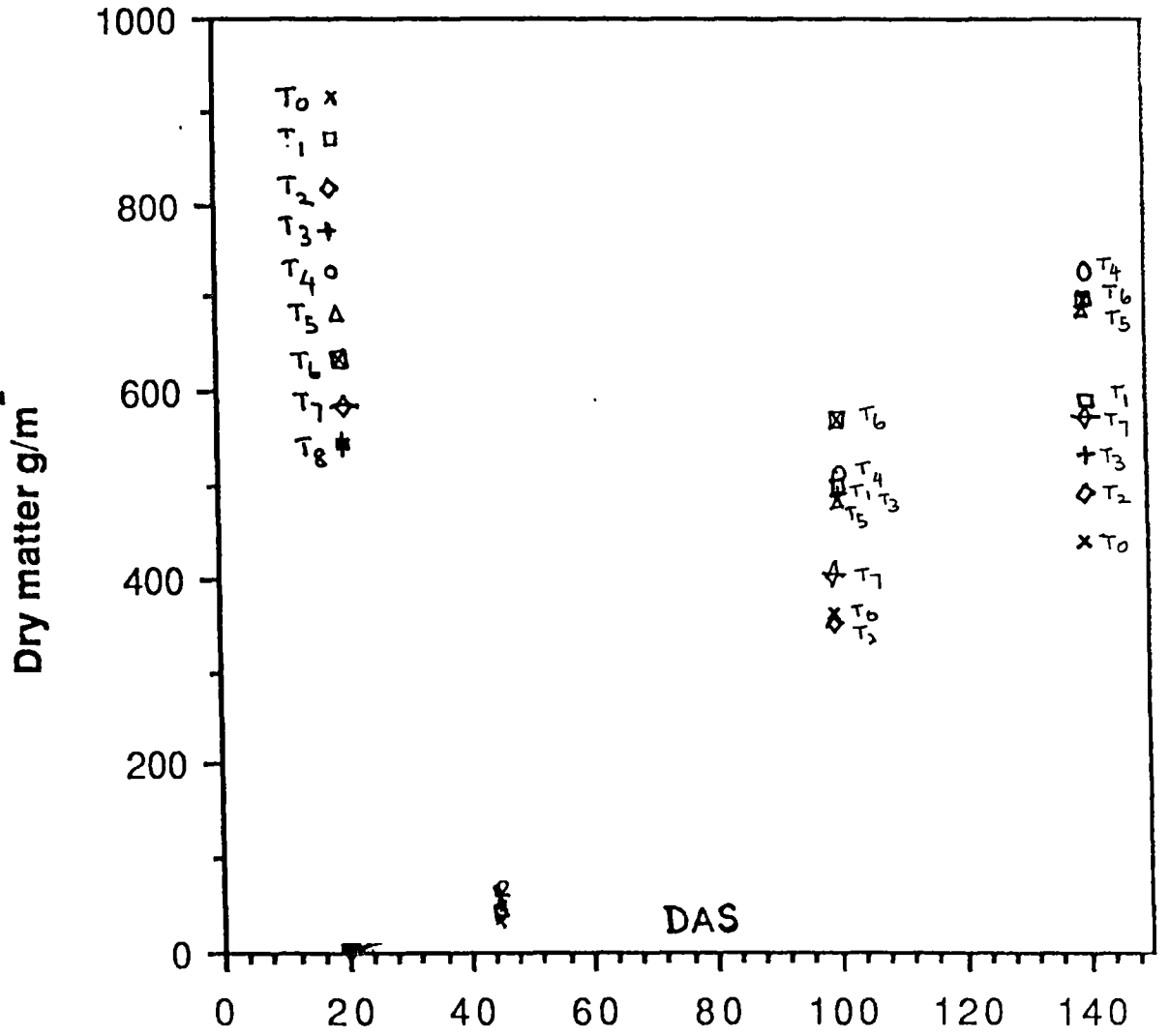
The significantly lower dry matter production in response to (To) treatments and (Lo) treatments was evident after the second sampling and Mendham and Russell (1987) also observed that this barley variety responded positively to both lime and N applications. According to the results obtained it is not only the amount of N that may have an effect on plant growth but also the way N is applied (eg. with or without nitrapyrin as a single, or split, application) could also be important.

Figure 9.1 (a-c): The effect of N/NI (T) treatments on dry matter production of barley.

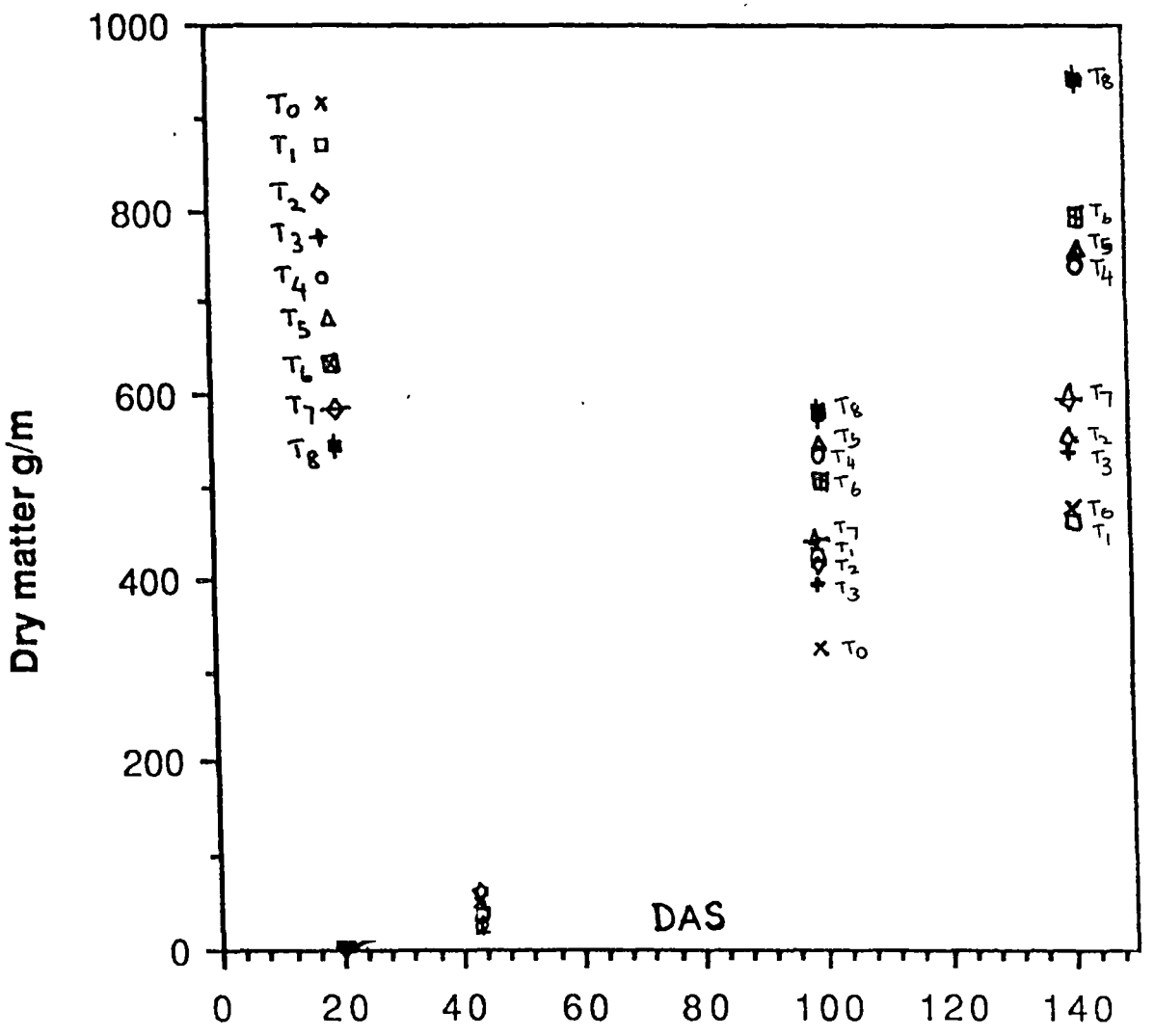
a. in unamended (non limed -Lo) soil



b. With low rate of lime application (Li=2.5mt/ha)



c. With high rate of lime application (Lii-7.5 mt/ha)



Simple regressions (Appendix 18) of the data plotted in Figure 9.1 show the rates of top dry matter production. It is clear that addition of lime and nitrogen (L and T treatments) resulted in marked increases in the rate of dry matter production compared to that of the control (ToLo). The trends of dry matter production in response to both rates of liming (Li and Lii) appear to be very similar.

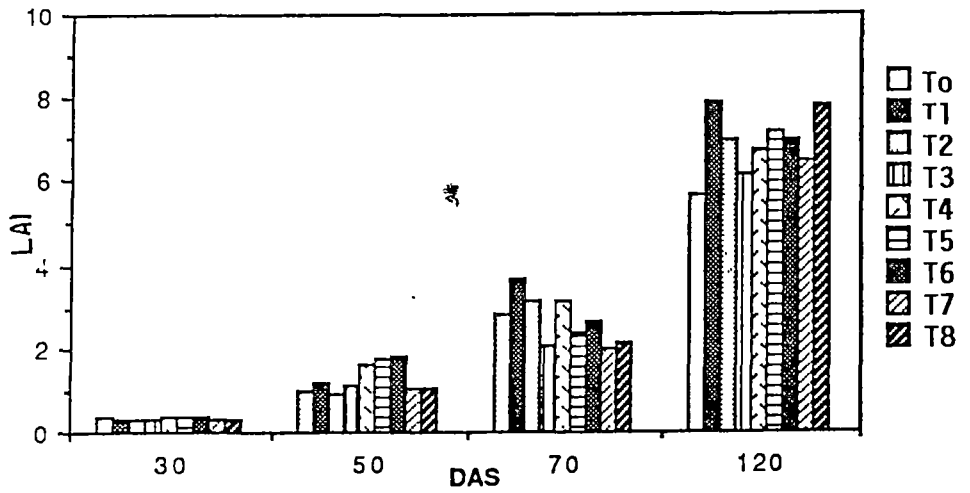
It should be noted that although the linear regression of growth curves of sigmoidal nature do not cover the various growth aspects of the later stages adequately, the technique is used here since only the dry matter production which level out towards the later stages of growth has been compared. According to the regression equations in Appendix 18, the rates of dry matter production in all N/NI treatments (T1 to T8) were greater than that of T0 regardless of lime application (Lo, Li and Lii). The split N/NI treatment (T8) gave the highest dry matter production rate within each lime treatment. The rates of dry matter production in response to low N treatments without lime (Lo/T1, T2 and T3 =50kg N/ha) were not markedly different from those in response to higher rates of N at the higher rate of liming application (Lii/ T4, T5, T6 and T8). In contrast there were marked differences in the rates of dry matter production in response to low versus high N in limed soil (Li and Lii). Interestingly, the rate of dry matter production in high lime (Lii) plots with low N (Figure 9.1c) were less than corresponding treatments of low lime (Li) plots (Figure 9.1b). When the amount of added nitrogen was increased from 50 to 100 kg N/ha the increase in rates of dry matter production in Li plots was greater than that of Lii plots. This suggests that although the response of the tested barley variety to N may be enhanced by liming but the effect may be reversed by excessive application of lime. Therefore, while supporting the observation by previous workers (Mendham and Russell, 1987) that both lime and N application can improve dry matter production of barley cv. Triumph, the present results also underline the need to workout a balance between these two amendments for optimum dry matter production.

Leaf area Index (LAI): The observations on dry matter production were further supported by the leaf area indices shown in Figure 9.2(a,b,c).

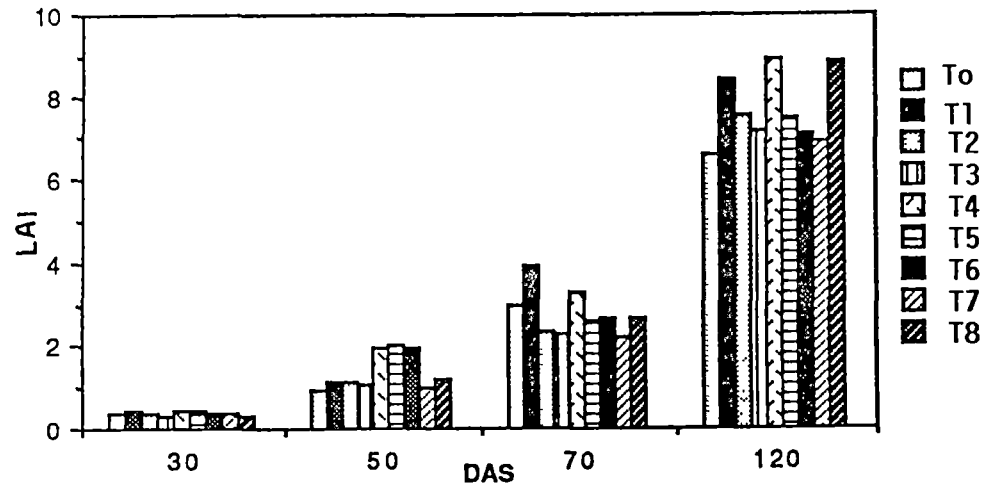
As in the case of dry matter production the LAI did not show treatment differences in the initial stages but with increasing plant growth, the effects of different N/NI treatments on LAI were apparent. On the other hand liming was found to have significantly affected the LAI of the crop from the very first stage of sampling (30 DAS). Since LAI is a direct indicator of effective photosynthetic area of the plant, the changes observed due to lime and N/NI treatments can be related to the dry matter production capability of the plants.

Figure 9.2 (a–c): Leaf area index during various stages of plant growth.

a.in non limed soil (Lo)



b. in plots with low rate of liming (Li)



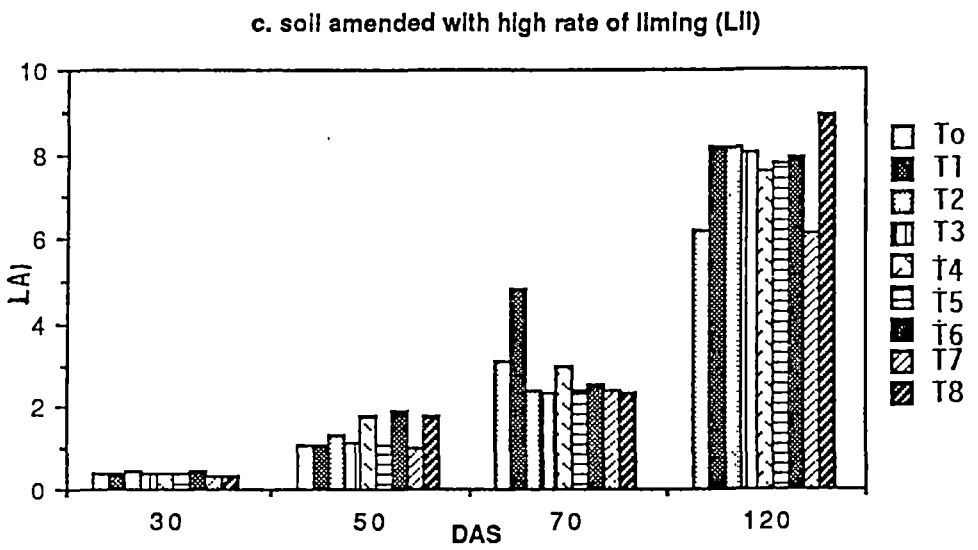
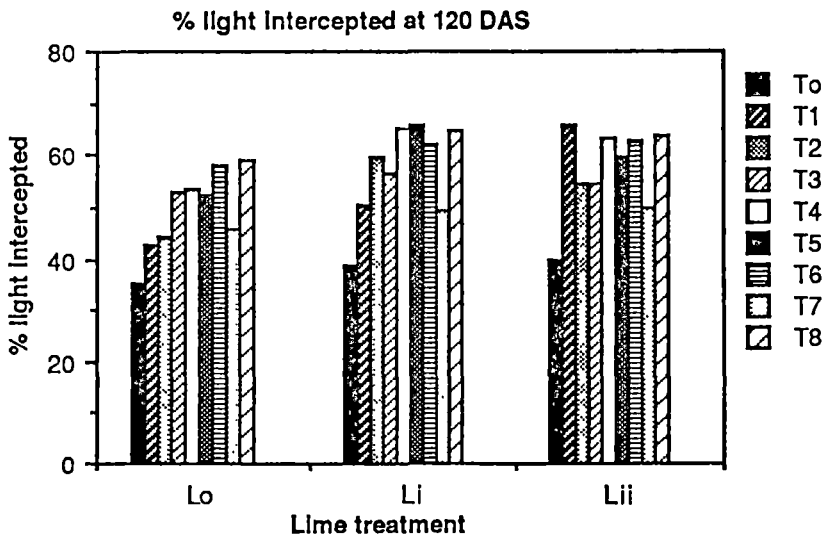


Fig. 9.2d: Light intercepted by the crop at 120 DAS (as a percent of light received at the time of estimation)



The LAI appears to be influenced by the application of nitrapyrin at 70DAS since it caused a reduction in LAI in limed and non limed plots (Lo,Li and Lii). However, as the growth progressed the effect of NI on LAI remained unchanged only in the nonlimed soil with low N treatment and in both N application rates of the low limed plots. The differences observed in LAI among the NI treatments receiving the two levels of N fertilizer rates and high liming rate at 70DAS diminshed by 120 DAS.

Since available soil nitrogen may contribute to excessive vegetative growth it might be assumed that harvest index (HI) (Chapter 5) would reflect grain yield decreases that may occur on account of prolific vegetative growth. However, as Riggs et al., (1981) pointed out, higher grain yields of recently developed barley cultivars are attributable to increases in harvest index rather than increases in total above-ground dry matter production. Therefore, the estimate of HI is given below with the data on yield components in order to clarify the yield and dry matter ratio relationships among the treatments.

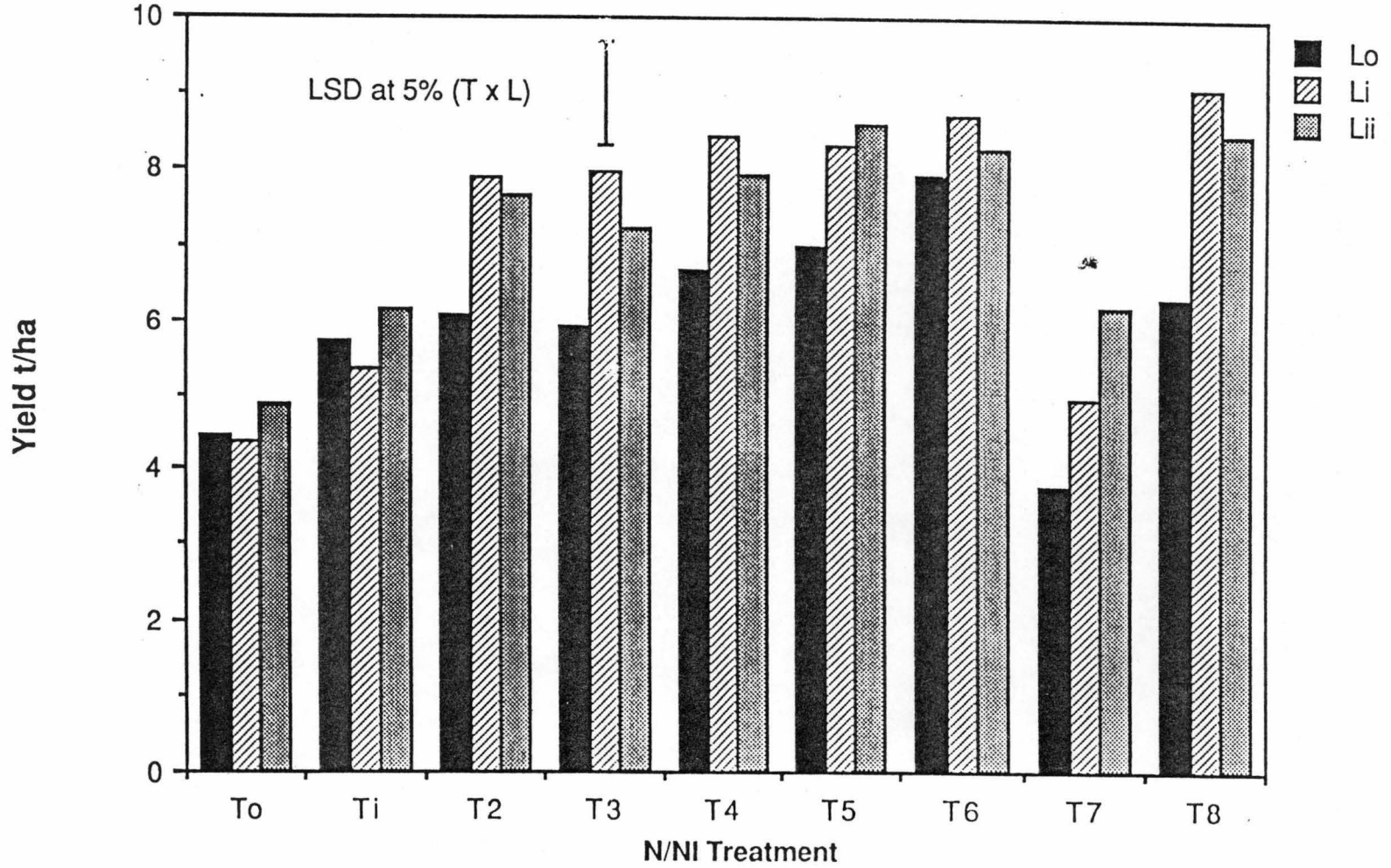
Grain and straw yield and harvest index.: Figures 9.3 (a-c) and Appendix 20 show the yield components and the estimated harvest index obtained from respective lime and N/NI treatments.

Grain production (Figure 9. 3a) responded markedly to L and T treatments. Although yield responses to different N/NI treatments (over all lime treatments) were evidently different, statistically significant ($p<0,05$) differences were obtained only between those involving high rates of urea N (T5, T6 and T8). The increased rate of nitrapyrin application to limed soil had no notable effect on grain production. The higher rate of N on limed soil (Li and Lii) resulted in comparatively higher grain yields with or without nitrapyrin. Dalal (1984) and Mendham and Russell (1987) also obtained improved crop performance following lime application to corn and barley respectively. It appears that under the conditions of present experiment application of nitrapyrin may be advantageous for forage production, only if the soil is unlimed and supplied with a higher dose of N fertilizer. Comparison of the grain yields of T6 (the high dose of NI) T8 treatments (split application) in respective liming levels shows that the effect of NI is better in unlimed soil and is comparable even to the split apllication of fertilizer N (Fig. 9.3a).

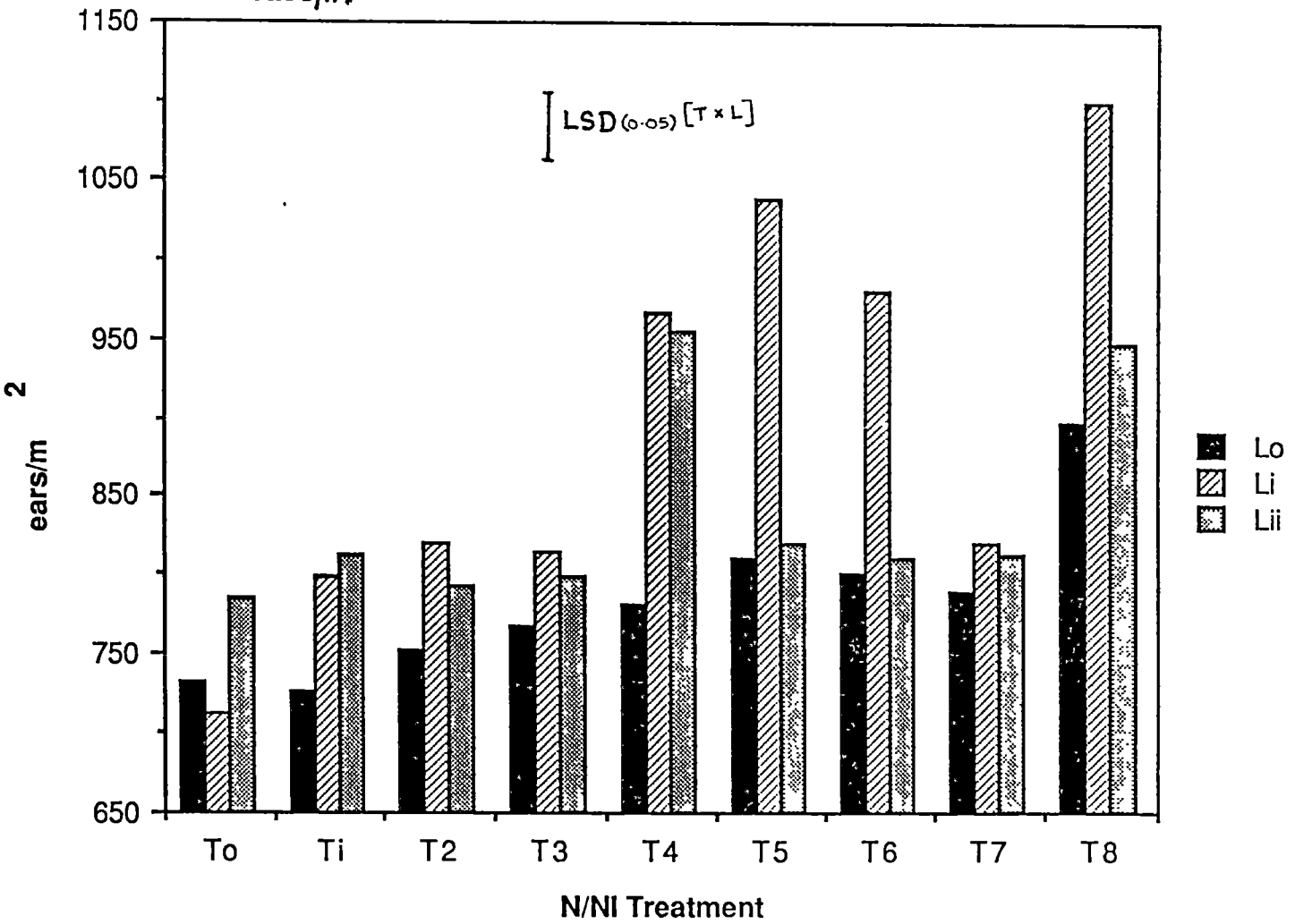
Straw production followed a similar trend to that of grain production in response to lime. However responses measured as straw dry matter production were significantly greater at the higher level of N application for each rate of liming (T4,T5, T6 and T8).

Figure 9.3 (a-d): Yield components of barley (cv. Triumph)

(a) Grain yield

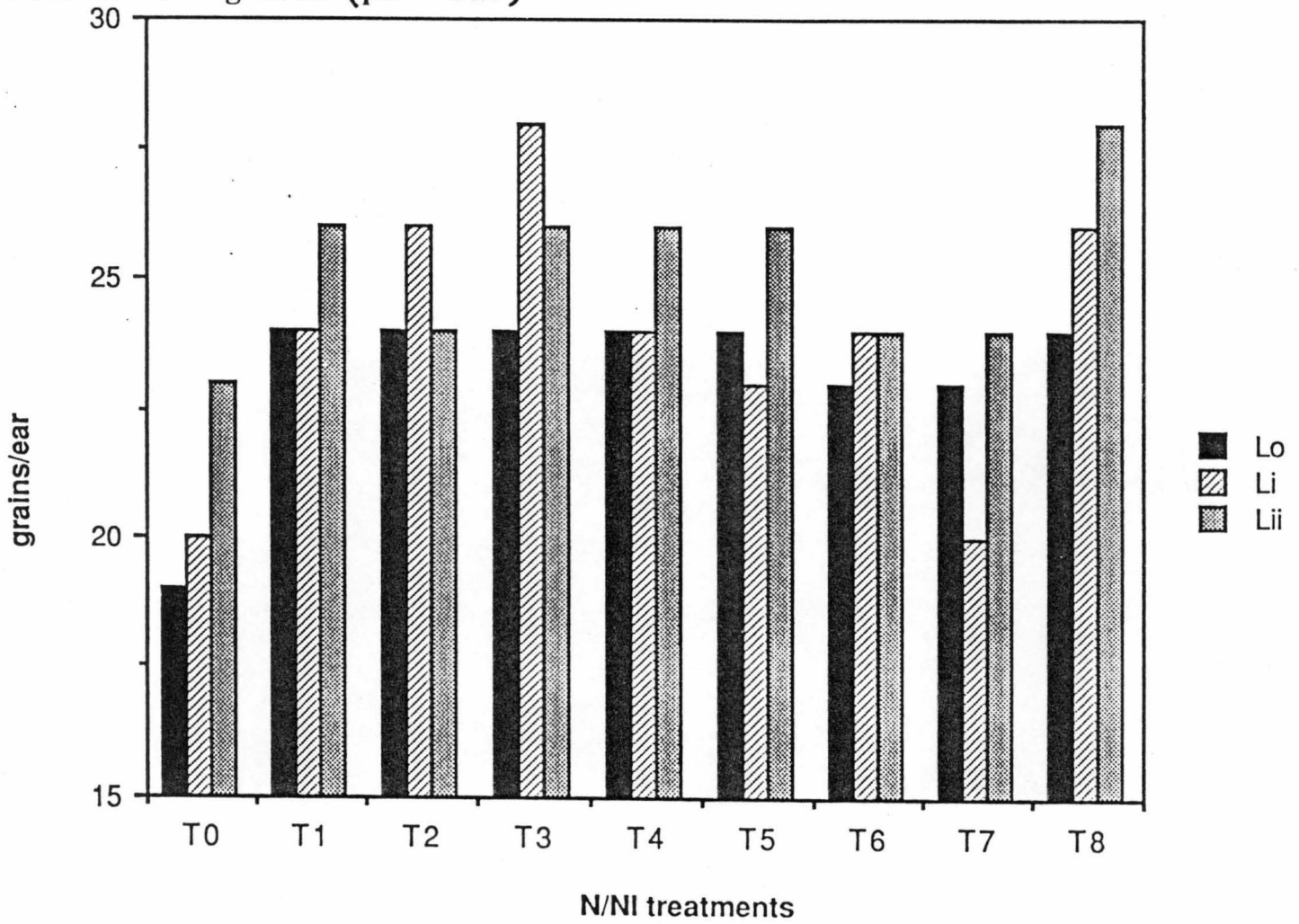


b. Number of ears/m^2



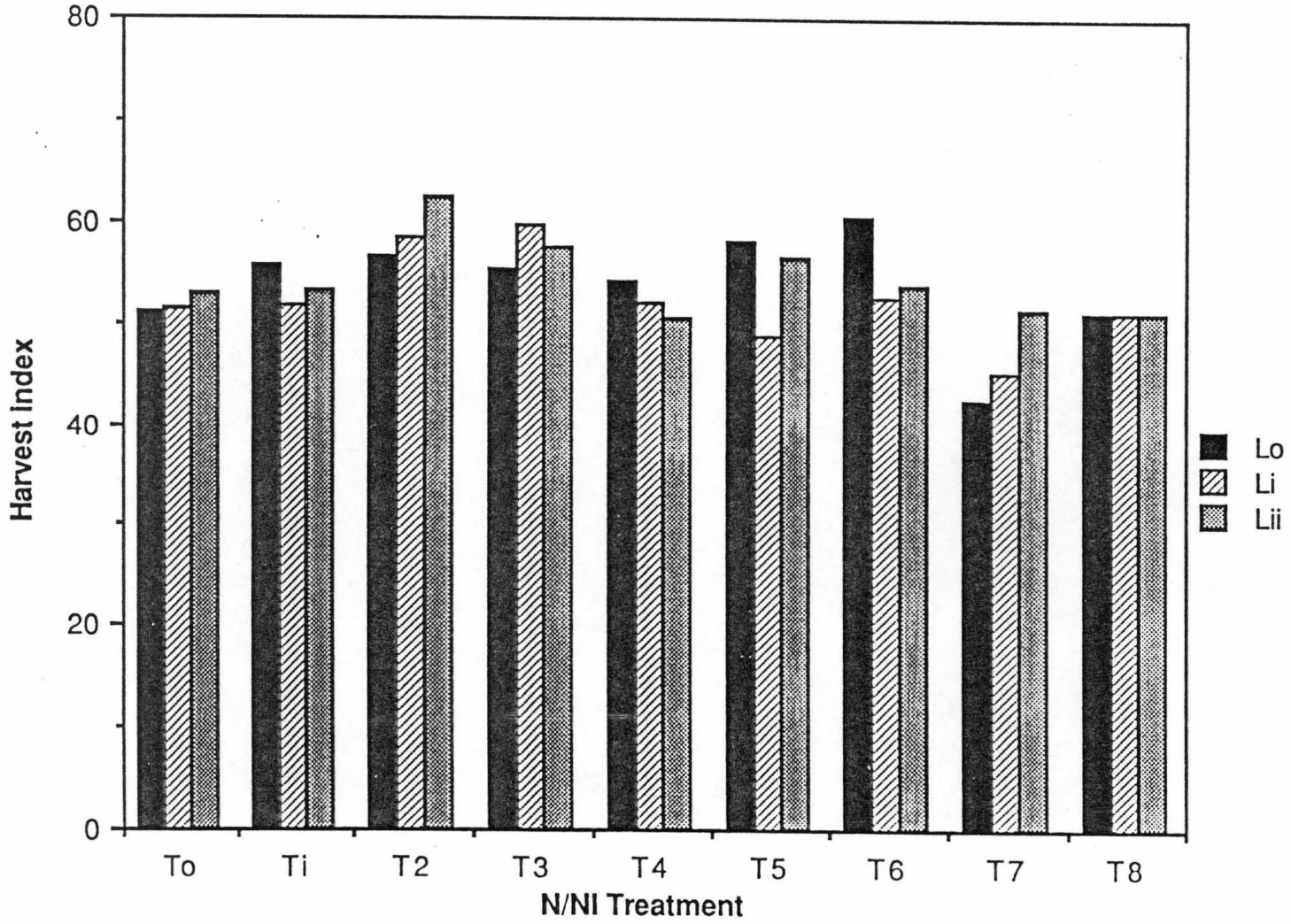
Lsd at $p<0.05$; (L) = 55.31 ; (N/NI)= 31.83 ; (L x N/NI)=40.21

c)Number of grains (per ear)



Lsd at $p<0.05$: (L) = 1.06 ; (N/NI)= NS ; (L x N/NI)=NS

d) Harvest Index



Variations in the harvest index were not significant at $p < 0.05$ level.

(Data are given in Appendix 20b on yield components)

Although yields were influenced by N/NI and L treatments the harvest indices did not differ significantly at $p \leq 0.05$. This may suggest that there was no "excessive" increase in production of vegetative parts relative to grain yield. This is important since it would mean that a farmer who needs to graze his crop early and then allows it to grow on to produce a high straw yield may not suffer seriously from loss of grain yield if fertilizer was applied at the rates used in this experiment.

Grain-N and Straw-N: Fairly specific grain nitrogen level (21.6g/kg) is an important aspect in barley grown for malting whereas grazing value is enhanced by a high level of N in the forage. Therefore differentiation of N concentration in above ground plant parts (i.e. grain vs. vegetative parts) may be a useful way of expressing N utilization by the plants in response to different treatments.

The nitrogen use efficiency index can be a useful parameter in studies of N utilization responses by plants to external factors that influence soil N availability (Craswell, 1987). The yield data for grain and straw and the total plant-N content (Appendix 20) were used to determine the nitrogen use efficiency (NUE) of the crop as per equations proposed by Bock (1984).

Higher rates of N application always produced the highest grain-N contents (Appendix 20). The control (To) gave the lowest grain-N content. Application of N as a topdressing at the rate of 50 kg N/ha at stem elongation (T7) was reflected in a notably poor grain-N content when compared to those treatments where an equal total amount of N was added at the time of sowing. Since the late topdressed application could not be incorporated into the soil it is possible that more N was lost by ammonia volatilization. However the split application of urea-N appeared to compare well and produced higher N uptake than any other treatment. Application of nitrapyrin did not produce significantly different responses ($p=0.05$) but there was a predictable trend with a slight increase in N content of grain at each N application rate when NI was applied (more details in Appendix 20).

The low rate of liming increased grain-N content in comparison to the Lo treatments but there was no further increase at the higher liming rate (Lsd at $p<0.05$ for N/NI = 40.56 and for L= 7.84). It is interesting to note that Dalal (1986), who worked on tropical acidic soils also found that application of lime had resulted in a significant increase in N content of corn. Liming with N application rates exceeding 50kg N/ha generally gave no significant responses in total N uptake. In the present experiment too there was significant effect of interaction of N/NI treatments with L on grain-N

Responses of Straw-N content was simialr to grain-N content in relation to N/NI and lime treatments. However, in this case there was no significant effect on straw N due to an interaction between the N/NI and L treatments.

Nitrogen use efficiency (NUE): A study of nitrogen use efficiency of the crop in relation to each treatment taking into account the N concentrations in grain and straw should further facilitate treatment comparisons.

The nitrogen use efficiency (NUE) was determined as the N recovery efficiency (RE) as well as the yield or "production" efficiency (YE). The definitions of each of these terms and equations to estimate NUE have been discussed by Bock (1984).

Neither fertilizer-N rates (from 50 kg and 100 N kg/ha) nor use of NI seem to have affected NUE. However the split application of urea resulted in a significantly enhanced grain-N content when compared to other treatments (To to T7). Liming on the other hand had a marked effect on grain NUE where the effect of the lower level of liming (Li) was evidently greater than that of the higher level (L2).

There appears to have been a significant interaction of N/NI and L only on grain-N recovery efficiency and grain yield efficiency. This may be rather unusual but only one cultivar was used in this experiment. Such a result would need to be clarified by further experimentation. The effect of liming on straw N recovery was similar to that observed for grain N recovery.

A comparison of yield efficiencies due to different treatments showed the two rates of urea-N application did not increase the yield efficiencies proportionately. Hence it would be worthwhile to evaluate the economics of the higher rates of N used in this experiment. Liming significantly promoted yield efficiencies with respect to grain and straw production. However the increase in liming rate from 2.5 to 7.5mt/ha did not cause much further improvement in cumulative yield efficiencies..

Since recovery efficiency and yield efficiency of the crop were not significantly affected by nitrapyrin application and comparatively higher efficiencies were obtained with split application of urea-N and liming the advantage of using a nitrification inhibitor with urea in the manner in which it was applied in this experiment is doubtful. On the other hand the injection of a nitrification inhibitor into soil using specially designed equipment (as in some countries) may have different results.

Soil N transformation: Further understanding of the effect of individual treatments on plant N utilization may be gained by studying changes in the available N levels in soil the during the growing season. This would also help to assess the extent of N lost or immobilized (temporarily unavailable to plants) during this period.

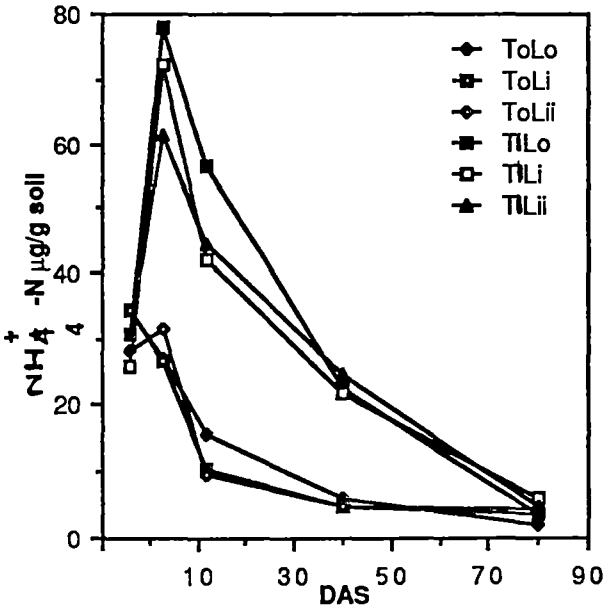
The level of soil mineral N was regularly determined (along with plant analysis) so that any effects of lime and nitrapyrin on soil N availability could be better understood.

Soil mineral N (ammonium and nitrite/nitrate N) changed during the growing season as shown in Figure 9.4(a -f) and the Appendix 21-23.

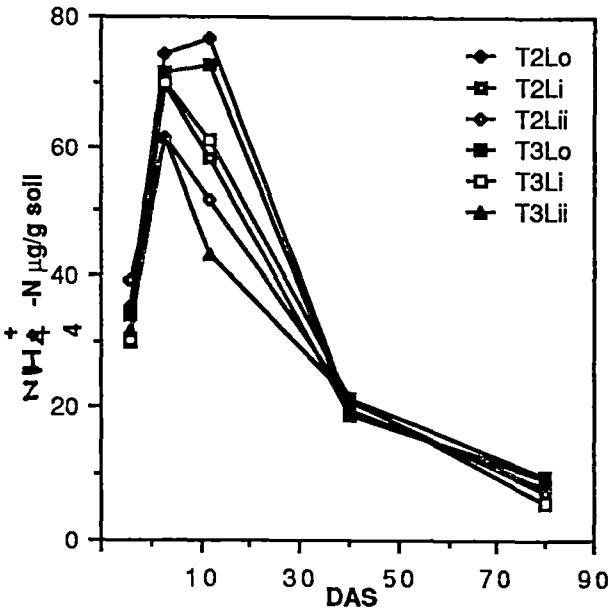
Figure 9.4 (a-e): Change in ammonium N content in top soil (at three L treatments) in (2x μg/g soil)

(For the explanation of T and L symbols please refer to Table 9.1).

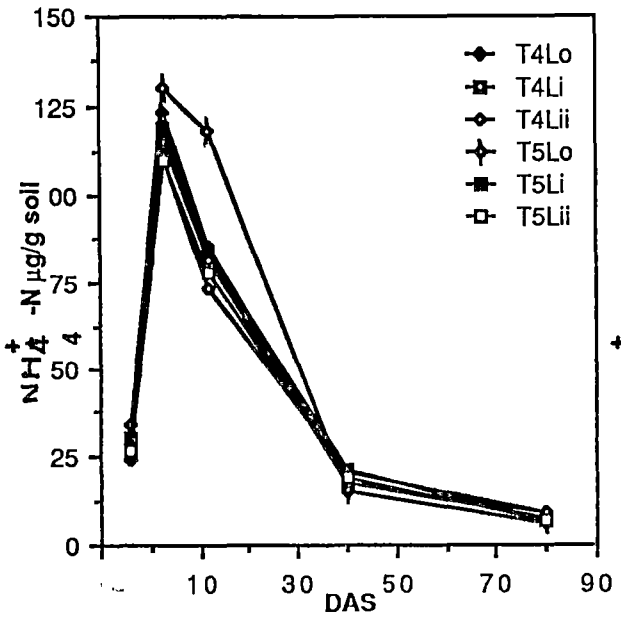
a. In (To) and (Tl) plots



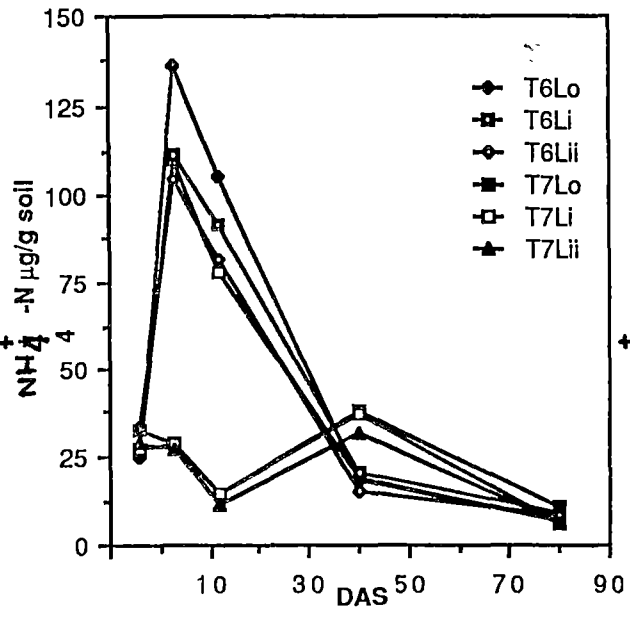
b.In (T2) and (T3) plots



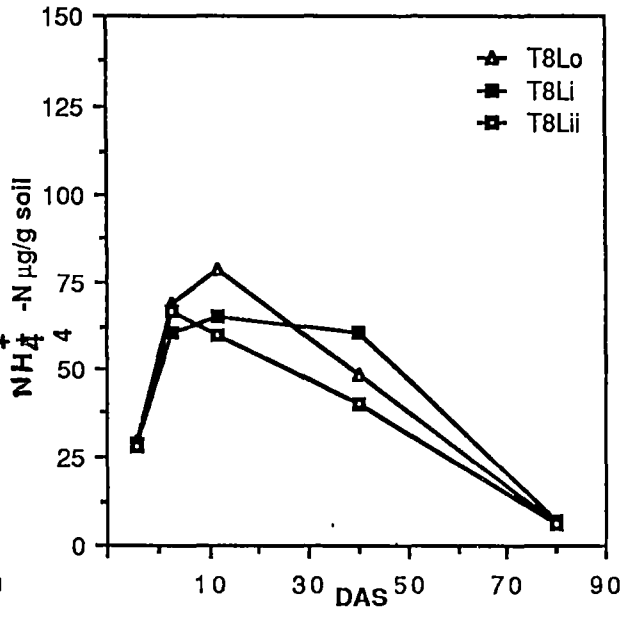
c. (T4) and T (T5) plots



d. (T6) and (T7) plots



e. (T8)plot



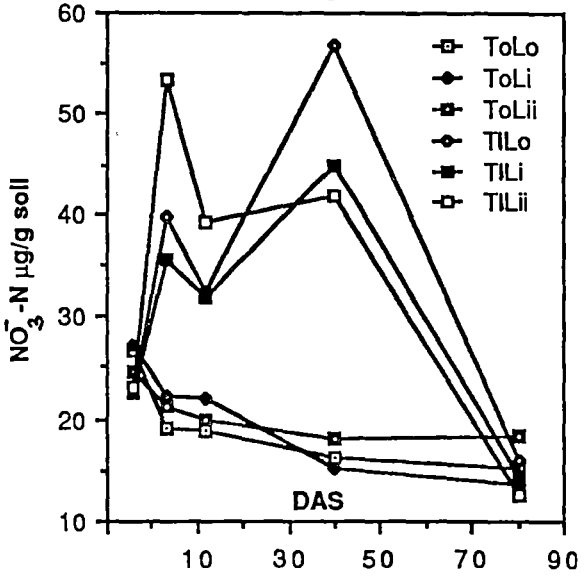
See Appendix 21 for Lsd (p=0.05) values.

It was found that ammonium-N concentration in soil before sowing was not significantly affected by liming. This is different from the results obtained from the experiment carried out under glasshouse conditions (Chapter 4) where the level changed markedly. The differences between field and glasshouse conditions include those due to temperature moisture and better mixing of lime with soil in the pot experiment. As revealed by the soil sampling carried out on 22 June 87, the application of urea (on 16 June 1987) resulted in a surge in the level of ammonium-N. Even as early as the first sampling, ammonium-N levels were significantly affected by the L treatments (Lsd at $p \leq 0.05 = 7.08$) and the N/NI treatments. However the effects of N/NI treatments at 6 DAS were due rather to differences in amount of N applied rather than to differences resulting from N transformation.

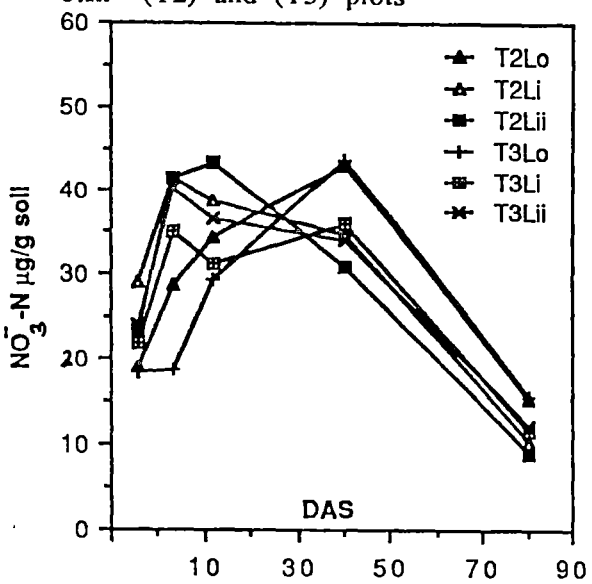
Soil specimens collected subsequently at 13 DAS contained a lower level of ammonium-N (Figure 9.4 a-f). Since there was no plant emergence at this stage the decline must have been due to a nett effect of N transformation processes, ie. the nett effect of acontributions from urea hydrolysis, N-mineralization and immobilization, ammonia volatilization, and leaching. Nitrification, on the other hand, may not have been significant at this stage as there was no significant increase in soil nitrate-N levels in the period between sowing and first sampling on 6 DAS. However this experiment was designed to assess only the nett level of mineral N remaining at different sampling times and the effects of individual processes were not measured. (Nevertheless, data for ammonia volatilization from limed plots were measured. The comparison of these values should be considered only in relative terms as the method employed would not be as sensitive as those described in Chapter 2). Later in the season, evidently, nitrification and plant absorption would have contributed to decline in ammonium-N levels. The LSD's ($p=0.05$) given in Appendix 21 show that treatment significantly affected ammonium-N concentration at later sampling times (13 and 42 DAS) for L treatments while the effects of N/NI treatments were significant ($p=0.05$) until 83 DAS. Except at 13 DAS there was no significant interaction of lime with N/NI treatments on ammonium-N recovered from in soil. Although there were significant differences in ammonium-N following the application of different rates of urea-N, nitrapyrin did not significantly affect ammonium-N levels at a given level of urea-N (ie. within the 50kg urea N/ha group T1 versus T2 versus T3 and within the 100 kg N/ha group, T4 versus T5 versus T6). The results for 42 DAS show that except for T7 and T8 (50 kg N/ha added at early stem elongation) all plots had significantly lower levels of ammonium-N. By the last sampling ammonium-N levels in all plots were even lower than the levels measured at the beginning of the experiment.

Figure 9.5 (a-e): Change in nitrate/nitrite-N content in top soil (at three L treatments) in (2x µg/g soil). (For the explanation of T and L symbols please refer to Table 9.1).

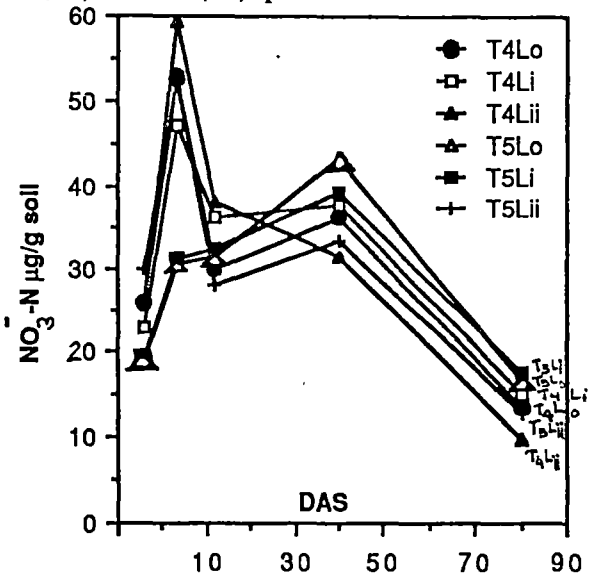
a. In (To) and (Tl) plots



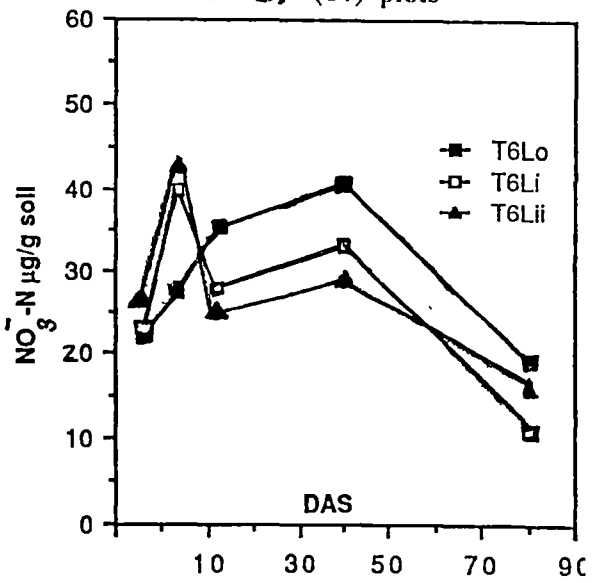
b.In (T2) and (T3) plots



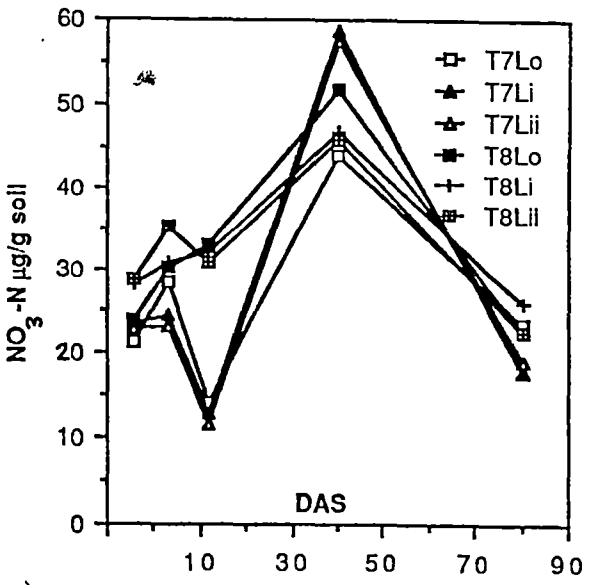
c.In (T4) and T (T5) plots .



d. In (T7) plots



e. In (T') and (T8).



(See Table 9.1 for explanation on T and L symbols and the Appendix 22 for - LSD values).

Ammonium-N concentration declined quite rapidly in the limed plots compared to the corresponding non-limed plots. Increased nitrification (Adam and Martin, 1984) as well as greater N uptake (higher plant N content) was consequence of better plant growth (higher dry matter yield) in the limed plots and is probably the major reason for the reduction of ammonium-N.

Unlike ammonium-N, the nitrate-/nitrate-N levels prior to sowing were significantly affected by liming [Appendix 22 and Figure 9.5(a-e)]

The amount of nitrate-N recovered from the surface layers during the present experiment was rather low when compared to the experiment conducted in the adjacent field in the previous year. Although no clear reason for this difference can be given it may be worth noting that the adjacent site carried a commercial oat crop during 1986/87 season which followed a chisel ploughing with 100kg/ha 16:19:0 N:P:K commercial fertilzer mixutre.

Figures 9.5 and Appendix 22 show that nitrate-/nitrite-N began to increase from as early as 6 DAS and presumably due toincreased nitrification. Since there was no plant growth at the first two sampling stages after sowing, and no rainfall occurred to cause removal of nitrate-N by leaching or denitrification, the increased levels during this period may fairly well represent net nitrification in this soil. The build-up of nitrate-N evidently differed due to L and N/NI treatments (Lsd at p=0.05= 2.78 and 7.27 respectively (Appendix 22). There was no significant interaction between L and T on nitrate-/nitrite-N levels except at 42 DAS.

Figure 9.5 (a-e) shows that except for T0, T1 and T7 other N/NI plots had their peak level of nitrate-N between the second and third sampling times. Since both T7 and T8 received urea-N at the rate of 50 kg/ha at the time of tillering (35 DAS) the relatively high nitrate-/nitrite-N level found at a later sampling time was understandable.

Another notable feature was that although nitrate-/nitrite-N in Lo plots increased relatively slowly when compared to corresponding Li and Lii plots higher levels of nitrate-/nitrite-N in non limed plots remained for a longer period than in the limed plots. This interesting difference may be due to the better plant growth observed in limed plots removing more nitrate-N. It is unclear whether this might also explain the observed greater increases in nitrate-/nitrite-N levels in the higher-urea-treated plots that were generally followed by an equally rapid decline in nitrate-/nitrite-N levels when compared to the slower rates of change in the corresponding low-urea treatments.

Although neither plant performance nor ammonium-N levels were found to respond significantly to nitrapyrin application the comparison of nitrate-/nitrite-N levels in specimens collected at 6DAS and 13 DAS revealed that the effect of nitrapyrin on nitrate-/nitrite-N was significant. For example, at the low rate of urea,

the higher application (T3) of nitrapyrin effectively reduced the formation of nitrate-/nitrite-N as opposed to the T1 plots at 6DAS. A similar result was observed in the case of the higher NI rates with the higher rate of urea (T6 had significantly less nitrate-/nitrite-N levels than T4 and T5 at 6 DAS and 13 DAS). It is important to note that this effective reduction in nitrate formation was achieved only by applying nitrapyrin at a rate four times greater than the recommended rate of application (however, NI mixed with urea was applied manually rather than by the reportedly more appropriate injection technique using special equipment). There was no significant interaction of lime with other treatments on soil nitrate-/nitrite-N levels.

Only differences in nitrate-/nitrite-N concentration reflected an influence of nitrification inhibitor in this soil possibly because the ammonium-N levels had been affected by other processes such as immobilization and ammonia volatilization thereby masking the inhibitor effect. Nitrate-N in this soil remains fairly stable in the absence of leaching except under extremely wet conditions [as indicated by very low denitrification potential (Chapters 4 and 6)]. The fact that differences in the ammonium-N concentration did not indicate any influence of nitrification inhibitor also underlines the importance of including both ammonium and nitrate-N levels in equations used to calculate the effectiveness of inhibitor, as proposed by Saharawat, (1984) (Section 2.3 of Chapter 2).

Moreover, the comparisons of mean nitrate-/nitrite-N levels shown in Figure 9.5 reveal that neither the increase in level of urea from 50 to 100 kg N/ha nor the increase in liming from 2.5 to 7.5mt/ha resulted in proportionate increases in the levels of nitrate-/nitrate-N at 6 DAS (for example, compare mean values for T1 with T4 or Li with Lii at 6DAS). It is possible that the higher pH and high ammonium-N concentrations in plots receiving higher rates of urea promoted increased ammonia volatilization so that proportionately less ammonium-N was available for nitrification.

Data for ammonia volatilization are shown in Figure 9.6 (no measurements were carried out on NI-treated plots). Loss of ammonia gradually declined from 2 DAS to 6 DAS.

It is clear that loss of N as ammonia occurred largely during the first few days after fertilizer application. Although the method employed to determine ammonia loss was not very sensitive it nevertheless provided a basis of comparison and also indicated an important factor in the overall N budget of the soil. As expected, the extent of ammonia volatilization was affected by liming and by the rate of urea application (Figure 9.6). But the differences due to lime soon disappeared whereas the effect of different rates of urea was still evident at 6DAS.

During the six-day period after fertilizer application at least 12% , 22.5% and 26.7% respectively of added urea-N was lost as ammoina respectively from Lo, Li and Lii plots at the higher rate of urea (T4), whilst, at least 18%, 27.5% and 34% of

added urea-N was lost from Lo, Li and Lii plots at the lower rate of urea (T1). Considering the trend of increased ammonia volatilization with increased urea addition and liming further investigation using an improved technique such as the micro-meteorological method of Denmead (1985) in a larger scale trial would certainly be useful.

From the point of view of N supply to the crop, the most important aspect to know is the amount of mineral-N available at any given time. Because of N transformation processes and simultaneous N uptake by plants, measured amounts of total mineral N, represent the nett effect of all these activities at a particular time. Assuming that any N input to the system other than by fertilizer (eg. rain and irrigation water) is either negligible or uniform for for a relatively small area we may consider the level of mineral N as follows:

Mineral N remaining in soil = [Original N + N added + mineralized-N] - [N immobilized+ N loss (ie. ammonia volatilization, leaching, denitrification, N uptake by plants)].

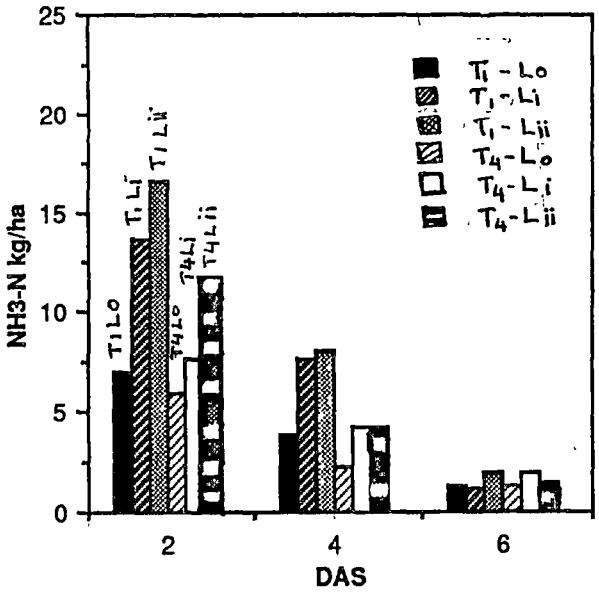
(see Appendix 23)

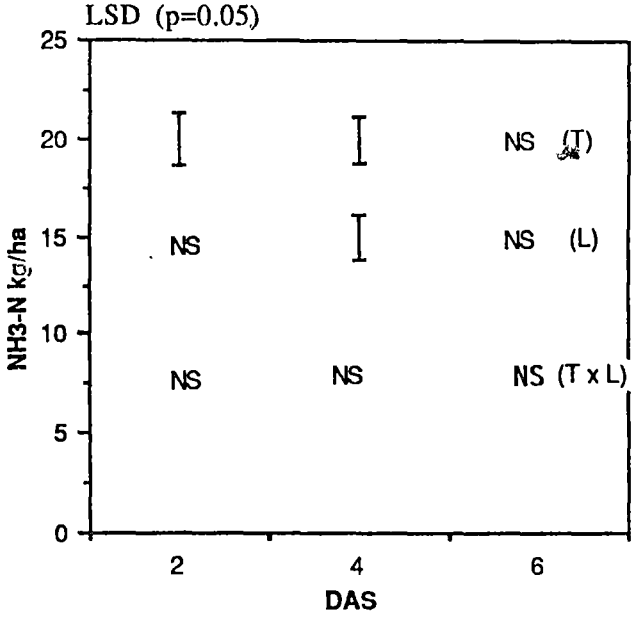
The total mineral-N remaining was estimated by addition of measured ammonium-Nand nitrate-/nitrate-N (Appendix 23) at each sampling time. The analysis of variance indicated that at the time of sowing pre-treatment with lime had not affected the total soil mineral-N content. Since there was no plant growth the absence of significant differences between different lime treatments indicates that there was no significant nett loss of mineral-N from soil due to liming. Subsequent addition of urea-N obviously caused variation in the level of total mineral-N in samples collected at 6 and 13 DAS in accordance with the rates of urea. There was no significant effect of NI at these times on total mineral N (ie. for 50 kg N/ha- T1; T2; T3 and 100 kg N/ha- T4; T5; T6). The differences between effects of the two rates of urea application diminished as the season progressed probably because more nitrogen was absorbed or lost from the mineral-N pool of the plots that received the higher rate of urea. At 42 DAS the highest total mineral-N content was recovered from the T8 and T7 plots which received 50 kg N/ha at the time of stem elongation.

Although liming did not cause a significant difference to the initial total mineral-N content of the soil prior to the addition of fertilizer-N, differences began to appear progressively thereafter (13, 42 and 83 DAS). For example the differences among limed and non-limed plots was more evident during the mid-season period with Lo plots having greater N content than Li and Lii plots. This may have been due to the greater removal of N by more vigorously growing plants and also due to ammonia volatilization from limed plots.

Diagram 2:

Figure 9.6: Ammonia volatilization* from selected N/NI treatments (T1 and T4) at three rates of liming (Lo, Li and Lii)





Thus the growth of barley (cv. Triumph) responded to both lime and N/NI applications. Although there was improved plant growth response to liming in general the low rate of liming (2.5 mt/ha to raise the pH to 5.8-6) appeared to be more appropriate for grain yield than the higher rate (7.5 mt/ha giving a pH of 6.6-6.8) at both rates of urea application. This is in good agreement with the recommendations of the Tasmanian Department of Agriculture Advisory Service for other barley varieties grown locally and also with the results of Mendham and Russell (1985) with the same barley variety in the Elliot area (kraznosem) in the north-west of Tasmania. NI at the levels used in the present experiment did not have a significant effect on yield components or straw production

The results suggest that the application of lime as well as urea could be useful practices in the soil used for this experiment, The application of lime at moderate rates (2.5mt/ha) appears to be better for dry matter production (eg. straw production or for grazing) than the higher rate of liming employed here. Addition of urea as a split application was much superior to the single dressing of the same quantity of urea. There was no clear advantage of using nitrapyrin with urea for dry matter production.

It is possible that initially, the effect of N/NI treatments was not apparent because the soil had enough native available N to meet plant demand (Figure 9.4 and 9.5). However, with progressive plant growth the advantage of an additional N supply was evident.

Nitrogen use efficiency (NUE) followed a similar trend to the growth responses due to lime and N/NI treatments. Amendment with nitrapyrin did not produce any significant change in plant growth response (also Chapter 2. Rajendra Prasad et al., 1978; Slangen and Kerkhoff, 1984).

As no investigations were carried out on the influence of other soil factors that may have been affected by liming (such as Ca, Mg and P availability, and possible reduction in level of active Al and Mn), the results suggest only that liming resulted in improved crop performance. The possibility of involvement of other factors is hightened by the fact that although ammonia volatilization was greater in limed soil than in non-limed soil, better plant growth occurred in the limed plots. Hence the improved recovery efficiency and yield efficiency used to describe NUE may be the nett end-effect of better plant growth andincreased N uptake.

A comparison of split fertilizer application with other N/NI treatments indicated that for grain yield the best results were obtained from split application of urea to limed plots to raise soil pH to 5.8-6.0. Grain yields from split application of urea to non-limed plots were not significantly different from those obtained in response to to a similar amount of N added as a singles dose to limed plots (Li). Hence, even without liming, split application of N could be beneficial. Although plant performance was not affected by the use of nitrapyrin, it did seem to influence nitrate-N production

when used under non-limed conditions at a high rate (four times higher than recommended). However this effect was not significant in limed plots suggesting that the effectiveness of nitrapyrin in inhibiting nitrification may have been masked by increased nitrate formation due to liming. Hence, indications are that if urea is applied as a single dose without nitrification inhibitor, performance can be improved by liming the soil to a higher pH while a split application could cut down the need for increased lime (i.e 2.5 vs.7.5 mt/ha). When low rates of N are employed delaying application until late tillering may reduce grain and straw production markedly probably because of the lower number of tillers initiated due to short supply of N in the earlier growth stages.

The absence of any distinct advantage of using nitrapyrin at either recommended or higher rates underlines the need to compare all fertilizer and soil management options on economic as well as agronomic criteria with any new or introduced cultivar. However, the results reported here need to be treated conservatively as only one soil type and crop combination was tested over one season. Confirmation of these results following trials on a wider range of soils over several seasons isrequired.

In conclusion it is evident that moderate liming (2.5mt/ha) conferred a definite advantage for performance of the barley variety (cv.Triumph) whereas no such clear advantage can be seen following the use of nitrapyrin. Because of the relative increase in ammonia volatilization in limed soils it appears that the better crop performance observed in limed soil may be due in part to change in other factors (such as P availability, increased Ca and Mg supply and reduced Al and Mn toxicities) apart from changes due to N transformation processes. The strongly duplex character of the soil used also appears to have restricted leaching loss of nitrate-N again limiting any advantage of using nitrapyrin to restrict nitrification in this soil. The observed significant changes in nitrate-N but not in ammonium-N levels due to nitrapyrin treatments, may also be because little nitrate-N was lost (by leaching or denitrification) from the soil system rather than an effect of nitrapyrin. Therefore, if the use of nitrapyrin has to be justified on agronomic terms investigations should be carried out in a soil environment where leaching is likely to be significant.

CHAPTER TEN

10. Concluding statements and indications for further research

Urea is fast becoming the world's no 1 nitrogen supplement for agricultural crops for the reasons outlined in the literature review of this thesis (Section 2.1.6, Chapter 2). This is compelling justification of research on the effects of various agronomic practices on the fate of urea-N under different environmental conditions and also on the response of different crops to urea-N supplements in terms of nitrogen use efficiency and yield.

After deciding to investigate the suitability and/or performance of urea as a supplementary N source it was necessary to select agricultural management practices relevant to the study that might be expected to influence N transformation activity and thereby crop performance. Review of the literature revealed that although lime is used primarily to ameliorate low soil pH, to overcome Al and/or Mn toxicities or to supplement low Ca and Mg in soils, the practice may also bring about marked changes in soil microbial activity with effects on N transformation. Hence lime application was selected as a variable in this study. As lime may also encourage autotrophic nitrification in acid soils, a chemical claimed to inhibit the process, namely the nitrification inhibitor nitrapyrin was also selected as a treatment variable. Finally the effects of these two variables were evaluated by comparing soil mineral-N availability and crop performance in response to single and split applications of urea at different rates in a field experiment.

The selection of a high yielding barley variety (cv. Triumph) as the test crop was based on the knowledge that this crop had responded to lime and nitrogen applications under local conditions and also because nitrogen nutrition is an important determinant of crop quality with different requirements for forage versus malting grain production.

A further requirement was access to an area of soil with moderate N transformation activity and especially nitrification potential to justify the use of lime and nitrapyrin treatments. As indicated in Chapter 4, where the screening process of selecting a suitable soil type for this research is described, the chosen soil in field condition was moderately "active" in terms of N transformation. In the process of evaluating the soil type it was noted that although some soils were similar in apparent physical properties they were markedly different with respect to N transformation activities.

A comparison of urea with other common N fertilizers (eg. calcium nitrate and ammonium nitrate) revealed that urea did not perform well as a supplementary source of N in the soil selected for this study. In the experiment described in Chapter 5 the barley variety (cv.Triumph) gave higher yields in response to equivalent rates of N

supplied as fertilizers other than urea confirming that the application rates of urea (with no other amendments) were well below that necessary to produce the potential maximum yield of crop This justifies investigation of management practices in attempts to improve crop production, particularly agronomic practices that may improve performance of urea in terms of yield and quality of product, nitrogen use efficiency of crop and availability of nitrogen in soil.

In a glasshouse incubation experiment carried out to study the effect of lime and nitrapyrin on the fate of urea-N in the absence of plant growth (Chapter 6) it was found that both lime and nitrapyrin could significantly affect the native-N transformation process. Liming stimulated an increase in native soil nitrate-N levels, prior to the addition of urea, to a level higher than that recovered from non-limed soil, presumably due to increased mineralization of organic-N and subsequent nitrification of ammonium-N.

Following urea application there was increased urease activity in limed soil but urease activity was not affected notably by nitrapyrin. Evidently the limed soil provided a better environment for soil heterotrophs capable of producing urease in the presence of urea. The absence of a significant effect of nitrapyrin on urease activity is in accord with results of earlier workers (Chapter 2). Although the analytical techniques employed and the number of replications used were inadequate to detect a small change in the total N pool, a declining trend in the organic-plus-fixed forms of N due to liming was noted. This decline was also considered to be due to increased mineralization of organic-N in limed soil .

A separate incubation experiment (Chapter 6) carried out to assess the effects of lime and nitrapyrin treatments on denitrification (under optimum conditions of abundant nitrate supply and anaerobic conditions) confirmed the observation made during the earlier screening study (Chapter 4) that the denitrification potential of the test soil was rather low. Even so it was clear that liming increased denitrification potential, while nitrapyrin effectively reduced denitrification, in non-limed soil. The effect of nitrapyrin on denitrification was reduced by liming. Since these observations were made in the presence of an abundant supply of nitrate-N the possibility that the observed inhibition of denitrification was a secondary effect would not arise. In fact this observation supports the earlier findings of Mills et al., (1976), and McElhannon and Mills, (1981) that nitrapyrin could effectively inhibit denitrification.

The level of ammonium-N and nitrate-N measured in the glasshouse experiment described in Chapter 6 showed that liming markedly increased nitrification rate but that this was slowed down in the presence of nitrapyrin. However the degree of inhibition of nitrification due to nitrapyrin was reduced with liming of the soil. In this experiment the effectiveness of nitrapyrin was determined in terms of % inhibition over a period of 45 days. Because of conflicting observations/proposals in the

literature (Section2.6 of Chapter 2) the term "effectiveness" was clarified by taking into consideration more precisely the time factor (relative to plant growth). A longer period of incubation resulted in a decline in inhibition in limed soil probably because of the faster recovery of nitrifiers under the higher pH conditions. But after about 45 days of incubation the rate of nitrate formation in limed soil had slowed down and was slightly lower than in non-limed soil. This may be because relatively more ammonium-N was available for nitrifiers in non-limed soil compared to limed soil and also because nitrapyrin by that time was not present in sufficient quantities for effective inhibition. Observations during this experiment gave rise to concern that product-related properties of the inhibitor might influence its effectiveness. Hence some product-related characteristics of nitrapyrin were investigated further (Chapter 9).

The effects of lime and nitrapyrin were further investigated in a short-term glasshouse experiment described in Chapter 7. Liming caused positive responses in the early stages of plant growth whereas there was no detectable response to nitrapyrin. A four-fold increase in the rate of nitrapyrin application produced no toxic effects but none were noted during 45 days of plant growth. This contrasted with some reports of nitrapyrin toxicity (Section2.3 of Chapter 2 1978).

In the present study both liming and incorporation of nitrapyrin affected microbial activity as measured by soil respiration rate. In a relatively short term incubation study (120hrs) liming increased the rate of carbon dioxide production above that of non-limed soil. The addition of nitrapyrin further amplified carbon dioxide production in limed soil. The latter effect can be explained as due to a more favourable pH for microbial activity whereas further increase attributed to nitrification inhibitor may be due to the inhibition of autotrophic nitrifiers that would otherwise have consumed part of the available carbon dioxide and to probable existence of heterotrophic strains capable of utilizing nitrapyrin or its hydrolysis products for their carbon requirements. These observations supported the conclusion that nitrification in the soil used in this study is due to autotrophic rather than heterotrophic nitrifiers since heterotrophic nitrification should not increase as a result of liming nor should it be inhibited by nitrapyrin.

The results of the field experiment described in Chapter 9 highlight a positive interaction of lime and nitrapyrin on plant growth and composition of inorganic soil nitrogen under field conditions. Confirming the results obtained from the glass house experiment (Chapter 6) liming again showed a marked influence on plant growth. Urea application significantly improved crop performance but amendment of urea with nitrapyrin made little or no difference to crop growth. However since lime is known to affect other growth-influencing soil characteristics (such as P availability, Ca and Mg supply, Al and Mn toxicities) and they were not examined in the present

investigations it is possible to conclude only that lime application resulted in improved plant growth. The possibility that factors other than N availability may have influenced the observed improvement in crop growth is underlined by the fact that lime caused increased ammonia volatilization from urea-fertilized plots with significant loss of N (ie. less available N) from the soil. This might have been expected to have been followed by reduced plant growth. But a completely opposite result suggests that other factors may have influenced plant growth. Although a similar relationship between inorganic N and plant growth was observed on limed plots later in the season this could have been due to entirely different reasons such as the uptake of more N as a result of better plant growth in the limed soil. Hence investigation of possible effects of lime on other growth factors is necessary in order to decide whether or not increases in N-recovery efficiency and yield efficiency are affected directly or whether they are better interpreted as a side effect of the improved crop growth.

Nitrapyrin did not affect plant growth at either of the concentrations used in this field experiment. This supports earlier observations by other authors cited in the literature review (Chapter 2 :section 2.3)..

As revealed in the results of the present series of laboratory and glasshouse studies and also because of the often-reported greater success of nitrification inhibitors in pot experiments than in experiments conducted in the field, a separate study was carried out to investigate certain product-related characteristics of nitrapyrin that could affect its activity in soil (Chapter 8). The results of leaching experiments with nitrapyrin and fertilizer-N in soil columns indicated that movement of nitrapyrin was very slow when compared to the rapid rate of leaching of added urea and the slower movement of ammonium-N. Hence there was early separation of urea and later ammonium-N from the nitrapyrin with which they were added at the top of the column. Probably this allowed nitrifiers in the lower part of the column to use ammonium-N . Since urea moved very rapidly with moving water the application of N as urea with nitrapyrin may not be a suitable practice unless incorporated deep into the soil and subsequent leaching/irrigation is minimal or well-controlled. This may explain the often-reported differences in the effectiveness of nitrapyrin between laboratory/glasshouse and field experiments. In the former the urea plus nitrapyrin has been mixed well with soil and no leaching has usually been allowed while the low effectiveness in field plots with unsatisfactory inhibition of nitrification may be due to leaching effects as discussed above (see Chapter 6 vs. Chapter 9; Slangen and Kerkhoff, 1984). There may be other mechanisms for loss of nitrogen (Chapter 2).

Nitrapyrin added to soil subsequently exposed to wind or direct air flow, was lost quite rapidly when compared to the rate of loss in a still air environment. An increase in temperature from 10^{o} to 25^{o}C also accelerated nitrapyrin loss. Such losses were attributed mainly to volatilization losses, to hydrolysis of nitrapyrin to 6-

hydroxy picolonic acid and also to possible utilization by microbes. These results emphasize the importance of incorporation of nitrapyrin deep into the soil with fertilizer. Preferably this application should be carried out when the temperature is low (ie. spring or late winter).

It is fairly general experience that attempts to answer questions by research usually result in a proliferation of further questions. The present studies have left many questions unanswered and have highlighted other aspects that might be investigated. Some of these aspects are discussed below.

In spite of much work in the past, further studies are needed under controlled environment conditions on the influence of different N fertilizers on plant growth in terms of their associated specific ions, effects on soil pH, and the extent of losses (mobility) for each form of fertilizer-N. It is also desirable to further clarify either by nutrient culture or sand culture techniques the influence of ammonium-N and nitrate-N on the growth of test plants. Such studies could benefit immensely from the use of labelled N compounds.

As discussed earlier in this thesis, liming affected the N transformation process in the acidic soil used in this project. Increases in urease activity, reduction of mineralizable-N, and increased nitrate-N levels supported this conclusion. Further to this is the observation of increased heterotrophic microbial respiration in limed soil compared to non-limed soil. The increase in nitrification due to liming indicated that nitrate production was due to autotrophic nitrifiers and therefore that nitrapyrin can be effective in this soil. In view of this it is felt that a study using pure cultures of nitrifiers would help to clarify the behaviour of nitrifiers in relation to nitrification inhibitor and pH. The present results for nitrapyrin suggest that its effectiveness may change over a period of incubation due to environmental factors that influence the recovery/activity of nitrifiers. Such studies could also aid an understanding of the influence of various soil factors such as a organic matter, soil clay fraction and inorganic elements (eg. Cu, Ni) on the effectiveness of nitrapyrin (Chapter 2. sect. 2.3 and the Addendum).

Further investigation would have the objective of more precise specification of the inhibitory role of nitrapyrin since effective inhibition of nitrification would be very useful in certain situations. For example in an environment where nitrate-N leaching is not significant but denitrification is, or in situations where the promotion of ammonium-N could lead to increased ammonia volatilization. Such an environment could occur under flood irrigation as in paddy (rice) cultivation on clayey soils (less leaching) where anaerobic microsites may develop (Savant and De Datta, 1980).

It appears that even if nitrapyrin does reduce nitrification better in limed soils as suggested by some workers the effect is evidently short-lived because of rapid recovery and proliferation of nitrifiers at higher soil pH. Hence, more relevant studies

for agriculturists would permit a description of nitrapyrin effectiveness over a time period that will take practical plant factors into consideration, such as the time necessary for the crop to rapidly absorb soil N. It is further suggested that experiments to ascertain the effects of pH and nitrapyrin over different time intervals should be conducted with axinic cultures of nitrifiers as there could be many complicating interactions when these reactions take place in soil environments.

It would be interesting to investigate whether any strains of microorganisms are capable of utilizing nitrapyrin or its hydrolyzsis products as their carbon sources. The observed increase in carbon dioxide production in nitrapyrin-treated soil (Chapter 7) suggest that microbes with such a capability may exist. Such information would be useful in better understanding of the loss of nitrapyrin from soil.

The relative mobility of nitrapyrin appears to be a significant factor in its practical effectiveness. This inhibitor moves at a much slower rate even than ammonium-N through soil columns while urea (and nitrate-N) moved very rapidly. Therefore, even though nitrapyrin may persist for a sufficiently long time, leaching may remove urea and ammonium away from the inhibitor thus reducing its practical effectiveness and nullifying its persistence qualities. Hence the recommendation to use nitrapyrin may be justified only after due assessment of all the factors influencing its effectiveness. In the light of results of the present study it could be useful in situations where nitrification is rapid and the rate of leaching is slow, the cation exchange capacity of the soil is high or when the potential loss by denitrification is significant.

The use of tracers could be useful in further study of salient points highlighted in this research project, such as the pattern of distribution of nitrapyrin, ammonium-N, nitrate-N and urea, by making it possible to follow complications due to N transformations that may take place simultaneously during the leaching process, as well as facilitating quantitative evaluation of the fate of soil N as was attempted in the experiment described in Chapter 6.

During the designing of the experiment described in Chapter 6 several problems related to sampling technique were addressed. A modified Bodman and Colman (1943) column was found to be best for this type of investigation. It is felt that in choosing the type of column used for such studies microbial aspects of the reactions involved should be taken into consideration since these may affect the outcome significantly. For example, anaerobic conditions that may develop within conventional closed columns could promote denitrification.

In conclusion, while the results obtained from a single season of field experiments may be indicative, it is essential to repeat such experiments over several seasons to evaluate the reproducibility of the results obtained before firm conclusions can be drawn.

BIBILIOGRAPHY AND APPENDICES

Bibliography

Abdul Rahman, M.S. (1988) The effect of defoliation for winter forage on recovery and grain yield of barley and oat in Tasmania. The Ph.D Thesis, University of Tasmania, Hobart, Tasmania, Australia.

Adams, J.A. (1986) Nitrification and ammonification in acid forest litter and humus as affected by peptone and ammonium amendment. Soil Biol. Biochem. 18(1): 45-51.

Adams F., and Martin, J.B. (1984) Liming effects on nitrogen use and efficiency. In: R.D. Hauck (ed.), Nitrogen in Crop Production: 417-426.

Adams M.A. and Attiwill, P.M. (1986) Nutrient cycling and nitrogen mineralization in eucalypt forests of South-East Australia., ii. Indices of nitrogen mineralization. Plant Soil 92: 341-362.

Addiscot, T.M. and Wagenet, R.J. (1985) Concepts of solute leaching in soils: a review of modelling approches. J. Soil Sci.36: 411-424.

Addiscott, T.M. (1977) A simple computer model for leaching in structured soils. J. Soil Sci 28: 554-563.

Adkins, A.M. and Knowles, R. (1986) Denitrification, denitrifying bacteria, and iron reduction in a soil supplemented with sulfide and acetylene. Can. J. Soil Sci.66: 633-639.

Agebede, O.O. (1987) Response of barley seedlings to nitrogen andphosphorous rates ion soils with various fertility levels. Soil Sci.143 (3): 192-197.

Agriculture Group Symposium: Agriculture and Water Quality-Part 1.(1984) J. Sci. Food Agric. 35: 855-862.

Agriculture Group Symposium: Agriculture and Water Quality-Part 1.(1984) J. Sci. Food Agric. 35: 849-854.

Ahamad Z., Kai, H. and Harada, T. (1972) Effects of nitrogenous form on immobilization and release of N in soil. J.Fac. Agri. Kyusm Uni.17: 49-65.

Albert E.E. and Spomer, R.G. (1985) Dissolved nitrogen and Phosphorous in runoff from waterseds in conservation and conventional tillage. J. of Soil and Water Conservation40: 153-157.

Allison,F.E. (1973) Soil organic matter and its role in crop production. Elsevier publication, Amesterdam.

Amberger, A. (1983) Ways to control the availability, turnover and losses of mineral fertilizer N in soils. In,'Efficient Use ofFertilizers in Agriculture', Proc. Symp. on Res. into Agrotechnical Methods Aiming at Increasing the Productivity of Crops-Co-efficients for the most Efficient Use of Fertilizer Nutr. and Means for Reducing the losses of Nutrients to the Environment under the conditions of Intensive Agriculture.Martinus Nijhoff Pub.145-170.

Anderson, J.P.E. (1982) Soil respiration. In Methods of Soil Analysis, Part 2, pp. 831-866 Agronomy No.9 , 2nd. edition, A.L. PAge, R.H. Miller, and D.R. Keeney (eds.), Am. Soc. Agron. and Soil Sci. Soc. Am.,Madison, Wisconsin, USA.

Armstrong, A.S.B. (1983) Nitrous oxide emission from two sites inSouthern England during winter 1981/1982. J.Sci. Food Agic. 34: 803-807.

Arnon, D.I. (1934) Ammonium and nitrate nitrogen nutrition of barley at different seasons in relation to H ion concentration, Manganese, Copper and and oxygen supply. Soil Sci. 44: 91-120.

Arnon, D.I. (1937) Ammonium and nitrate nitrogen nutrition of barley at different seasons in relation to hydrogen-ion concentration, manganese, copper, and oxygen supply. Soil Sci. 4491-120.

Aslam M., Huffaker, R.C. and Rains, D.W. (1984) Early effects of salinity on nitrate assimilation in barley seedlings. Plant Physio.76 (2): 321-325.

Aulakh, M.S., Rennie, D.A. and Paul, E.A. (1984) Acetylene and N-serve effects upon N2O emissions from NH4+ and NO3- treated soils under aerobic and anaerobic conditions. Soil Biol. Biochem.16 (4): 351-356.

Aulakh, M.S. and Rennie, D.A. (1985) Azide effects upon N2O emission and transformation of N in soils. Can. J. Soil Sci.65: 205-212.

Aulakh, M.S. and Rennie, D.A. (1985) Gaseous nitrogen losses from conventional and chemical summer fallow. Can. Soil Sci.65: 195-203.

Avnimelech, Y. and Laher, M. (1977) Ammonia volatilization from soils:equilibrium considerations. Soil Sci. Soc. Am. J. 41: 1080-1084.

Axley, J.H. and Legg, J.O. (1960) Ammonium fixation in soils and the influence of pottasium on nitrogen availability from nitrate and ammonium sources. Soil Sci. 90: 151-156.

Aydeniz, A., Hatipoglu, F. and Aktas, M. (1976) The effects of N-Serve on the health of barley, maize and Vetch. In,' Fertilizer and the plant health':Proc. of the 12th. College of the intermediate potash.77-83.

Azhar, E.S., Verne, R., Sandra, M.and Verstraete, W. (1986a) Binding of nitrite-N in polyphenols during nitrification. Plant and Soil94: 369-382.

Azhar, El. Sayed, O van Cleemput, and Verstraete, W. (1986b) Nitrification mediated nitrogen immobilization in soils. Plant and Soil94: 401-409.

Azhar, El Sayed, Vandenabeele, J. and Verstraete, W. (1986c) Nitrification and organic nitrogen formation in soils. Plant and soil94: 383-399.

Bacon P.E., McGarity J.W., Hoult, D. and Alter, D. (1986) Soil mineral N concentration within cycles of flood irrigation. i. Effect of rice stubble and fertilization management. Soil Biol. Biochem.18 (2): 173-178.

Baily, L.D. and Beauchamp, E.G. (1973) Effects of moisture, added NO3 and macerated roots on NO3 transformation and redox potential on surface and subsurface soils. Can J. Soil Sci.53: 219-224.

Balakrisnan, S., and Eckenfelder, W.W. (1969) Nitrogen relaationships in biological treatment processes. iii. Denitrification in the modified activated sludge process. Water Res.3: 177.

Balasubramanian V. and Kanerio, Y. (1976) Denitrification potential and pattern of gaseous loss in tropical Hawaiian soils. Tropi. Agri. 53292-303.

Baldwin, I.T., Olson, R.K. and Reiners, W.A.(1983) Protein binding phenolics and the inhibition of nitrification in sub-alpine balsam fir soils. Soil Biol. Biochem. 15: 419-423.

Balwinder Singh and Bajwa, M.S. (1986) Studies on the leaching of urea in sodic soils. J. Agric. Sci., Camb.vol, 106: 323-330.

Barraclough, D., Hyden, M.J. and Davies, G.P. (1983) Fate of fertilizer nitrogen applied to grassland. i. Field leaching results. J. Soil Sci.34: 483-497.

Barry, D.A., Rose, C.W., Saffigna, R.G. and Parlange, J.Y. (1985) Interpretation of leaching under multiple fertilizer applications. J. Soil Sci.36: 9-20.

Batholomew, W.V. (1965) Mineralization and Immobilization in the decomposition of plant and animal residues. In, 'Soil nitrogen.' W.V. Batholomew and F.E Clark (eds.) Agronomy 10: 285-306.

Belser, L.w. (1979) Population ecology of nitrifying bacteria. Ann. Res. Microbiol.33: 309-333.

Belser, L.W. and Schmidt, E.L. (1981) Inhibitory effect of nitrapyrin on three genera of ammonia-oxidizing nitrifiers. Applied and Environ, Microbiol.vol. 41 (3): 819-821.

Bhuiya, Z.H. and Walker, N. (1977) Autotrophic nitrifying bacteria in acid tea soils from Bangladeh and Sri Lanka,J. of Appl. Bacteriology 42: 253-257.

Bijay-singh and Sekhon G.S. (1982) The influence of different moisture regimes on leaching of nitrate in columns. Polish J. of Soil Sci.15 (2): 129-132.

Bingeman, C.W., Vamer, J. E. and Martin, W.P. (1953) The effect of theaddition of organic materials on the decomposition of an organic soil, . Soil Sci. Soc. Am. Proc.17: 34-38.

Bjarnason, S. (1987) Immobilization and remineralization of ammonium and nitrate after addition of different energy sources to soil,. Plant and Soil97: 381-389.

Black, A.S.and Waring, S.A.(1972) Ammonium fixation and availability in some cereal producing soils in Queensland,. Aust.J. Soil Res. 10: 197-207.

Black, A.S., Sherlock, R.R., Cameron, K.C., Smith, N.P. and Goh, K.M. (1985) Comparison of three field methods for measuring ammonia volatilization from urea granules broadcast on to pasture. J.Soil Sci.36: 271-280.

Black A.B and Waring, S.A. (1976a) Nitrate leaching and Adsorption in a krasnozem from redland bay, Queensland i.Leaching of banded ammonium nitrate in a horticultural rotation. Aust. J. Soil Res.14: 171-180.

Black A.B. and Waring, S.A. (1976b) Nitrate leaching and Adsorption in a krasnozem from redland bay, Queensland ii.Soil factors influencing adsorption. Aust. J. Soil Res. 14: 181-188.

Black, A.S. and Waring, S.A. (1976c) Nitrate leaching and adsorption in a krasnozem from Redland Bay, Qld. iii. Effect of nitrate concentration on adsorption and movement in soil columns. Aust. J. Soil Res.14: 189-195

Blackmer, A.M. and Bremner, J.M. (1978) Inhibitory effect of nitrate on reduction of N2O to N2 by soil microorganisms. Soil Biol. Biochem.10: 187-191.

Blackmer, A.M., Bremner, J.M. and Schmidt, E.L. (1980) Production of N2) by ammonium oxidizing chemoautotrophic microorganisms in soils. Appl. and env. microbiol. 40: 1060-1066.

Blackmer, A.M. and Cerrato, M.E. (1986) Soil properties affecting formation of nitric oxide by chemical reactions of nitrite. Soil Sci. Soc. Am. J. 50: 1215-1218.

Bock, E. (1978) Lithotrophic and chemoorganotrophic growth of nitrifying bacteria. In Microbiolgy 1978. (ed.) Schlessinger, D. American Society of Microbiology, Washington DC, pp. 310-314.

Bock, B.R. (1984) Efficient use of nitrogen in cropping systems. In: Nitrogen in crop production(ed.) R.D.Hauck: 272-294.

Bock, B.R., Lawrence, J.E., and Williams, H.M. (1981) Relative mobility of Dicyandiamide, ammonium and urea by mass flow in soils. In Proc. Technical workshop on Dicyandiamide (ed.) R.D.Hauck and H. Behnke: 25-37.

Bodman, G.B. and Colman, E.A.(1943) Moisture and energy conditions during downward entry of water into soils. Soil Sci. Soc. Am. Proc. 8: 116-122.

Boerner R.E.J. (1982) Fire and nutrient cycling in temperate ecosystem . Bio Science 32: 187-192.

Bole, J.B. and Gould, W.D. (1986) Overwinter losses of mitrogen-15 labelled urea fertilizer. Can. J. Soil Sci. 66: 523-520.

Bollag, J.M., Orcutt, M.L. and Bollag, B. (1970) Denitrification by isolated bacteria under various environmental conditions. Soil Sci. Soc. Am. Proc. 34: 875-879.

Boon, B. and Laudelout, H. (1962) Kinetics of nitrite oxidationby Nitrobacter wingrodskyi, Biochem. J.85: 440-444.

Boto, K. Saffigna, P. and Clough, B. (1985) Role of nitrate in nitrogen nutrition of the mangrove Avicennia marina. Marine Ecology, vol 21: 259-265.

Bouwmeester, R.J.B., Vlek, P.L.G. and Stumpe, J.M. (1985) Effect of enviornmental factors on ammonia volatilization from a urea-fertilized soil. Soil Sci. Soc. Am. J. 49: 376-381.

Bower, C.A. (1951) Availability of ammonium fixed in difficultly exchangable form by soils of semiarid region. Soil Sci. Soc. Am. Proc.15,: 119-122.

Boyd S.A. and Mortland, M.M. (1985) Urease activity on a clay-Organic complex. Soil Sci. Soc. Am. J.49: 619-622.

Breitenbeck, G.A., Blackmer, A.M., and Bremner, J.M. (1980) Effects of different nitrogen fertilizers on emmision of nitrous oxide from soil, Geophysics Res. Lett.,7: 85-88.

Breitenbeck, G.A. and Bremner, J.M.(1987) Effects of storing soils at various temperatures on their capacity for denitrification. Soil Biol. Biochem19: 377-380.

Bremner, J.M. and Shaw, K. (1958) Denitrification in soil. ii. Factors affecting denitrification. J. of Agric. Sci,: 51.

Bremner, J.M. and Douglas, L.A. (1971) Inhibition of urease activity in soils. Soil Biol. Biochem.3: 297-307.

Bremner, J.M. and Douglas, L.A. (1973) Effects of some urease inhibitors on urea hydrolysis in soils. Soil Sci. Soc. Am. Proc.,37: 225-226.

Bremner, J.M. and Mulvany, R.L. (1978) In: Soil enzymes (ed. R.G. Burns) Urease activity in soils: 149-196.

Bremner, J.M., Blackmer, A.M. and Bundy, L.G. (1978) Problems in use of nitrapyrin (N-Serve) to inhibit nitrification in soils. Soil Biol. Biochem. 10: 441-442.

Bremner, J.M. and Blackmer, A.M.(1978) Nitrous oxide: Emission from soils. Science 199: 295-296.

Bremenr, J.M. (1982) Total Nitrogen. In, Methods of Soil Analysis, Part 2, pp. 1149-1176. Agronomy No.9 , 2nd. edition, A.L. Page, R.H. Miller, and D.R. Keeney (eds.), Am. Soc. Agron. and Soil Sci. Soc. Am., Madison, Wisconsin, USA.

Bremenr, J.M. (1982) Inorganic forms of nitrogen. In Methods of Soil Analysis, Part 2, pp. 1179-1232 Agronomy No.9 , 2nd. edition, A.L. Page, R.H. Miller, and D.R. Keeney (eds.), Am. Soc. Agron. and Soil Sci. Soc. Am.,Madison, Wisconsin, USA.

Briggs, G.G. (1975) The behavior of the nitrification inhibitor 'N-Serve' in broadcast and incorperated applications to soil. J. Sci. Food and Agric. Sci.22: 73-81.

Britenbeck G.A., Blackmer, A.M. and Bremner J.M. (1980) Effects of differential nitrogen fertilizers on emmission of N2O from soil. Geophys. Res. Letter, 7: 85-88.

Broadbent, F.E., and Clark, F. (1965) Denitrification,. In,'Soil Nitrogen', (eds. W.V. Batholomew and F.E. Clark)Agronomy: 10: 344-359.

Broadbent, F.E. and Carlton, A. B. (1978) Field trials with isotopically labelled nitrogen fertilizer. In,'Nitrogen in the environment' (eds. D.R. Nielsen, and J.G. MacDonald) 1-63.

Broadbent, F.E., Tyler, K.B. and Hill, G.N. (1957) Nitrification of ammoniacal fertilizers in some Califonian soils.Hilgradia27: 247-267.

Broadbent, F.E., Nakashima, T. and Chang, G.Y. (1985) Performance of some urease inhibitors in field trials with corn. Soil Sci. Soc. AM. J. 49: 348-351.

Broadbent F.E., Hill, G.N. and Tyler, K.B. (1958) Transformation and measurment of urea in soils. SSSA. Proc.22: 303-307.

Bundy, L.G. and Bremner, J.M. (1973) Inhibition of nitrification in soils. Soil Sci. Soc. Am. Proc. 37: 396-398.

Bundy, L.G.and Bremner, J.M. (1974a) Effects of nitrification inhibitors on transformations of urea nitrogen in soils. Soil Niol. Biuochem.6: 369-376.

Bundy, L.G. and Bremner, J.M. (1974b) Efects of urease inhibitors on nitrification in soils. Soil Biol. Biochem. 6: 27-30.

Burns, I.G. (1975) An equation to predict the leaching of surface-applied nitrate. J. Agric. Sci. 85: 443-454.

Burns, R.G. (1982) Location and a poosible role in microbial ecology . Soil Biol. Biochem. 12: 423-427.

Byrnes, B.H., Savant, N.K. and Craswell, E.T. (1983) Effect of aurease inhibitor Phenyl Phosphorodiamidate on the efficiency of urea applied to rice. Soil Sci. Soc. Am. J. 47: 270-274.

Cady, F.B. and Batholomew, W.V. (1960) Sequential products of anaerobic denitrification in Norfolk soil material. Soil Sci. Soc. Am. Proc.24: 477-482.

Cameron, K.C. and Haynes, R.J. (1986) Retention and movement of nitrogen in soils. In: Mineral nitrogen in plant soil-systems(ed.) R.J.Haynes: 166-241.

Campbell, N.E. R., and Aleem, M.I.H. (1965) The effect of 2 chloro,6 trichloromethyl pyridine in the chemoautotrophic metabolism of nitrifying bacteria. 1. Ammonium and hydroxylamine oxidation by Nitrosomanas. Antonie van Leeuwenhock31: 124-130.

Campbell C.A. (1978) Soil organic carbon, nitrogen and fertility. In,' Soil organic Matter', (eds. M. Schnitzer and S.U. Khan), 173-271.

Campbell, C.A., Myers, R.J.K. and Weier, K.L. (1981) Potentially mineralizable nitrogen, decomposition rates and their relationship to temperature for five Queensland soils. Aust. J. Soil Res.19: 323-332.

Campbell, C.A., Jong, R.De and Zentner, R.P. (1984) Effect of cropping, summer fallow and fertilizer nitrogen on nitrate-nitrogen lost by leaching on a brown chernozemic loam. Can. J. Soil Sci. 64: 61-74.

Campbell, C.A., Leyshon, A.J., Ukrainetz, H. and Zentner, R.P. (1986) Time of application and source of nitrogen fertilizer on yield, quality, nitrogen recovery and net returns for dryland forage grasses. Can. J. Plant Sci.66: 915-931.

Carter, M.R. and Rennie, D.A. (1984a) Dynamics of soil microbial biomass N under zero and shallow tillage for spring wheat, using N-15 urea. Plant and Soil,76: 157-164.

Carter, M.R. (1986) Microbial Biomass and mineralizable N in Solonetzic soils; influence of gypsum and lime amendments. Soil Biol. Biochem18: 531-537.

Carter, M.R. and Rennie, D.A. (1984b) Nitrogen transformation under zero and shallow tillage. Soil Sci. Soc. Am. J. 48: 1077-1081.

Catchpoole, V.R., Harper, L.A. and Myers, R.J.K. (1981) Annual losses of ammonia from a grazed pasture fertilized with urea. Proc. XIV Inter. Grassland Cong.Lexington, KY.: 344-347.

Cervelli, S., Nannipieri, P., Giovaninni, G. and Perna, A. (1975) Jack bean urease inhibition by substantial urease. Rest. Biochem.Physiol.5: 221-225.

Chalk, P.M. and Smith, C.J. (1983) Chemodenitrification, . In, 'In gaseous loss of nitrogen from plant-soil systems'.(eds. J.R.Freney and J.R. Simpson): pp. 65-89.

Chalk, P.M., Keeney, D.R. and Walsh, L.M. (1975) Crop recovery and nitrification of fall and spring applied ammonia. Agrono. J. 67: 33-37.

Chancy, H.F. and Kamprath, E.J. (1980) Relationship between organic matter content and nitrapyrin rate required to inhibit nitrification. Agron Abstr.166.

Chancy, H.F. and Kamprath, E. J. (1987) Effect of nitrapyrin rate on nitrification in soils having different organic matter contents. Soil Sci.144(1): 29-35.

Chao, T. and Kroontje, W. (1964) Relationships between ammonia volatilization, ammonia concentration and water evaporation. Soil Sci. Soc. Am. Proc.28: 393-395.

Chapman, H.D. and Pratt, P.F. (1961) Methods of analysis for soils, plants, and waters. University of California, USA,

Chew, W.Y., Williams, C.N. and Chesters, A. (1976) studies on the availability to plants of soil nitrogen in Malaysian tropical pligotrophic peat. I. Effect of liming and pH, II. Effect of N,P,K, and micronutrients. Trop. Agric.53: 69-87.

Chin, W. and Kroontje, W.(1963) Urea hydrolysis and subsequentloss of ammonia. Soil Sci. Soc. Am. Proc.27: 316-318.

Christensen, S. (1985) Denitrification in an acid soil: Effects of slurry and potassium nitrate on the evolution of nitrous oxideand on nitrate-reducing bacteria. Soil Biol. Biochem17 (6): 757-764.

Clark, F.E. (1962) Losses of N accompanying nitrifications. In: Transactions of International Soil Conference, New ZelandIV and V: 173-176.

Clark, F.E. and Paul, E.A. (1970) The microflora of grassland. Adv. Agronomy22: 375-435.

Clarke, A.L. and Russell, J.S. (1977) Crop sequential practices. In; (eds. J.S. Russell and E.L. Greacen),"Soil factorsin crop production in a semi-arid environment" 279-300.

Colbourn, P. and Dowdell, R.J. (1984) Denitrification in fieldsoils. Plant and Soil6: 213-226.

Colbourn, P. (1985) Nitrogen losses from the field: denitrification and leaching in intensive winter cereal production in relation to tillage method of a clay soil. Soil Use and Management 1(4): 117-120.

Cooper, G.S. and Smith, R.L. (1963) Sequence of products from denitrification in some diverse western soils. Soil Sci. Soc. Am. Proc.27: 659-663.

Cosgrove, D.C., Benton Jones, J. and Mills, H.A. (1985) Influence of NO3 and NH4 on Kjeldhl N, NO3-N and Snapbean pod yield. HortScience20(3): 427-429.

Craswell, E.T., and S.A. Waring,. (1972a) Effect of grinding on the decomposition of soil organic matter, ii. Oxygen uptake and nitrogen mineralization in vergin and cultivated cracking clay soils. Soil Biol. Biochem.4: 453-442.

Craswell, E.T. and Waring, S.A. (1972b) Effect of grinding on the decomposition of organic matter. ii. Oxygen uptake and nitrogen mineralization in virgin and cultivated cracking clay soil. Soil Biol. Biochem.4: 435-442.

Craswell, E.T. (1973) Ph.D. Thesis. Uni. of Queensland.

Craswell, E.T. and Martin, A.E. (1975) Isotopic studies of the nitrogen balance in a cracking clay.i. Recovery of added nitrogen from soil and wheat in the glasshouse and gas lysimeter. Aust. J. Soil Res.13: 43-52.

Craswell, E.T. (1973) The nitrogen balance of the soil-plant system with special reference to wheat and black earth soils.

Craswell, E. T. and Mason, L.R. (1976) Factors affecting nitrogen mineralization. Queensl. Wheat Res. Inst. Annu. Rep. 1975/76: 41-42.

Craswell, E.T. (1978) Some factors influencing denitrification and nitrogen immobilization in a clay soil. Soil Biol. Biochem.10: 241-245.

Craswell E.T. and Waring, S.A. (1972) Effect of grinding on the decomposition of organic matter. i. The mineralization of organic N in relation to soil type. Soil Biol. Biochem.vol. 4,: 427-434.

Craswell E.T., Saffigna, P.G. and Waring, S.A. (1970) The mineralization of organic N in dry soil aggregates of differentsizes. Plant Soil33: 383-392.

Crutzen, P.J. and Ehhalt, D.H. (1975) Effect of nitrogen fertilizers and combustion on the statospheric ozone layer. Ambio 6: 112-117.

Curtin, D. and Smillie, G.W.(1986) Effects of liming on soil chemical characteristics and grass growth in laboratory and long term field-amended soils; i. Soil chemistry. Plan and Soil95: 15-22.

Dalal, R.C. (1975) Urease activity in some Trinidad soils. Soil Biol. Biochem.7: 5-8.

Dalal, R.C. (1986) Yield response of corn to lime and urea application on an acidic tropical soil. Commun. in Soil Sci. and Plant Anal. 17 (7): 789-798.

Davidson, E.A., Swank, W.T. and Perry, O.T. (1986) Distinquishing between nitrification and denitrification as sources of gaseous N production. Appl. and Environ. Microbiol. 52(6): 1280-1286.

Davey and Simpson (1987) Liming, water deficits and C2H2 reduction by Subterranean Clover. In the Proceedings of the 4th Agronomy Conference held at La Trobe University, Melbourne, Victoria, Aug 1987. pp 259.

De Datta, S.K. (1981) Principals and practices of rice production, Wiley Publish. N.Y.

De Man, W. and Dondeyne, P. (1985) Effect of nitrogen fertilization on protein content, total fatty acid and composition of barley (Hordeum vulgare L.) grains. J.Sci. Food Agic.(36): 186-190.

Delune, R.D. and Patric, W.H. (1970) Urea conversion to ammonia in water-logged soils. SSSA proc.34: 603-607.

Denmead, O.T., Freney, J.R. and Simpson, J.R. (1976) A closed ammonia cycle within a plant canopy. Soil Biolo. Biochem.8: 161-164.

Denmead, O.T., Simpson, J.R. and Freney, J.R. (1977) A directfield measurement of ammonia emission after injection of anhydrous ammonia. Soil Sci. Soc. Am. J. 41: 1001-1004.

Denmead, O.T. (1983) Micrometerological methods for measuring gaseous losses of nitrogen in the field. In,' Gaseous losses ofnitrogen from plant and soil systems', (eds. J.R. Freney / J.R.Simpson): 133-157.

Douglas, L.A. and Bremner, J.M. (1971) A rapid method of evaluating different compounds as inhibitors of urease activity in soils. Soil Biol. Biochem. 3: 309-315.

Dowdell, R.J. and Cannell, R.Q. (1975) Effect of ploughing anddirect drilling on soil nitrate content. J.Soil Sci. 26: 53-61.

Dowdell, R.J. and Webster, C.P. (1984) Effect of drought and irrigation on the fate of N applied to cut permanant grass swards in lysimeters, i. experimental design and crop uptake. J. Sci. Food Agric. 35: 1092-1104.

Dowson, R.W. and Murphy, K.L. (1973) Factors affecting biological denitrification of waste water. In,'Advances in water pollution research' (ed. S.H. Jenkins): 671-680.

Duggins, J.A. (1984) Autotrophic and heterotrophic nitrification in acid forest soils from Hubbard brook, New Hampshire. Ph.D. thesis, Yale University.

Duxbury, J. (1986) Advantages of the acetylene method of measuring denitrification. In: Field measurement of dinitrogen fixation and denitrification(ed.) R.D. Hauck and R.W. Weaver: SSSA Special publ. No. 18.

Ernst, J.W. and Massey, H.F. (1960) The effects of several factors on volatilization of ammonia formed from urea in the soil. Soil Sci. Soc. Am. Proc.24: 87-90.

Fenn, L.B. and Escarzaga, R. (1976) Ammonia volatilization from surface application of ammonium compounds on calcareous soils. v. Soil water content and method of nitrogen application. Soil Sci. Soc. Am.J. 40: 537-541.

Fenn, L.B. and Kissel, D.E. (1975) Ammonia volatilization from surface applications of ammonium compounds on calcareous soils.iv. Effect of calcium carbonate content. Soil Sci. Soc. Am. Proc.39: 631-633.

Fenn, L.B. and Kissel, D.E. (1976) The influence of cation exchange capacity and depth of incorporation on ammonia volatilization from ammonium compounds applied to calcareous soils. Soil Sci. Soc. Am. J.40: 394-398.

Fewson, C.A. and Nicholas, D.J.D. (1961) Nitrate reductase from Pseudomanas aeroginosa. Biochem. Biophysics Acta 49: 355-349.

Fillery, I.R.P. (1979) Denitrification in soils under low oxygen or anaerobic environemnts. Diss. Abstr. Int. Diss. Abstr. Int. B 40: 1529.

Fillary, I.R.P. (1983) Biological denitrification. In, 'Gaseous loss of nitrogen fromplant-soil systems'. (eds. J.R. Freney and J.R. Simpson): 33-63.

Fillery, I.R.P. and De Datta, S.K. (1986) Ammonia volatilization from nitrogen sources applied to rice fields. i. Methodology, ammonia fluxes and nitrogen-15 loss. Soil Sci. Soc. Am. J. 50: 80-86.

Fillery, I.R.P., Roger, P.A. and De Datta, S.K. (1986) Ammonia volatilization from nitrogen sources applied to rice fields: ii. Floodwater properties and submerged photosynthetic biomass. Soil Sci. Soc. Am. J. 50: 86-91.

Firestone, M.K. (1982) Biological denitrification. In: Nitrogen in agricultural soils, (ed.) F.J Stevenson, ASA monograph no.22: 289-326.

Firestone, M.K., Smith, M.S., Firestone, R.B. and Tiedje, J.M. (1979) The influence of nitrate, nitrite and oxygen on the composition of gaseous products of denitrification in soil . Soil Sci. Soc. Am. J.43: 1140-1144.

Fliermans, C.B. and Schmidt, E.L. (1975) Autoradiography and immunoflorescence combined for autechological study of single cell activity with Nitrobacter as a model system Appl. Microbiol. 30:676-684.

Focht, D.D. (1974) The effect of temperature, pH and aeration onthe production of nitrous oxide and gaseous nitrogen-a zero order kinetic model. Soil Sci,118: 173-179.

Focht, D.D. (1978) Methods for analysis of denitrification in soils. In,' Nitrogen in the environment, ' (eds. D.R. Nielsen and J.G. MacDonald)2: 433-490.

Focht D.D. and Verstraete, W. (1977) Biochemical ecology of nitrification and denitrification. Advances in Microbiolo. Ecology 1: 135-214.

Ford, G.W. and Greenland, C.J. (1968) The dynamics of partly humidified organic matter in some arable soils. Trans. 9th. Int. Congr. Soil Sci.2: 403-410.

Forest, W.W. and Walker, D.J. (1971) The generation and utilization of energy during growth. Adv. Microb. Physiol. 5: 213-274.

Frankenberger (Jr.), W.T. and Johanson, J.B. (1982) Effect of pH on enzyme stability in soils. Soil Biol. Biochem. 14: 433-437.

Fredrickson, J.K., Fkoehler, E. and Cheng, H.H. (1982) Availability of N-15 labelled nitrogen in fertilizer and wheat straw to wheat in tilled and non till soil. Soil Sci. Soc. Am. J. 46: 1218-1222.

Freney, J.R. (1964) An evaluation of occurring fixed ammonium in soils. J. Agri. Sci.63:: 297-303.

Freney, J.R. Denmead, O.T. and Simpson, J.R. (1979) Nitrous oxide emission from soils at low moisture content, Soil Biol. Biochem. 11: 167-173.

Freney, J.R., Denmead, O.T., Watanabe, I. and Craswell, E.T. (1981) Ammonia and nitrous oxide losses following applicationsof ammonium sulphate to flooded rice. Aust. J. Agri. Res.32: 37-45.

Freney, J.R. and Simpson, J.R. (1983) Gaseous loss of nitrogenfrom plant-soil systems (eds.) Martinus Nijhoff/Dr W. Junk publishers, Netherland.

Freney, J.R. Simpson, J.R. and Denmead, O.T. (1983) Volatilization of ammonia. In; ' Gaseous loss of nitrogen from plant-soil systems' (eds. J.R.Freney and J.R. Simpson): 1-32.

Freney, J.R., Simpson, J.R., Denmead, O.T., Murihead, W.A. and Leuning, R.(1985) Transformations and transfers of nitrogen after irrigating a cracking clay soil with a urea solution. Aust. J. Agric. Res., 36: 685-694.

Galstyan, A Sh. (1958) Soil enzymes Sorbshch. Lab. Agrokhim., Akad. Nauk Arm 5SR2:. 19-25

Gamble, T. N., Betlach, M.R. and Tiedje, J.M. (1977) Numerically dominant denitrifying bacteria from world soils. Appl. Environ. Microbiol.33: 926-939.

Gasser, J.K.R. (1964) Urea as a fertilizer. Soils Fert.27: 175-180.

George, U.S. and Antoine, A.D. (1982) Denitrification potentialof a salt marsh soil: effect of temperature, pH and substrate concentration. Soil Biol. Biochem.14: 117-125.

Geronimo, J., Smith, Jr. L.L. and Stockdale, G.D. (1973) Effect of site of exposure to nitrapyrin and 6-chloropicolonic acid on growth of cotton and wheat seedlings. Agron. J. 65; 692-693.

Geronimo, J., Smith, Jr. L.L., Stockdale, G.D. and Goring, C.A.I. (1973) Comparative phyto-toxicity of nitrapyrin and its principal metabolite 6-chloropicolonic acid. Agron. J. 65

Gilliam, J.W., Dasberg, S., Lund, L.J. and Focht, D.D. (1978) Denitrification in four California soils: Effect of soil profile characteristics. Soil Sci. Soc. Am. J.61-66.

Gilliam, J. W. and Gambrell, R.P. (1978) Temperature and pH as limiting factors in loss of nitrate from saturated Atlantic Coastal Plain soils. J. Envion. Qual. &: 524-532.

Gilmour J.T. (1984) The effects of soil properties on nitrification and nitrification inhibition. Soil Sci. SOc. Am. J.48: 1262-1266.

Golden, D.C., Sivasubramaniam, S., Sivapalan, S. and Wijedasa, M.A. (1981) Inhibitor effects of commercial pottasium chlorideon the nitrification rates of added ammoniumsulphate in an acidred yellow podsolic soil. Plant and Soil.59: 147-151.

Goh, K.M. and Haynes, R.J. (1986) Nitrogen and Agronomy practice. In, Mineral nitrogen in plant-soil system, (ed. R.J. Haynes), pp. 379-468.

Gomez, K.A. and Gomez A.A. (1984) Statistical Procedures for agricultural research. International Rice Research Institute, John Wiley & Sons, New York, USA.

Goodroad, L.L. and Keeney, D.R. (1984) Nitrous oxide production in aerobic soils under varying pH, temperature and water content. Soil Biol. Biochem.16 (1): 39-43.

Goring, C.A.I. (1962a) Control of nitrification by 2-Chloro-6-(trichloromethyl) pyridine. Soil Sci.93: 431-439.

Goring, C.A.I. (1962b) Control of nitrification of ammonium fertilizers and urea by 2 Chloro-6-(trichloromethyl pyridine. Soil Sci.93: 211-218.

Goring, C.A.I. (1972) Agricultural chemicals in the environment: A quantitative viewpoint p.783-863. In Goring C.A.I. and Hammaker, J.W. (eds.) Organic chemicals in the soil environment, vol. 2 Marcel Deckker, Inc., New York.

Goyal, S.S. and Haffaker, R.C. (1984) Nitrogen toxicity in plants. In,' Nitrogen in crop production' (ed. R.D. Hauck) ASA/CSSA/SSSA.: 97-112.

Gould, W.D., Hagedorn, C., McCready, R.G.C. (1986) Urea transformation and fertilizer efficiency in soil. Adv. Agronomy 40: 209-238.

Guthrie, T.F. and Bomke, A.A. (1981) Effect of low temperature and nitrification inhibitor on urea hydrolysis. Canadian J. Soil Sci.61: 529-532.

Hageman, R.H . (1980) Effect of form of nitrogen on plant growth. In: Nitrification Inhibitors -potential and limitations.(ed.)Meisinger: SSSA Special publication, 38.

Hageman, R.H. (1984) Ammonium versus nitrate nutrition of higher plants. In; Nitrogen in crop production, (ed. R.D. Hauck),ASA, CSSA, SSSA, publication, : 67-95.

Hanks, R.J., James, D.W. and Watts, D.W. (1983) Irrigation management and crop production as related to nitrate mobility. In,'Chemical mobility and reactivity in soil systems',SSSA Special publication number 11,: 141-152.

Hanson, R.G., Maedy, S.R. and Jenetes, C.E. (1987), Effect of anhydrous ammonia with and without nitrapyrin applied fall and spring on corn yield. Plant anal., 18(4): 387-403.

Harmsen, G. W. and van Schreven, D.A. (1955) Mineralization oforganic nitrogen in soil. Adv. Agron. Vol. 7,: 300-383.

Harper, L.A., Catchpoole, V.R., Davis, R. and Weier, K. (1983)Ammonia volatilization: Soil, plant and microclimate effects on diurnal and seasonal fluctuations. Agron. J.75: 212-218.

Hauck, R.D. (1972) Synthetic slow release fertilizers and fertilizer amendments. In,' Organic Chemicals in the Soil Environment' (eds.C.A.I. Goring and J.W.Hamaker) 2633-690.

Hauck, R.D., and Bremner, J.M. (1976) Reply to real and imaginary source of error in agronomic research involving N-15. Soil Sci. Soc. Am. J.42: 175-176.

Hauck, R.D. (1980) Mode of action of nitrification inhibitors.In,'Nitrification inhibitors-potentials and limitation' (ed.J.J. Meisinger)ASA/SSSA publ.: 19-32.

Hauck, R.D. (1983) Agronomic and technological approches to minimizing gaseous nitrogen losses from croplands. In, ' Gaseous losses of nitrogen from plant soil-systems'.285-311.

Hauck, R.D. (1984) Significance of N fertilizer microsite reactions in soils . In: Nitrogen in crop production (ed.) R.D.Hauck, ASA-CSSA, SSSA joint publ.: 507-519.

Hauck, R.D. (1986) Field measurment of denitrification-An Overview . In: Field measurment of dinitrogen fixation and denitrification(ed.) R.D. Hauck and R.W. Weaver: SSSA Special Publi. No. 18.

Haynes, R.J. (1986a) Origin, distribution and cycling of nitrogen in terrestrial ecosystems. In, Mineral nitrogen in soil-plant system, (ed. R.J. Haynes),pp 1-51.

Haynes, R.J. (1986b,) The decomposition process, mineralization,immobilization, humus formation and decomposition,. In, Mineral nitrogen in soil-plant system, (ed. R.J. Haynes),pp. 52-109.

Haynes, R.J. (1986c) Nitrification. In,"Mineral nitrogen in the plant-soil system'Academic press (Lon): 127-157.

Haynes, R.J. and Sherlock, R.R. (1986) Gaseous losses of nitrogen. In,' Mineral nitrogen in plant-soil systems' (ed. R.J. Haynes)242-302.

Hendrickson L.L. and Keeney, D.R. (1978) A bioassay to determine the effect of organic matter and ph on the effectiveness of nitrapyrin (N-Serve) as a nitrification inhibitor. S.Biol. Biochem11: 51-55.

Hendrickson L.L., Keeney, D.R., Walsh, L.M. and Liegel, E.A. (1978a) Evaluation of nitrapyrin as a means of improving nitrogen efficiency in irrigated sands. Agro. J. 70: 699-704.

Hendrickson, L.L., Walsh, L.M. and Keeney, D.R. (1978b) Effectiveness of nitrapyrin in controlling nitrification of fall and spring applied anhydrous ammonia. Agron. J.70: 704-708.

Hendrickson, L.L. and Keeney, D. R. (1979a) Effect of some physical and chemical factors on the hydrolysis of nitrapyrin (N-serve) Soil Biol. Biochem.11: 47-50.

Hendrickson, L.L., and Keeney, D.R. (1979b) A bioassay to determine the effect of organic matter and pH on the effectiveness of nitrapyrin (N-Serve) Soil Biol. Biochem. 11:51-55.

Hendrickson, L.L., Omholt, T.E. and O'Connor, M.J. (1987) Effect of Phenylphosphorodiamidate on immobilization and ammonia volatilization. Soil Sci. Soc. Am. J.51: 1067-1071.

Henninger, N.M. and Bollag, J.M. (1976) Effect of chemical inhibitors used as nitrification inhibitors on the denitrification process. Can. J. Microbiol. 22: 668-672.

Henster R.F. and Attoe, O.J. (1970) Rural sources of nitrate inwater. In: Nitrate and water supply: Sources and control (ed.) V.L. Snocyink: Proc. of 12th. Sanitary Engr. Conf., Urbana.

Hewitt, E.J. (1970) Physiological and biochemical factors which control the assimilation of inorganic N supplies by plants . In Nitrogen nutrition of plants (ed. E.A. Kirby) 68-82.

Hocking P.J., Steer, B.T. and Pearson, C.J. (1984) Nitrogen nutrition of non-leguminous crops; A review, part 1. Field Crop Abstracts, Aug.37 (80)

Hoffmann G. (1963) Urease: In 'Methods of enzymatic analysis' (ed. H.V. Bergmeyer) Academic, N.Y. 913-916.

Hooper, A.B. and Terry, K.R. (1977) Hydroxylamine oxidoreductase from Nitrosomanas; inactivation by hydrogen peroxide. Biochemistry 16: 455-459.

Hoyt, G.D., McLean, E.O., Reddy, G.Y. and Logan, T.J. (1977) Effects of soil cover crop, and nutrient source on movement of soil, water and nitrogen under simulated rain-slope conditions.J. Environ. Qual.6: 285-290.

Huber, D. M., Warren, D.W., Nelson, D.W. and Tsai, C.Y. (1977) Nitrification Inhibitors-New tolls for food production. Bio Science 27: 523-529

Huett, D.O. and Menary, R.C. (1980) Effect of Aluminium on growth and nutrient uptake of cabbage, lettuce and Kikku grass in nutrient solution. Aust. J. Agri. Res. 31: 749-761.

Hutchinson, G.L., Millington, R.J. and Peters, D.B. (1972) Atmospheric ammonia: absorption by plant leaves. Science175: 771-772.

Isirimah, N. O. and Keeney, D.R. (1973) Nitrogen transformations in aerobic and waterlogged Histosols. Soil Sci.Vol 115:123-129.

Ivarson, K.C. (1977) Changes in decomposition rate, microbial population and carbohydrate content of an acid peat bog after liming and reclamation. Can. J. Soil Sci. 57: 129-137.

Jackson, W.A., Rasmussen, L.E., Hause, A.W. and White, A.W. (1973) Nitrate in surface and subsurface flow from a small agricultural watershed. J. Environ. Qual.2: 480-482.

Jansson, S.L., Hallam, M.J. and Batholomew, W.V. (1955) Preferential utilization of ammonium over nitrate by microorganisms in the decomposition of oat straw. Plant Soil6:382-390.

Jansson, S.L. (1958) Tracer studies on nitrogen transformationin soil with special attention to mineralization-immobilizationrelationships,. In; Analas of Royal Agri. Col. of Sweden, 24;: 101-361.

Jansson, S.L. (1963) Soil Sci., 95: 31-37.

Jansson, S.L. and Persson, J. (1982) Mineralizationand immobilization of soil nitrogen,. In, Nitrogen in agricultural soils, (ed. F.J. Stevenson),A.S. A. Agronomy monograph 22,: pp. 229-252.

Jarvis, S.C. (1984) The responses of two genotypes of white clover to addition of lime to an acid permanent grassland soil. J.Sci. Food Agricul. 35: 1149-1158.

Jenkinson, D.S., Fox, R.H. and Rayner, J.H. (1985) Interactions between fertilizer nitrogen and soil nitrogen-the so called 'priming effect'. J.Soil Sci. 36;: 425-444.

Jones, R.H. (1973) The effect of excess manganese on nitrate assimilation in lettuce. Ph.D. thesis. University of Tasmania, Hobart, Australia.

Jensen, H.E. (1973) Pottasium-calcium exchange on a montmorillonite and a kaolinte clay, ii. Application of double layer theory, . Agrochimia,17:: 191-201. 206. Johnsson, H., Bergstrom, L. and Jansson, P. (1987) Simulated nitrogen dynamics and losses in a layered agricultural soil. Agriculture, Ecosystems and Environment,18: 333-356.

Kallilo, H., Linko, R.R., Tikanmaki, E. and Puntari, I. (1980) Effect of nitrapyrin on nitrapyrin residues and nitrate content in red beet roots fertilized with urea. J. Sci.Fd. Agric.31: 701-708.

Kallilo, H., and Sandholm, J. (1982) Distribution of nitrapyrin[2-Choloro-6-(trichloromethyl)-pyridine] and 2 (Chloromethyl)-pyridine in red beet treated with nitrapyrin. J.Sci. Fd. Agric.33: 451-455.

Kapusta, G. and Varsa, E.C. (1972) Nitrification inhibitors do they work? Down to Earth 28: 21-33.

Keeney, D.R., Fillery, I. R.P. and Monx, G.P. (1979) Effect of temperature on the gaseous nitrogen products of denitrification in a silt loam soil. Soil Sci. Soc. Am. J.43: 1124-1128.

Keeney, D.R. (1980) Factors affecting the persistence and bioactivity of nitrificantion inhibitors.: 33-46. In: (ed. J.J. Meisinger) Nitrification inhibitors-Potentials and Limitations, Special Publication, Soil Sci. Soc. Am. USA.

Keeney, D.R. (1982) Nitrogen management for maximum efficiency and minimum pollution.pp. 605-641. In, Nitrogen in agricultural soils' (ed.F.J. Stevenson), Agronomy monograph 22, ASA,

Keeney, D.R. and Nelson, D.W. (1982) Nitrogen-Inorganic forms. In (eds. Al. Page, R.H. Miller and D.R. Keeney) Methods of soil analysis, pp.643-698. Part 2, Agronomy monograph no. 9.-ASA-SSSA, Madison, USA.

Keeney, D.R. (1986a) Inhibition of nitrification in soils. In 'Nitrification' (ed. J. Prosser) Special publication of the Soc. for General Microbiology vol.20:99-115.

Keeney, D.R.(1986b) Critique of the acetylene blockage technique for field measurement of denitrification.(p103-115)In Field measurment of dinitrogen fixation and denitrification (eds. R. D. Hauck and R.W. Weaver) SSSA Special Publi.No. 18:

Khan, M.F.A. and Moore, A.W. (1968) Denitrification capacity of some Alberta soils,. Can. J. Soil Sci.48: 89-91.

Khanif, Y.M, Van Cleemput, O. and Baert, L. (1984) Evaluation ofthe Burns model for nitrate movement in wet sandy soils. J. Soil Sci.35: 511-518.

Killham, K. (1986) Heterotrophic nitrification. Special publication of the Society for General Microbiology 20, Department of Soil Science, Uni. of Aberdeen AB 9, 2UE, UK.: 117-126.

Kowalenko, C.G., and D.R. Cameron. (1978) Nitrogen transformation in soil plant systems in three years of field experiments using tracer and non-tracer methods on an ammonium fixing soil. Can. J. Soil Sci.58: 195-208.

Kowalenko, C.G. (1978) Organic nitrogen, phosphorous, and sulphur in soils,. In, Soil organic matter, (eds.) M.Schnitzer and S.U. Khan.95-136.

Krishnapillai, S. (1979) Inhibition of nitrification by waste tea. Plant and Soil 51: 563-569.

Kucey, R.M.N. (1986) Effect of fertilizer form, method and timing of application on barley yield and N uptake under dryland conditions in southern Alberta. Can. J. Soil Sci.66: 615-621.

Kucey, R.M.N. (1987) Nitrogen fertilizer application practices for barley production under Southern Canadian Praidie conditions. Commun. in Soil Sci. Plant Anal.18(7): 753-769.

Kumar, V. and Wagenet, R.J. (1984) Urease activity and kinetics of urea transformation in soils. Soil Sci.137: 263-269.

Ladd, J.N. and Jackson, R.B. (1982) Biochemistry of Ammonification. In: Nitrogen in agricultural soils, (ed.) F.J Stevenson,ASA monograph no. 22: 173-228.

Ladd, J.N. and Paul, E.A. (1973,) Changes in enzymic activity and distribution of acid soluble amino acids nitrogen in soil during nitrogen immobilization and mineralization,. Soil Biol. Biochem5: 825-840.

Ladd, J.N., Oades, J.M. and Amato, M. (1981) Microbial biomass formed from C14 and N15 labelled plant material decomposing in soils in the field,. Soil Biol. Biochem.12: 185-189.

Ladd, J.N. and Amato, M. (1980) Studies of nitrogen immobilization and mineralization in calcareous soils. iv. Changes in the organic nitrogen of light and heavy subfractionsof silt and fine clay-size particles during nitrogen turnover. Soil Biol. Biochem. 12: 185-189.

Laidler, K.J. and Hoare, J.P. (1949) The molecular kinetics of urea-urease system. 1. The kinetics laws. J. Am. Chemical Soc.71: 2699-2702.

Lamb, J.A., Peterson, G.A. and Fenster, C.R. (1985) Wheat-fallow tillage systems effect on a newly cultivated grassland soil's nitrogen budget. Soil Sci. Soc. Am. J.49: 352-356.

Lance, J.C., Rice, R.C. and Whisler, F.D. (1978) Effects of vegetation on denitrification and phosphate movement during rapid infiltration of soil columns. J. Water Pollu. Control Fed.50: 2183-2188.

Landua, D.P. (1976) The effectiveness and persistence of a nitrification inhibitor, 2 Chloro-6 (tricholoromethyl) pyridine, in soils. Ph.D. Dissertation. Texas A&M Univ.

Laskowski, D.A. (1972) Influence of the environ. on N serve stability . Abstr. Am. Soc. Agron.97.

Laskowski, D.A. O'Melia, F.C., Griffith, J.D. Regoli, A.J. Youngson, C.R. and Goring, C.A.I. (1975) Effect of 2-chloro-6(trichloromethyl) pyridine and its hydrolysis product 6-chloropicolonic acid on soil microorganisms. J. of Environ. Quality. 4(3)412-417.

Laskowski, D.A. and Bidlack, D. (1977) Nitrification recovery after inhibition by nitrapyrin. Down to Earth33: 12-17.

Latey, J., Hadas, J. A., Valoras, N. and Focht, D.D. (1980) Effect of preincubation treatments on ratio of N2)/N2 evolution. J. Env. Qual.9: 232-235.

Lees, H. and Quastel, J.H. (1946) Biochemistry of nitrification in soil 2. The site of sol nitrification. Biochem. J. 40: 815-823.

Lees, H. (1963) Inhibitiors of nitrification. In,' Metabolic inhibitors' (eds. R.H. Hochester and J.H. Quastel), Comprehensive Treatise.2: 615-629.

Legg, J.O. and Meisinger, J.J. (1982) Soil nitrogen budgets. In:Nitrogen in agricultural soils, (ed.) F.J Stevenson,ASA monograph no.22: 503-566.

Lehr, I.J. and Van Wesemael, J.C. (1961) The volatilization ofammonia from lime rich soils,. Landbouwkd. Tijdschr.73: 1156-1168.

Lensi, R., Masurier, S., Gourbiere, F. and Josserand, A. (1986) Rapid determination of the nitrificationpotential of an acid format soil and assesment of its variability. Soil Biol. Biochem.18 (2): 239-240.

Lewis, D.C. and Stefanson, R.C. (1975) Effect of N-serve on nitrogen transformations and wheat yields in some Australian soils. Soil Sci. 119: 273-279.

Linn D.M. and Doran, J.W. (1986) Carbon dioxide and nitrous oxide production as regulated by water-filled pore space in tilled and nontilled soils. (Nihal Jayawardene per. commun.)

Lodhi, M.A.K. and Killingbeck, K.T. (1980) Allelopathic inhibition of nitrification and nitrifying bacteria in a ponderosa pine (pinus ponderosa Dougl.) community. Am. J. of Bot. 67: 1423-1429.

Loveday, J. (1955) Reconnaissance soil map of Tasmania, Hobart.Sheet: Zone 7; South K55.

Loveday, J. (1974) Methods for analysis of irrigated soils. Technical communication no. 54 of the Commonwealth Bureau of Soils, Australia.

Lynd, J.Q., Rieck, C., Barnes, D., Murray, D. and Santelmann, P.W. (1967) Indicator plant aberrations at threshold soil herbicid levels. Agron J. 59, 194-196.

Mahendrappa, J.K., Smith, R.L. and Chirstianson, A.T. (1966) Nitrifying organisms affected by climatic region in western United States. Soil Sci. Soc. Am. Proc.30: 60-62.

Mahendrappa, M.K. and Smith, R.L. (1967) Some effects of moisture on denitrification in acid and alkaline soils,. Soil Sci. Soc. Am. Proc.31 : 212-221.

Mahendra Singh, Yadev D.S. and Vinod Kumar. (1984) Leaching and transformation of urea in dry and wet soils as affected by irrigation. Plant and Soil81: 411-420.

Malhi, S.S. and Nyborg, M. (1983a) Field study of the fate of fall-applied N-15 labelled fertilizers in three Alberta soils. Agron. J. 75: 71-74.

Malhi, S.S. and Nyborg, M. (1983b) Release of mineral N from soils: influence of inhibitors of nitrification. 15 (5) 581-585.

Malhi, S.S. and Nyborg, M. (1986) Increase in mineral N in soilsduring winter and loss of mineral N during early spring in North-central Alberta. Can. J.Soil Sci., 66: 397-409.

Malhi, S.S. and Nyborg, M. (1988a) Effect of ATC, N-Serve 24-E, and thiourea nitrification inhibitors on yield and N uptake of barley fertilized fall-applied N. Plant and soil 105: 223-229.

Malhi, S.S. and Nyborg, M. (1988b) Control of fertilizer nitrogen: Effect of inhibitors, banding and nesting. Plant and Soil 107; 245-250.

Martens, D.M. and Bremner, J.M. (1984) Urea hydrolysis in soils: Factors influencing the effectiveness of phenyl phosphorodiamidate as a retardant. Soil Biol. Biochem.16 (5): 515-519.

Martikainen, P.J. (1985) Nirification in forest soil of different pH as affected by urea, ammonium sulphate and potassium sulphate. Soil Biol. Biochem.17(3): 363-367.

Martin, A.E., Gilkers, R.J. and Skjemstad, J.O. (1970) Fixed ammonium in soils developed on some Queensland phyllites and its relation to weathering. Aust. J. Soil Res.8: 71-80.

Martin, A.E. and Cox, J.E. (1956) Nitrogen studies on black soils from the Darling Downs, Queensland. i. Seasonal variations in moisture and mineral nitrogen fractions. Aust. J. Agric. Res.7: 169-183.

Martin, J.P. and Chapman, H.D. (1951) Volatilization of ammonia from surface fertilized soils. Soil Sci. 71: 25-34.

Mashhady, A.S. (1983) Loss of nitrogen from ammoniacal fertilizers incubated under different soil and temperature conditions. Cpmmun. in Soil Sci. Olant Anal.14(9): 877-891.

Mateson, P.A. and Vitousek, P.M. (1981) Nitrogen mineralization potentials following clearcutting in the Hoosier natural forest, Indiana,. Forest Sci.27(4): 781-791.

Matin, A. (1978) Organic nutrition of chemolithtrophic bacteria. Annu. Rev. Microbiol., 3: 433-468.

Matzel, W., Lippold, H. and Heber, R. (1979) Nitrogen uptake and balance of the urea, urea with urease inhibitor, and ammonium nitrate applied to spring wheat at stem elongation growth stage. In Symp. Use of Isotopes and Radiation in research on soil-plant relationships67-82.

May, P.B. and Douglas, L.A. (1976) Assay for soil urease activity. Plant Soil45: 301-305.

Maynard, D.N., Barker, A.V., Minotti, P.L. and Peck, N.H. (1976) Nitrate accumulation in vegetables. Adv. Agron. 28: 71-118.

Maynard, P.A. and Baker, A.V. (1979) Regulation of nitrate accumulation in vegetables. Acta Hortic. 93: 153-161.

McCall, P.J. and Swann, R.L. (1978) Nitrapyrin volatility from soil. Down to Earth 34: 21-27.

McClung, G., Wolf, D.C. and Foss, J.E. (1982) Nitrification inhibition by Nitrapyrin and Etradiazole in soils amended with sewege sludge compost. Soil Sci. Soc. Am. J. 47: 75-80.

McClung, G. and Franklenberger, W.T. (1985) Soil Nitrogen transformation as affected by salinity . Soil Sci. 139 (5), 405-411.

McDowell, L.L. and McGregor, K.C. (1980) Nitrogen and phosporous losses in runoff from no-till soybeans. Trans. ASAE23: 643-648.

McDowell, L.L. and McGregor, K.C. (1984) Plant nutrient lossesin runoff from conservation tillage corn. Soil and Tillage Res. 4: 79-91.

McElhannon W.S., and Mills, H.A. (1981) Inhibition of denitrification by nitrapyrin with field-grown sweet Corn. J.Am. Soc. Hort. Sci.106 (5): 673-677.

McElhannon W.S., Mills, H.A. and Bush, P.B. (1984) Simazine and atrazine-suppression of denitrification. HortScience, 19: 218-219

McGarity, J.W. (1961) Denitrification studies on some South Australian soils. Plant and Soil14: 1-21.

McGarity, J.W. (1962) Effect of freezing of soil on denitrification. Nature196,: 1342-1343.

McGarity, J.W. and Myers, R.J.K. (1968) Denitrifying activity in solodized solonetz soils of eastern Austalia. Soil Sci. Soc. Am. Proc. 32: 812-814.

McHarness, D.D. and McCarty, P.L. (1973) Field study of nitrification with submerged filters,. Environ. Protection Agency Tech. Serv.EPA-R273-158.

McInns, K.J., Ferguson, R.B., Kissel, D.E. and Kanemasu, E.T. (1986) Field measurements of ammonia loss from surface application of urea solution to bare soil. Agron. J.78: 192-196.

McKell, C.M. and Whalley R.D.B. (1964) Compatibility of 2chloro-6-trichloromethyl pyridine with *Medicago sativa* inocultated with *Rhizobium melioti*. Agron. J. 56: 26-28.

Meisinger, J.J. (1980) Potentials and Limitations of Nitrification Inhibitors, SSSA Special Publi.

Mendham, N.J. and Russell, J. (1987) Management package for high yielding cereals. In Proc. of Aust. Barley Technical Sympo. 11-15th. 1987. Wagga Wagga, Australia, N.S.W. 159-163.

Mennering, J.V. and Fenster, C. R. (1983) What is conservation tillage ?J.Soil and Water Cons. 38141-143.

Merdith, F.I., Mills, H.A. and Dull, G.G. (1984) Nitrogen concentration and NH4/ NO3 ratio effects on ammino acid pattern and protein quality. HortScience19 (1): 88-90.

Meyer, M.W. (1973) Absorption and release of ammonia from and tothe atmosphere by plants. Ph.D. thesis Univ. of Mayland, College Park.

Meyer, R.D., Olson, R.A. and Rhoades, H.F. (1961) Ammonia losses from soils. Agron. J.66: 355-358.

Mikkelesen, R.L., Jarrell, W.M., Letey, J. and Whaley, S. (1986) Effect of terrazole on nitrogen transformation and movement in irrigated corn. Soil Sci.Soc.Am.J.50: 1471-1478.

Mills, H.A. and McElhannon, W.S. (1984) Terrazole suppression of denityrification. HortScience, 16: 54-55.

Mishustin, E. (1926) Zur Frage von der Nitrite-bidung durch meatrophe Bakterien. Ber. Bakterol.-Agron. Sat.42: 28.

Misra, C., Nielsen, D.R. and Biggar, J.W. (1974) Nitrogen transformation in soil during leaching. ii. Steady state nitrification and nitrate reduction. Soil Sci. Soc. Am. Proc.38: 294-299.

Moe, P.G. (1967) Nitrogen losses from urea as affected by altering soil urease activity. Soil Sci. Soc. Am. Proc.31: 380-382.

Monreal, C.M., McGill, W.B. and Nyborg, M. (1986) Spatial heterogenity of substrate effects on hydrolysis, immobilization and nitrification of urea N, Can. J. of Soil Sci. 66 (3): 499-511.

Morrill, L.G. and Dawson, J.E. (1967,) Patterns observed for theoxidation of ammonium to nitrate by soil organisms. Soil Sci. Soc. Am. Proc. vol 31: 757-760.

Morris, D.R., Boonkerd, N and Vasuvat, Y. (1980) Effects of N-serve on soybeans and soil nitrogen transformations. Plant and Soil 57: 31-39.

Mortland, M.M. (1966) Ammonia interaction with soil materials. In; (eds. M.H. McVickar, W.P. Matin, I.E. Miles, // H.H. Tucker) Agricultural anhydrous ammonia, Technology and use; Proc. Sympo.ASA & ASSS: 29-30.

Mortland, M.M. and Wolcott, A.R. (1965) Sorption of inorganic nitrogen compounds by soil materials. In; Soil Nitrogen (eds. W.V. Batholomew and F.E. Clark),ASA; Agronomy no.10: 150-197.

Mosier, A.R., Meyer, W.S. and Melhuish, F.M. (1986) Effect of irrigation method on the recovery of N-15 fertilizer in a slowly permeable clay soil cropped with maize. Aust. J. Soil Res.24: 1-10.

Mosier, A.R., Parton, W.J. and Hutchinson, G.L. (1983) Modellingnitrous oxide evolution from cropped and soils. In Environmental Biogeochemistry35: 229-241.

Mullison, W.R. and Norris, M.G. (1976) A review of the toxicological, residual and environmental effects of nitrapyrin and its metabolite 6-chloropicolonic acid.

Mulvaney, R.L. and Bremner, J.M. (1981) Control of urea transformations in soils. In; Soil Biochemistry (eds. Paul E.A.& J.N. Ladd)vol. 5: 153-196.

Murakami, T.,Owa, N. and Kumazawa, K. (1987) The effects of soilcondition and nitrogen form on N2) evolution by denitrification. Soil Sci. Pl. Nutri.33(1): 35-42.

Myers, M.G. and McGarity, J.W. (1968) Urease activity in profiles of five great soil groups from northern New South Wales. Plant Soil 28: 25-27.

Myers, R. J. K. (1974) Soil processes affecting nitrogenous fertilizers,. In; Fertilizers and the environment (ed. D.R.Leece), pp13-26.

Myres, R.J.K and McGarity, J.W. (1972) Denitrification in undisturbed cores from a solodized solenetz B horizon, . Plant and Soil37-81.

Myres, R.J.K., Campbell, C.A. and Weier, K.L. (1982) Quantitative relationship between net nitrogen mineralization and soil moisture content in soils. Can. J. Soil Sci. 62: 111-124.

Nannipieri, P., Ceccanti, B., Cervelli, S. and Sequi, P. (1978) Stability and kinetic properties of humus-rease complexes. Soil Biol. Biochem. 10: 143-147.

Nelson, D.W. and Huber, D,M, (1980) Performance of nitrification inhibitors in the Midwest (east), In Meisinger, J.J. (ed.), Nitrification inhibitors-Potentials and limitation, Publi. Number 38, 75-88, Madison Wisconsin 53711. Am Doc. of Agron. and Aoil Sci. Soc. of Am.

Nelson, D.W. (1982) Gasous losses of nitrogen other than through denitrification. In,' Nitrogen in Agricultural Soils' Agronomy MonographNo. 22: 327-363.

Neilson, K.F., Warder, F.G., and Hinman, W.C. (1967) Effect of chemical inhibition of nitrification on phosporus absorption by wheat. Can. J.Soil Sci. 47: 65-71.

Neilsen, G.H. and MacKenzie, A.F. (1977) Soluble and sediment nitrogen losses as related to land use and type of soil in Eastern Canada. J. Environ. Qual. 6: 318-321.

Nielsen, D.R., and Biggar, J.W. (1981) Implications of the vadose zone to water resources management. In, ' National Research Council, Geophysics Study Commission'. National academy of Sci. Washington, D.C.

Nielsen, D.R., Wierenga, P.J. and Biggar, J.W. (1983) Spatial soil variability and mass transfers from agricultural soils. In,'Chemical mobility and reactivity in soil systems',SSSA Special publication number 11: 65-78.

Nommik, H. (1956)Investigation on denitrification in soil. Acta. Agri. Scand.6: 195-226.

Nommik, H. (1965a) Ammonium fixation and other reactions involving a nonenzymatic immobilization of mineral N in soil,. In; Soil nitrogen (ed. W.V.Batholomew and F.E.Clak) Agronomy 10: 198-258.

Nommik, H. (1965b) Fixation of ammonia by organic fraction of the soil. Acta. Agri.Scand.13: 371-390.

Nommik, H. and Nilsson, K.O. (1963) Fixation of ammonia by the organic fraction of the soil. Acta. Agric. Scand. 13: 205-219.

Nommik, H. and Vahatras, K.(1982) Retention and fixation of ammonium and ammonia in soils. In, 'Nitrogen in agricultural soils' (ed. F.J.Stevenson) ASA monograph no 22,: 123-171.

Nor, Y.M. (1982) Soil urease activity and kinetics. Soil Biolo. Biochem. 14: 63-65.

Northcote, K.H. (1979) Factual key for the recognition of Australian soils,. 2nd. editionRellim. Adelaide.

Nyborg, M. and Hoyt, P.B. (1978) Effects of soil acidity and liming on mineralization of soil nitrogen. Can J. Soil Sci.Vol.58: 331-338.

O'Hara, G.W. and Daniel, R.M. (1985) Rhizobial denitrification: A review. Soil Biol. Biochem17(1): 1-9.

O'Toole, P. and Morgan, M.A. (1984) Thermal stabilities of urease enzymes in some irish soils. Soil Biol. Biochem. 16 (5): 471-474.

O'Toole, P., McGarry, S. J. and Morgan, M.A. (1985) Ammonia volatilization from urea-treated pasture and tillage soils: effects of soil properties. J. Soil SCi.36: 613-620.

Oades, J.M. and Ladd, J.N. (1977) Biochemical properties: carbon and nitrogen metabolism. In ' Soil factors in crop production in semi-arid environment'. (eds. J.S. Russell and E.L. Greacen)127-160.

Ogunkunle, A.O. and Beckett, P.H.T. (1988) The efficiency of pot trials, or trials on undisturbed soil cores, as predictors of crop behaviour in the field. Plant soil 107: 85-93.

Okereke, G.U. and Meints, V.W. (1985) Immediate immobilization of labeled ammonium sulfate and urea nitrogen in soils. Soil Sci.140 (2): 105-109.

Olson, R.A. and Kurtz, L.T. (1982) Crop nitrogen requirements, utilization and fertilization. In: Nitrogen in agricultural soils, (ed.) F.J Stevenson,ASA monograph no. 22.

Olson, R.V. and Reiners, W.A. (1983) Nitrification in subalpine balsam fir soils: tests for inhibitory factors. Soil Biol. Biochem. 15: 413-418.

Olson, R.V. (1987) Effects of field fertilizer practices on labeled ammonium-nitrogen transformations and its urtilization by winter wheat.

Oluobi, A., Hedlin, R.A. and Cho, C.M. (1986) Crop utilization and soil retention of nitrogen from N15 -labelled urea, calcium nitrate, and ammonium sulphate in several Manitoba soils. Can. J. Soil Sci.6:661-671.

Onikura, Y., Yoshino, T. and Maeda, K. (1975) J. Sci. Soil Manure (Jpn) . 46255-259.

Onken, A.B. (1980) Performances of nitrificaton inhibitors in the Southwest. In,'Nitrification inhibitors-Potential and limitations' (ed. J.J. Meisinger) ASA/SSSA: 119-129.

Osborne, C.J. (1977) Chemical fractionation of soil nitrogen in six soils from southern New South Wales. Aust. J. Soil Res.15,: 159-165.

Osborn, C.J. (1977b) Some effects of the nitrification inhibitor 2-chloro-6-trichloromethyl -pyridine on the use of fertilier nitrogen and the growth of two wheat varieties. Austr. J. Exp. Agri. and Animal Husb. 17: 645-651.

Overrein, L.N. and Moe, P.G. (1967) Factors affecting urea hydrolysis and ammonia volatilization in soil. Soil Sci. Soc. Am. Proc.31: 57-61.

Owens, L.B. (1981) Effects of nitrapyrin movement in soil columns. J. Environ, Qual.10: 308-310.

P age, A.L., Miller, R.H. and Keeney, D.R. (1982) Methods of soil analysis, Part 2-Chemical and Microbiological properties. Second edition. ASA, SSA publication Number 9, Madison, Wisconsin, USA.

Painter, H.A. (1977) Microbial transformation of organic nitrogen. Prog. Water Technolo.8(4/5): 3-29.

Pang, P.C., Cho, C.M. and Hedlin R.A. (1975) Effects of pH and nitrifier population on nitrification of band applied and homogenously mixed urea nitrogen in soils. Can. J. Soil Sci. 55: 15-21.

Papendick, R.J. and Cook, R.J. (1974) Plant water stress and the development of Fusarium foot rot in wheat subjected to different cultural practices. Phytopathology 64; 358-363.

Patten, D.K., Bremner, J.M. and Blackmer, A.M. (1980) Effects of drying and air-dry storage of soil on their capacityfor denitrification of nitrate. Soil Sci. Soc. Am. J.44: 67-70.

Payne, W.J. (1981) Denitrification. John Wiley and Sons, New York.

Pearson, R.W. and Adams, F. (1967) Soil acidity and liming. ASA monograph 12: American Society of Agronomy, Madison Wisconsin, USA.

Peck, A.J. (1984) Recent advancesin soil salinity research. Aust. Soc. Soil Sci. Conf. Proc. Bisbane.

Pedrazzini, F.R., and Tarsitano, R. (1986) Ammonia volatilization from flooded soil following urea application. Plant and Soil 49: 101-107.

Pedrazzini, F.R., Tarsitano, R. and Nannipieri, P. (1987) The effect of phenyl phosphorodiamidate on urease activity and ammonia volatilization in flooded rice. Biology and fertility of soils 3(3): 183-188.

Pel'tser, A.S. (1972) Kinetics of C-14 urea decomposition depending on soil moisture, addition of toxic chemicals, liming and soil pH. Sov. Soil Sci. 5: 571-576.

Peters, D.B. (1965) Water availability. In C.A. Black (ed.) Methods of Soil Analysis. Part 1. 279-285. Agronomy Series Number 9, American Soc. of Agronomy, Madison, Wisconsin, USA.

Pettit, N.M., Smith, A.R.J., Freedman, R.B. and Burns, R.G. (1976) Soil urease: activity, stability and kinetic properties. Soil Biol. Biochem.8: 479-484.

Pinkerton, A. and Simpson, J.R. (1986) Responses of some crop plants to correction of subsoil acidity. Aust. J. Expt. Agricul. 26: 107-113.

Poth, M and Focht, D.D. (1985) N-15 kinetic analysis of N2O production by Nitrosomonas europea: an examination of nitrifier denitrification. Appl. Environ. Microbiol., 49: 1134-1141.

Poth, M. (1986)Dinitrogen production from nitrite by a Nitrosomonas isolates. Appl. Environ. Microbiol. 52 (4): 957-959.

Polglase, P.J., Attiwill, P.M. and Adams, M.A. (1986) Immobilization od soil nitrogen following wildfire in two eucalyp forests of south-eastern Australia. Acta Œcologica,Œcol. Plant., vol7 (21): 261-271.

Powell S.J. (1986) Laboratory studies of inhibition of nitrification. In Prosser, J.I. (ed.) Nitrification, p. 79-98, special publications of the soc. for general microbiol. Vol. 20, IRL press, Washington DC. USA.

Powell, S.J. and Prosser, J.I. (1986a) Effect of copper on inhibition by nitrapyrin of growth of nitrosomanas europea.Curr. Mikrobiol. 14177-179.

Powell, S.J. and Prosser, J. I. (1986b) Inhibition of ammonium pxidation by nitrapyrin in soil and liquid culture. Appl. and environ. microbiolo.vol. 52 (4): 782-787.

Powell, S.J. and Prosser, J.I. (1985) The effect of nitrapyrin and chloropicolonic acid on ammonium oxidation by Nitrosomanas europea. FEMS Microbiol. Lett. 28: 51-54.

Powlson, D.S. (1980) Effect of cultivation on the mineralizationof nitrogen in soil,. Comm, Plant & Soil,57: 151-153.

Prakasa Rao, E.V.S. and Rajendra Prasad. (1980) Nitrogen leaching losses from conventional and new nitrogeneous fertilizers in low-land rice culture. Comm. in Soil and Plant Anal.57

Prasad, R., Rajale, G.B. and Lakhdive, B.A. (1971) Nitrification retarders and slow-release nitrogen fertilizers. Adv. in Agron.23: 337-.

Prasad, R and Turkehead, B.B. (1971) Relative efficiency of nitrogen fertilizer for 'Ganga 101' maize (Zea mays L.) as influenced by rainfall. Indian J Agri. Sci. 41: 485-489.

Prasad, R. (1979) Increasing fertilizer nitrogen efficiency with slow release fertilizers and nitrification inhibitors. Fet. News 4: 25-32.

Prasad, R. (1982) The use of nitrification inhibitors and slow-release nitrogen fertilizers for manipulation of the growth and yield of rice.

Pratt, P.F. (1984) Nitrogen use and nitrate leaching in irrigated agriculture. In: Nitrogen in Crop Production (ed.) R.D.Hauck319-333.

Preez, C.C. du and Burger, R. du T. (1987) Laboratory measurments ofammonia volatilization from five nitrogen containing fertilizers after surface application at different rates on a neutral to alkaline soil.)S. Afr. Plant Soil. 4(1)17-20.

Prior, S.D. and Dalton, H. (1985) The effect of copper ions on membrane content and methane monooxygenase activity in methanol-grown cells of Methylococcus capsulatus (Bath.) J. Gen. Microbiol., 131: 155-163.

Prosser, J.I. (1986a) Nitrification, Special publication of the Soc. for general microbiology.

Prosser, J.I. (1986b) Experimental and theoretical models of nitrification. In, Nitrification (ed. J.I. Prosser) Special publication of the Soc. for general microbiology. 63-78

Quastel, J.H. (1965) Soil metabolism,. Ann. Rev. Plant Physiology 16: 217-240.

Rachhpal-Singh and Nye, P.H. (1984) The effect of soil pH and high urea concentrations on urease activity in soil. J. of soil Sci.35:519-527

Rachinsky, V.V. and Pel'tser, A.S.(1965) The kinetics of urea decomposition in soil. Dokl. TSKhA109: 75-78.

Rafii, Z.E., Evarard, T.O., Rockwell, J.C. and Balty, A. (1984) Nitrification inhibiton properties of etradiazol. In: Antimicrobials and Agriculture: The proc. of the 4th. International Symposium on Antibiotics in Agriculture, (ed.) M.Woodbine; 45-62.

Raison, R.J. and Mc Garity, J.W. (1978) Effect of plant ash onnitrogen fertilizer transformation and ammonia volatilization. Soil. Sci. Soc. Am. J.vol. 42 (1): 140-143.

Rajendra Prasad, Rajale, G.B and Lakhdive, B. (1971) Nitrification retarders and slow-release nitrogen fertilizers. Advs. in Agronomy 23; 337-383.

Rao, M.S.,. (1977) Adsorption of urea and urease activity in some Trinidad soils. Ph.D. thesis,Univ. of the West Indies,.

Rasmussen, R.A. and Kahlil, M.A.K. (1986) Atmospheric trace gases: trends and distribution over the last decade. Science 232: 1632-1624.

Raynolds, C.M., Wolf, D.C. and Armbruster, J.A. (1985) Factors related to urea hydrolysis in soils. Soil Sci. Soc. Am. J.49: 104-108.

Reddy, Prasad B.V., Dhanaraj, P.S. and Naryan Rao, V.V.S. (1984) Effects of insecticides on soil microorganisms. In,'Insecticide Microbiology' (ed. N.Y. Berlin)169-201.

Redeman, C.T. and Clark, H.W. (1967) Fate of 2-chloro-6-(trichloromethyl) pyridine in the rat. J. Agri. food chem.vol. 15 (6): 1127-1128.

Redemann, C.T., Meikle, R.W. and Widofsky, J.G. (1964) The loss of 2-chloro-6-(trimethyl)-pyridine from soil. IJ. Agricultural and food chem. vol. 12 (3): 207-209.

Reid, R. F. and Waring, S.A. (1979) Nitrogen transformations in a soil of the lower Burdekin, Queensland. i. Mineral nitrogen and redoc potential and nitrogen loss under rice. Aust. J. Exper. Agric. and Animal Husb.19: 732-738.

Rennie, D.A., Racz, G.J. and McBeath, D.K. (1976) Nitrogen losses. In,' Proceedings of Western Canada Nitrogn Symp. Calgary, Alberta, Canada,325-353.

Rennie, R.J. and Rennie, D.A. (1973) Standard isotope versus nitrogen balance criteria for assessing the efficiency of nitrogen sources for barley. Can. J. Soil Sci.53: 73-77.

Reuss, J. O., and Smith, R.L. (1965) Chemical reactions of nitrites in acid soils. Soil Sci. Soc. Am. Proc. 29: 267-270.

Reynold, C.M., and Wolf, D.C. (1987) Effect of soil moisture and air relative humidity on ammonia volatilization from surface-applied urea. Soil Sci.143 (2): 144-152.

Rieck, C.E. and Lynd, J.Q. (1967) Parameters of chlorinated pyridine toxicity to Robinia pseudoacacia. Agron. J. 59: 507-509.

Riggs, T.J., Hanson, P.R., Start, N.D., Miles, D.M., Morgan, C.L. and Ford, M.A. (1981) Comparison of spring barley varieties grown in England and Wales between 1880 and 1980. J. Agricul. Sci. Cambrid. 97: 599-610.

Robinson, J.B. (1963) Nitrification in a New Zealand grassland soil. Plant and Soil. XiX (2): 173-183.

Rodgers, G.A. (1984) Action of nitrification inhibitors. In: Antimicrobials and Agriculture (ed.) M. WoodbineThe proceedings of the 4th. International Symposium on Antibiotics in Agriculture: Benefits and Malefits: 33-43.

Rodgers, G.A. and Ashworth, J. (1982) Bacteriostatic action of nitrification inhibitors. Can. J. Microbiolo. 28: 1093-1100.

Rodgers, G.A. (1984) Inhibition of soil urease activity by aminocresols. Plant and Soil 79: 155-158.

Rodgers, G.A., A. Penny, F.V. Widdowson and M.V.Hewitt. (1985) Effects of nitrification inhibitors on uptakes of mineralized nitrogen and on yields of winter cereals grown on sandy soil after ploughing lod grassland: J.Sci. food Agric. 36: 915-924.

Rodgers, G.A., A. Penny, F.V. Widdowson and M.V.Hewitt. (1987) Tests of nitrification and urease inihibitors, when applied with either solid or aqueous urea, on a light sandy soil. J.Agr. Sci. Camb.108: 109-117.

Rodgers, G.A. and Pruden, G. (1984) Field estimation of ammonia volatilization from N-15 labelled urea fertiliser. J. Sci. Food Agic.35: 1290-1293.

Rodrigues, G. (1954) Fixed ammonia in tropical soils,. J. Soil Sci.5: 264-274.

Rolston, E.E., Hoffman, D.L. and Toy, D.W. (1978) Field measurement of denitrification i. Fux of N2 and N2O. Soil Sci. Soc. Am. J.42: 863-869.

Rolston, D.E. (1986) Limitations of the Acetylene Blockage Technique for field measurement of denitrification. (pp. 93-102) In Field Measurement of dinitrogen fixation and denitrification (eds. R. D. Hauck and R.W. Weaver), SSSA special publication; no. 18. Soil Sci. Soc. of Am., Inc. Madison, WI., USA.

Rolston, D.E. and Cerevelli, S. (1980) Denitrification as affected by irrigation frequency and applied herbicides. Proc. and Report of Meeting on Agrochemical Residue-Biota Interactions FAO/IAEA, 1978.

Rose, C.W., Chichester, F.W., Williams, J.R. and Ritchie, J.T. (1982) A contribution to simplified models of field solute transport. J.Environ. Qual.2: 292-295.

Rosolem, C.A. and Machado, J.R. (1985) Efficiency of nitrogen fertilization in sweet sorghum. Commun. in soil Sci. Plant Anal.vol 16 (7): 693-706.

Russell J. Thorp, J. and Mendham, N.J. (1987) Liming and nutrition of intensively managed barley. In Proc. of Aust. Barley Technical Symposium 11-15th Oct. 1987 Wagga Wagga, Australia, N.S.W. 145-158.

Ryan, J., Curtin, D. and Safi, I. (1981) Ammonia volatilization from surface-applied wastewater sludge. J. water pollut. control fed.47: 386-393.

Ryden, J.C. and Rolston, D.E.,. (1983) The measurement of denitrification. In,' Gaseous loss of nitrogen from plant soil systems, (eds. J.R. Freney, J.R. Simpson)Martinus Nisjhoff (Hague): 91-132.

Saffigna, P.G., Cole, A.L., McMahon, G. and Prove, B. (1984a) Evaluation of the usefulness of in situ field soil cores measuring solute movement in a vertisol. In The properties & utilization of cracking clay soils (eds.) McGarity, J.W. Hoult,E.M., So H.B. ; Proceedings of symposium, Armidale, Aug. 1981 : 181-183.

Saffigna, P.G., Cole, A.L., Strong, W.M. and Waring, S.A. (1984b) The effect of stubble on N15 fertilizer nitrogen balance and immobilization in a vertisol during the summer fallow. In " The properties and utilization of cracking clay soils", (eds. J.W. Mc Garity, E.H. Hoult, and H.B. So, Proc. Symp. Armidale August 1981, Rev. Rur. Sci. 5

Safigna, P.G. (1976) Fertilizer nitrogen balance and transformations in Queensland wheat soils using N-15. M.Agri. Sci. Thesis, Uni of Queensland.

Sahrawat, K.L., (1980) On the criteria for comparing the ability of compounds for retardation of nitrification in soil. Plant and Soil 55: 47-19.

Sahrawat, K.L. (1981) Karanja (Pongamia glabra Vent.) as source of nitrification inhibitors. Ferti. News, March,29-33.

Sahrawat, K.L. (1984) Effects of temperature and moisture on urease activity in semi-arid tropical soils. Plant and Soil 78:401-408.

Sahrawat, K.L. and Keeney, D.R. (1985) Perspective for research on development of nitrification inhibitors. Comm. Soil Sci. and Pl. nutr.16(5): 517-525.

Saharawat, K.L., Keeney, D.R. and Adam, S.S. (1987) Ability of nitrapyrin, dicyandiamide and acetylene to retard nitrification in a mineral and an organic soil. Plant and Soil 101: 179-182.

Salvas, P.L. and Taylor, B.F. (1980) Blockage of methanogenesis in marine sediments by the nitrification inhibitor 2-chloro, 6 trichloromethyl) pyridine. Curr. Microbiolo. 4: 305-308.

Sander, G. and Paralange, J.Y. (1984) Water and air movement in soils: an application of brutsaert's and optimization techniques. Soil Sci. 138(3); 198-202.

Sarathchandra, S.V. (1978) Nitrification activities and the changes in the populations of nitrifying bacteria in soil perfused at two different H-ion concentrations. Plant Soilvol. 50: 99-111.

Savant, N.K., and De Datta, S.K. (1982) Nitrogen transformations in wetland rice soils,. Adv. in Agronomy 35: 241-302.

Savant, S.K. James, A.F. and McClellan, G.H. (1985) Effect of soil submergence on urea hydrolysis. Soil Sci.vol. 140 (2): 81-88.

Schimdt, E.L. (1954) Nitrate formation by a soil fungus. Science119: 187-189.

Schimdt, E.L. (1982) Nitrification in soil. In: Nitrogen in agricultural soils, (ed.) F.J Stevenson, ASA monograph no. 22: 252-288.

Schimel, D.S. and Parton, W.J. (1986) Microclimatic controls of nitrogen mineralization and nitrification in shortgrass steppe soils,. Plant and Soil93: 347-357.

Sen, H.S. and Bandyopadhyay, B.K. (1987) Volatilization loss of nitrogen from submerged saline soil. Soil Sci.143 (1): 34-39.

Shaviv, A., Hagin, J. and Neumann, P.M. (1987) Effects of a nitrification inhibitor on efficiency of nitrogen utilization by wheat and millet. Commun. in Soil Sci. Pl. Anal.18(8): 815-833.

Sherlock, R.R. and Goh, K.M. (1984) Dynamics of ammonia volatilization from simulated urine patches and aqueous urea applied to pasture. i. Field experiments,. Fertilizer Res.5: 181-195.

Silva, C.G. and Perera, A.M.A. (1971) A study of the urease activity in the rubber soils of Ceylon, Rubber Research Institute of Ceylon. RRIC Quat. J.vol. 47: 30-36.

Simpson, J.R., Freney, J.R., Murihead, W.A. and Leuning, R. (1985) Effects of Phenylphosphorodiamidate and Dicyandiamide on nitrogen loss from flooded rice. Soil Sci. Soc. Am J.vol 49 (6): 1426-1431.

Simpson, J.R., and Freney, J.R. (1974) The fate of fertilizer nitrogen under cropping systems,. In,'Fertilizers and the environment' (ed. D.R. Leece)Aust. Insti. Agric. Sci. (NSW branch), Australia.

Sims, J.L. and MacKown, C.T. (1987) Ratw of nitrapyrin and soil pH effects on nitrification of ammonium fertilizer and growth and composition of burley tobacco. Commun. in Soil Sci. Plant Anal.18(12): 1479-1500.

Sivapalan, K., Fernando, V. and Thenabadu, M.W. (1985) N-mineralization in polyphenol rich plant residues and their effect on nitrification of applied ammonium sulphate.Soil Biol. Biochem. 17547-551.

Skinner, F.A. and Walker, N. (1961) Growth of nitrosomans europea in batch and contineous culture. Arch. Mikrobiol.38: 339-347.

Slangen, J.H.G. and Kerkhoff, P. (1984) Nitrification inhibitors in agriculture and horticulture: A literature review. Fert. Reser.5 (1): 1-76.

Smith, C.J. and Chalk, P.M. (1980) Gaseous nitrogen evolution during nitrification of ammonia fertilizer and nitrite transformations in soils. Soil Sci. Soc. Am. J.44: 277-282.

Smith, J.H. and Peterson, J.R. (1982) Recycling of nitrogen through land application land application of agricultureal, food processing and municipl wastes. In, ' Nitrogen in agricultural soils'(ed. F.J. Stevenson)ASA monograph no 22.: 791-832.

Smith, K.A. (1984) Leaching losses of nitrogen from agricultural land. In: Summary of paper presented at the Agricultue group symposiumJ.Sci.Food Agric, 35: 855.

Smith, M.S. and Tiedje, J.M. (1979) The effect of roots on denitrification. Soil Sci. Soc. Am. J.43: 951-955.

Soil Sci. Soc. Am. (1983) Chemical mobility and reactivity in soil systems. SSSA Special Publication number 11.Madison, Wisconsin.

Sowden, F.J., Chen, Y. and Schnitzer, M. (1977) The nitrogen distribution in soils formed under widely differeing climatic conditions. Geochim. Cosmochim. Acta41: 1524-1526.

Stanford. G., and Legg, J.O. (1984) Nitrogen and yield potential, . In,' Nitrogen in crop production' (ed. R.D. Hauck)ASA/CSSA/SSSA: 263-272.

Stark, J.C. and Brown, B.D. (1987) Estimating nitrogen requirements for irrigated malting barley, Hordeum Vulgaris L. stem nitrate, sufficiency level. Commum in Soil Sci. Anal. vol. 18 (4): 433-444.

Starr, J.L. (1983) Assessing Nitrogen movement in the field. In,'Chemical mobility and reactivity in soil systems'SSSA special publication, numser 11,: 79-92.

Starr, J.L. and Parlange, J.V. (1975) Nonlinear denitrification kinetics with continuous flow in soil columns. Soil Sci. Soc. Am. Proc.39: 875-880.

Stefanson, R.C. (1973) Evolution patterns of nitrous oxide and nitrogen in sealed soil plant systems. Soil Biol. Biochem.5: 167-169.

Stevenson, F.J. (1982a) Organic forms of soil nitrogen,. In; Nitrogen in agricultural soils, (ed. F.J. Stevenson)Agronomy monograph no.22,: 67-122.

Stevenson, F.J. (1982b) Origin and distribution of nitrogen in soil. In ; Nitrogen in Agricultural Soils (ed) F.J. StevensonASA monograph no. 22: 1-39.

Strong, W. M. (1986) Effects of nitrogen application before sowing. compared with effects of split applications before and after sowing, for irrigated wheat on the Darling Downs. Aust. J.Exp. Agic.vol 26: 201-207.

Sumner, R.E. and Reeves, N.G. (1966) The effect of iron oxide impurities on the positive and negative adsorption of chloride by kaolintes. J. Soil Sci.17: 274-279.

Swaider, J.M. (1985) Pumpkin response to nitrogen regime and nitrapyrin in dryland and irrigated cultures. Commun. in Soil Sci. Plant Anal. 16(5):525-538.

Swezey, A.N.L. and Turner, G.O. (1962) Crop experiments on the effect of 2-Chloro-6-trichloromethyl pyridine for the control of nitrification of ammonium and urea fertilizer. Agron. J. 54:532-535.

Tabatabai, M.A. and Bremner, J.M. (1972) Assay of urease activity in soils. Soil Biol. Biochem.9: 9-13.

Temple-smith, M.G. (1985) Soil acidification. In (ed.) J.V. Lovett, Proceedings of Scool of Soil Management, May 14-17, 1985, Uni. of Tasmania, Hobart.

Terman, G. L., Parr, J.F. and Allen, S.E.(1968) Recovery of nitrogen by corn from solid fertilizers and solutions. J. Agricul. and Food Chem. 16: 685-690.

Terman, G.L. (1979) Volatilization losses of nitrogen as ammoniafrom surface applied fertilizers, organic amendments, and crop residues. Adv. Agronomy 31: 189-223.

Terry, R.E., Nelson, D.W., Sommers, L.E. and Meyer, G.J. (1978) Ammonia volatilization from wastewater sludge applied to soil. J. Water Pollut. Control Fed. 50: 2657-2665.

Thompson, J.P. (1984) Residue decomposition and nitrogen mineralization in a black earth under aerobic and waterlogged conditions. In'The properties and utilization of cracking clay soils', (eds. J.W.McGarity, E.H. Hoult, and H.B. So)Proc.Symp. Armidale, August 1981, Rev. Rur, Sci. 5.

Theng, B.K. (1979) Formation and properties of clay polymer complexes- Elsvier Scientific Publication Comp. N.Y.

Timmons, D.R. (1984) Nitrate leaching as influenced by water application level and nitrification inhibitors. J.Environ. Qual.13: 305-309.

Tomar, J.S. and MacKenzie, A.F. (1984) Effects of catechol andp-benzo-quinone on the hydrolysis of urea and energy barriers of urease activity in soil. Can. J. Soil Sci.64: 51-60.

Topp, E. and Germon, J. (1986) Acetylene metabolism and stimulation of denitrification in an agricultural soil. Applied and Environ. Microbiolo. vol. 52 (4): 802-806.

Touchton, J.T., Hoeft, R.G., Welch, L.F. and Agryilan, W.L. (1979) Loss of nitrapyrin as affected by pH and temperature. Agron. J. 71: 865-869.

Tucker, B.M. (1985) Laboratory Proceedure for Solution Salts and exchangeble Cations in Soils. Division of Soils Technical Paper No. 47 CSIRO- Australia.

Underhill, S.E. and Prosser, J.I. (1985) Inhibition of nitrification by pottasium ethyl xanthate in soil and in liquid culture. Soil Biol. Biochem.17: 229-233.

Underhill, S.E. and Prosser, J. (1987) Surface attachment of nitrifying bacteria and their inhibition by pottasium ethyl xanthate. Microbial Ecol.14: 129-139.

Unger, P.W. and McCalla, T.M. (1980) Conservation tillage systems. Adv. Agron.33: 1-58.

Valis, I., and Jones, R.J. (1973) Soil Biolo. Biochem., 5: 391-398.

Vallis, I. and Gardener, C. J. (1984) Short-term nitrogen balance in urine-treated areas of pasture on a yellow earth in the subhumid tropics of Queensland. Aust. J. Exp. Agic. Anim. Husb.,24: 522-528.

Vallis, I., Harper, L.A., Catchpoole, V.R., and Weier, K.L. (1982) Volatilization of ammonia from urine patches in a subtropical pasture. Aust. J.Agric. Res.,33: 97-107.

Van Cleemput, O' and Patrick, W.H. Jr. (1974) Nitrate and nitrite reduction in flooded gamma-irradiated soil under controlled pH and redox potential conditions. Soil Biol. Biochem. 6: 85-89.

Van Cleemput, O. and Baert, L. (1984) Nitrite, a key compound in loss processes under acid conditions. Plant and Soil 76: 233-241.

Van de Dijk, S.J. and Troelstra, S.R. (1980) Heterotrophic nitrification in a heath soil demonstrated by an in-situ method. Plant and Soil,57: 11-21.

Varsa, E.C. Liu, S.L. and Kapusta, G. (1981) Effects of nitrification inhibitor on wheat yields and soil nitrogen retention. Down to Earth 37: 1-5.

Verstrate, W. and Alexender, M. (1972) Heterotrophic nitrification by Arthrobacter sp. J. Bacteriology 110: 955-958.

Vlek, P.L.G. and Craswell, E.T. (1979) Effect of nitrogen source and management on ammonia volatilization losses from flooded rice-soil systems. Soil Sci. Soc. Am. J. 17: 43.

Vlek, P.L.G., and Carter, M.F. (1983) The effect of soil environment and fertilizer modification on the rate of urea hydrolysis. Soil Sci.136: 56-63.

Vlek, P.L.G., Stumpe, J.M. and Byrnes, B.H. (1980) Urease activity and inhibition in flooded soil systems. Fertilizer Res.1: 191-202.

Voss, R.D. (1984) Potential use of urease inhibitors, In 'Nitrogen in crop production ' (ed.) R.D. Hauck, ASA, CSSA, SSSA publication.; 571-578.

Wada, K., and Harward, M.E. (1974) Amorphous clay constituents of soils. Adv. Agron.26: 211-260.

Wainwright, M. and Pugh, C.J. F. (1973) Effect of three fungicides on nitrification and ammonium in soil. Soil Biol. Biochem.5: 577-584.

Waksman, S.A. (1932)Principals of soil microbiology. 2nd ed. Williams and Wilkins, Baltimore.

Walker, D.W. and Woodson, W.R. (1987) Nitrogen rate and cultivar effects on nitrogen and nitrate concentration pf sweetpotato leaf tissue. Commun. in Soil Sci. Plant Anal.18 (5): 529-541.

Waelsch, H. (1972) Certain aspects of intermediary metabolism of glutamine, asperagine and glutathione. Adv. Enzymol. 13: 237-239.

Walker, M.E., Keiseling, T.C., Merchant, W.H. and Morey, D.D. (1979) Urea-ammonium sulfate and urea-ammonium phosphate evaluation as nitrogen sources in the southeast United States. Soil Science Society of Am. J. 43: 606-610.

Walker, N. and Wickramasinghe, K.N. (1979) Nitrification and autotrophic nitrifying bacteria in acid tea soils. Soil Biol. Biochem 11: 231-236.

Walkey A. and Black, C.A. (1934), An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 34: 29-38. Extracted from Soil and Plant Analysis by C.S. Piper(1974) pp 223-227.

Walsh, L. M. and Murdock, J.T. (1963) Recovery of fixed ammonium by corn in greenhouse studies. Soil Sci. Soc. Am. Proc. 27: 200-204.

Waring, S.A. and Bremner, J.M. (1964) Ammonium production in soil under waterlogged conditions as an index of nitrogen availability. Nature (Lon.)201: 951-952.

Waring, S.A. and Saffigna, P.G. (1984) Biological processes affecting nutrient element availability and distribution in Australian cracking clays. In,' The properties and utilization of cracking clau soils'. Reviews in Rural Science 5, Uni. of New England Armidale (eds. J.W. McGarity, E.H. Hoult, and H.B. So)187-204.

Waring, S.A., and Gilliam, J.W. (1983) The effect of acidity on nitrate reduction reduction and denitrification in Lower Coastal Plain soils. Soil Sci. Soc. Am. J.vol. 47: 246-251.

Waring, S.A. (1963) A rapid procedure for the estimation of nitrogen availability in soils. Ph.D. Thesis, Univ. of Queensland

Watson, C.J. (1986) Preferential uptake of ammonium nitrogen from soil by rye grass under simulated spring conditions. J.agric. sci., Camb.107: 171-177.

Watson, S.W. (1971) Reisolation of Nitrosospira briensis, . Arch. Mikrobiol.75: 179.

Watson, S.W. (1974) Gram-negative chemolithotrophic bacteria. Family I. p. 450-456. In, R.E. Buchnan and N.E. Wilkins (eds.) Bergey's manual of determinative bacteriology, 8th. ed. The Williams and Wilkins Co., Baltimore.

Webster, C.P. and Dowdell, R.J. (1986) Effect of drought and irrigation on the fate of nitrogen applied to cut permanent grass in lysimeter: Nitrogen balance sheet and the effect of sward destrcution and ploughing on nitrogen mineralization. J.Sci. Agric. 37: 845-854.

Weier, K.L. and Gilliam, J.W. (1986) Effect of acidity on nitrogen mineralization and nitrification in Atlantic Coastal Plain soils. Soil Sci. Soc. Am. J. 50: 1210-1214.

Westermann, R.L. and Tucker, T.C. (1979) In situ transformation of nitrogen -15 labelled materials in Sonoran desert soils,. Soil Sci. Soc. Am. J.43: 95-100.

Wetselaar, R. and Hutton, J.T. (1972) Ionic composition of rainwater at Katherine, N.T. and its part in the cycling of plant nutrients. Aust. J. Agric. Res. 14: 319-302.

Wetselaar, R. (1974) Increasing the effiency of nitrogeneous fertilizers and minimizing losses to the environment. In,' Fertilizers and environment' (ed. D.R. Leece),Aust. Inst. Agric. Sci. (NSW branch), Australia.

Wetselaar, R. (1980) Nitrogen cycling in semi-arid region of tropical australia. In; Nitrogen cycling in west african ecosystems (ed. T Rosswall),157-169.

Wetselaar, R., and Ganry, F. (1982) Nitrogen balance in tropical agrosystems. Microbiology of Tropical soils, (eds. Y.R. Dommergues and H.G. Diem),1-35.

Wetselaar, R., Shaw, T., Firth, P., Oupathum, J. andThitspoca, M.(1977) Ammonia volatilization losses from variously placed ammonium sulphate under lowland rice field conditions in central Thailand. In,' Proc. Int. Seminar on Soil Environment and Fertility Management in Intensive Agriculture (SEFMIA)', Tokeyo, Japan, 1977: 282-288.

White, E.M. (1987) Straw and grain production in spring barley cultivars. J.Agri. Sci. Camb.108: 293-298.

Wickramasinghe, K.N. and Talibudeen, O. (1981) Denitrification in a very acid tropical soil. J. Soil Sci.32: 119-131.

Wickramasinghe, K.N., Rodgers, G.A. and Jenkinson, D.S. (1985)Nitrification in acid tea soils and a neutral grassland soil: effects of nitrification inhibitors and inorganic salts. Soil Biol. Biochem.17: 249-252.

Wiedenfeld, R.P. (1985) Nitrogen loss and plant responses of ficus benjamina to different fertilizer sources applied preplant. Hort Science 20 (4): 720-722.

Wijler, J. and Delwiche C.G. (1954) Investigations on the denitrifying process in soil . Plant and Soil 5: 155-169.

Wild, A. and Cameron, K.C. (1980) Soil nitrogen and nitrate leaching. In,' Soils and agriculture' (ed. P.B. Tinker)Soc. Chem Industry; Crit. Rep. Appl. Chem. Vol.2: 35-70.

Woldendorp, J.W. (1963) The influence of living plants on denitrification. Meded. Lane bouwhogesch. Wageningen63: 1-100

Wullstein, L.H. and Gillmour, C.M. (1969) Non enzymatic gaseous loss of nitrite from clay and soil systems. Soil Sci.97: 428-430.

Yadev, D.S., Vinod Kumar, Mahendra Singh and Relan, P.S. (1987) Effect of temperature and moisture on kinetics of urea hydrolysis and nitrification. Aust. J.Soil Res.25: 185-191.

Yadev, D.S., Viod Kumar and Mahendra Singh. (1986) Inhibition of Soil urease activity and nitrification with some metallic cations. Aust. J. Soil Res.24: 527-532.

Yamashita, K. (Quoted by Savant and De Datta, 1982) (1969) In, 'New Techniques in relation to Soil and Fertilizer'.84-100.

Yavinder-singh and Beuchamp, E.G. (1986) Nitrogen in,,, Soil Sci. Plant Anal. 17(12): 1369-1381.

Yeomans, J.C. and Bremner, J.M. (1985a) Denitrification in soil: Effects of Herbicides. Soil Biol. Biochem.17 (4): 447-452.

Yeomans, J.C. and Bremner, J.M. (1985b) Denitrification in soil:Effects of insecticides and fungicides. Soil Biol. Biochem.17 (4): 453-456.

Yeomans, J.C. and Bremner, J.M. (1987) Effects of Dalapon, Atrazine and Simazine on denitrification in soil. Soil Biol. Biochem.19(1): 31-34.

Yeomans, J.C. and Bremner, J.M. (1986) Effects of urease inhibitors on denitrification in soil. Commun. In Soil Sci. Plant Anal.17 (1): 63-73.

York, A.C and Tucker, M.R. (1977) Nitrification inhibitor evaluation in cotton ii. Inhibitor incorporation depth and N placement. Agron. J. 77: 407-411.

York, A.C. and Tucker, M.R. (1985) Nitrification inhibitor evaluation on cotton i. Rate and time of application. Agron. J. 77: 403-406.

Yoshino, T, and Dei, Y. (1974) Japan Agric. Res. Quart. 8137-141.

Young, J.L. and Aldag, R.W. (1982) Inorganic forms of nitrogen in soil. In: Nitrogen in agicultural soils, (ed.) F.J.Stevenson ASA monograph no. 22: 43-66.

Young, J.L. and Cattani, R.A. (1962) Mineral fixation of anhydrous NH3 by air-dry soils. Soil Sci. Soc.Am.Proc.26: 147-152.

Zantua, M.I., Dumenil, L.C. and Bremner, J.M. (1977) Relationships between soil urease activity and other soil properties. Soil Sci. Soc. Am. J. 41: 350-352.

Zantua, M.I. and Bremner, J.M. (1977) Stability of urease in soils. Soil Biol. Biochem.9: 135-140

Zadoks, J.C., Chang, T.T., Konzak, C.F. (1974) A decimal code for the growth stages of cereals. Weed Res. 14: 415-421.

LIST OF APPENDICES

Appendix 1: Selected bibiliography of studies of nitrapyrin and crop performances

(i).yield

rate of NI application % of added N	crop	nitrification inhibited or not	Field conditions denitr.	leaching	References.
Yield increased significantly					
3.3	corn	inhibited	-	+	Touchton et al.(1979).
2.0	corn	na	-	+	Prasad and Turkehead (1971).
5.0	corn	inhibited	-	+	Kapusta and Varsa (1972).
0.1-2.0	corn	na	-	+	Swezey and Turner (1962).
1.0	corn	inhibited	+	-	Hanson et al. (1987).
0.7-2.0	rice	na	-	-	Prasad, (1982).
5.0	sugar cane	inhibited	-	+	Prasad, (1979).
2.0	wheat	inhibited	-	+	Varsa et al. (1981).
Yield did not increased significantly					
0.6-1.2	corn	inhibited	-	-	Kapusta and Varsa, (1972).
0.8-3.3	corn	inhibited	-	-	Kapusta and Varsa (1972).
0.5	corn	inhibited	-	-	Taber and Peterson (1979).
1.0	corn	inhibited	-	-	Guthrie and Bomke (1981).
2.0	Barley	inhibited	-	+	Aydeniz et al. (1976).
5.0	wheat	not consistant	-	+	Rodgers et al. (1985).
2.0	wheat	inhibited	-	-	Olson (1987)

(ii) Phytotoxicity or reduced growth (mainly on seedlings) or reduced N use .efficiency.

NI level /kg soil	Crop	Reference/comments
12.5 mg	oats, tomatoes lettuce	Goring (1962b).
25mg	sugar beat wheat corn	
1mg	lucerne	MeKell and Whalley (1964).
	soy bean	Rieck and Lynd (1967)
5mg	rye grass clover	Osborn (1977b)
10mg	wheat	Osborn (1977b).
5ppm/soil sol.	Avecenea	Boto et al.(1985)-phtotoxicity and reduce N use.
10ppm(no added N)	Soybeans	Morris et al.,(1980)
1.1 & 2.2ppm	potatoes	Hendrickson et al.(1978a)-reduce yield and quality

n.a. not available

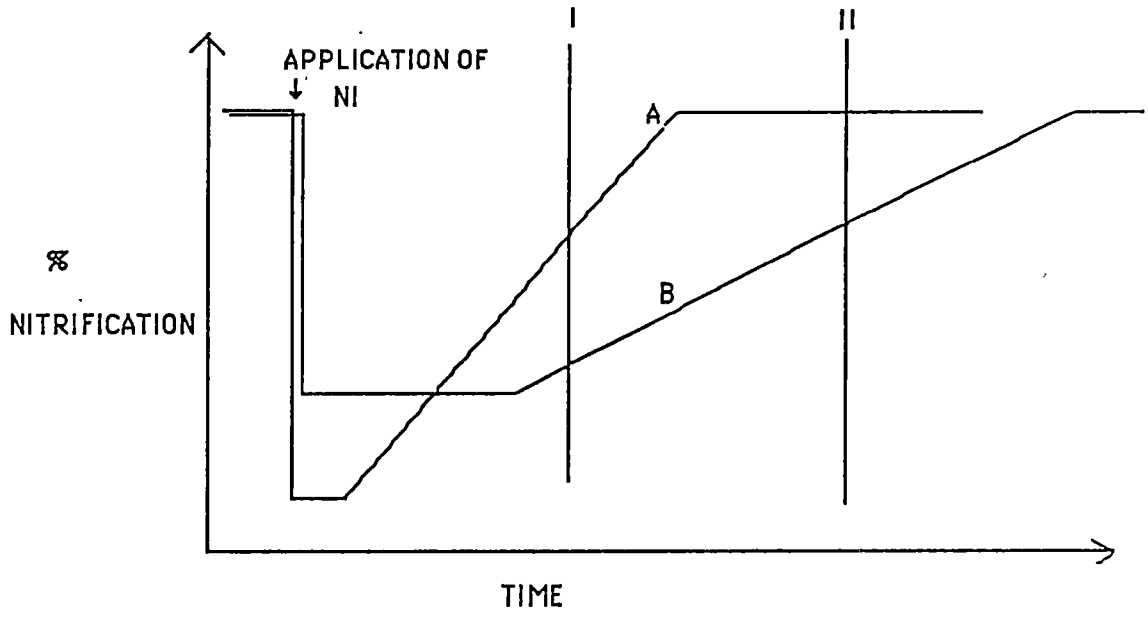
Appendix 2: Some properties of several commercial nitrification inhibitors.

Trade/common names	Chemical name	relative water solubility	volatility	comments/ (mfd. by)
N-serve Nitrapyrin	2-chloro 6 tri chloromethyl pyridine	low	moderate	first commercial inhibitor, Dow Chemical Co.USA.
DCD	C2H4N Dicyanadiamide	high	low	Chisso Corp, Japan & SKW, Trostberg
Etradiazole Terrazole D-Well	5 Ethoxy 3 trichloro methyl 1,2,4, tridiazole	high	moderate	Olin Matheson Chemical Co. now Uniroyal chemicals, USA
ATC	4 Amino 1,2,4, triazole HCL	high	low	
AM	2 Amino 4 Chloro 6-methylpyridine	low	moderate	Mitsui Toutsu Co Japan.
ST	Sulfathiazole			Mitsui Toatsu Co. Japan.

Appendix 3: Factors affecting the efficacy of nitrification inhibitors (NI) in soil.

Factor	Comments
primary factors:	associated with the properties of the compound
Specificity	Should inhibit the activity of ammonium oxidizers only
Relative mobility	Inhibitor leaching rate relative to ammonium-N
Persistance/volatility	Should remain in soil until plants can absorb N rapidly.
Water solubility	Influences the method of application,
Secondary factors:	associated with soil, climate, and management.
Soil factors	
pH	Affects persistance of some NI, but not nitrapyrin, influences the efficacy via the effect on nitrifier activity.
Adsorption	Sorption of NI on to soill colloids (especially organic matter) will affect persistance, may also influence by affecting nitrifier activity.
Moisture/aeration	Affects the mobility of NI, nitrifier activity may also change thus affecting NI efficacy.
Nitrifier population	The strain of active nitrifiers will respond differently to NI.
other factors:	
Temperature	Increase in temperature rapidly reduces the half-life of NI,and increases the activity of nitrifiers.
Rainfall	Influence via soil moisture/aeration status.
Form of fertilizer	Alkalinity and acidity produced during fertilizer transformation (eg. urea hydrolysis).
Mode of application	If broadcast possibility of increased volatilization loss, incorporation or injection may improve the efficacy.
Economy	Use depends on cost of NI compared to cost of additional N fertilizer necessary to increase crop production in similar proportions.

Appendix 4: Schematic diagram of nitrification inhibition at two different soil pH levels (A and B)

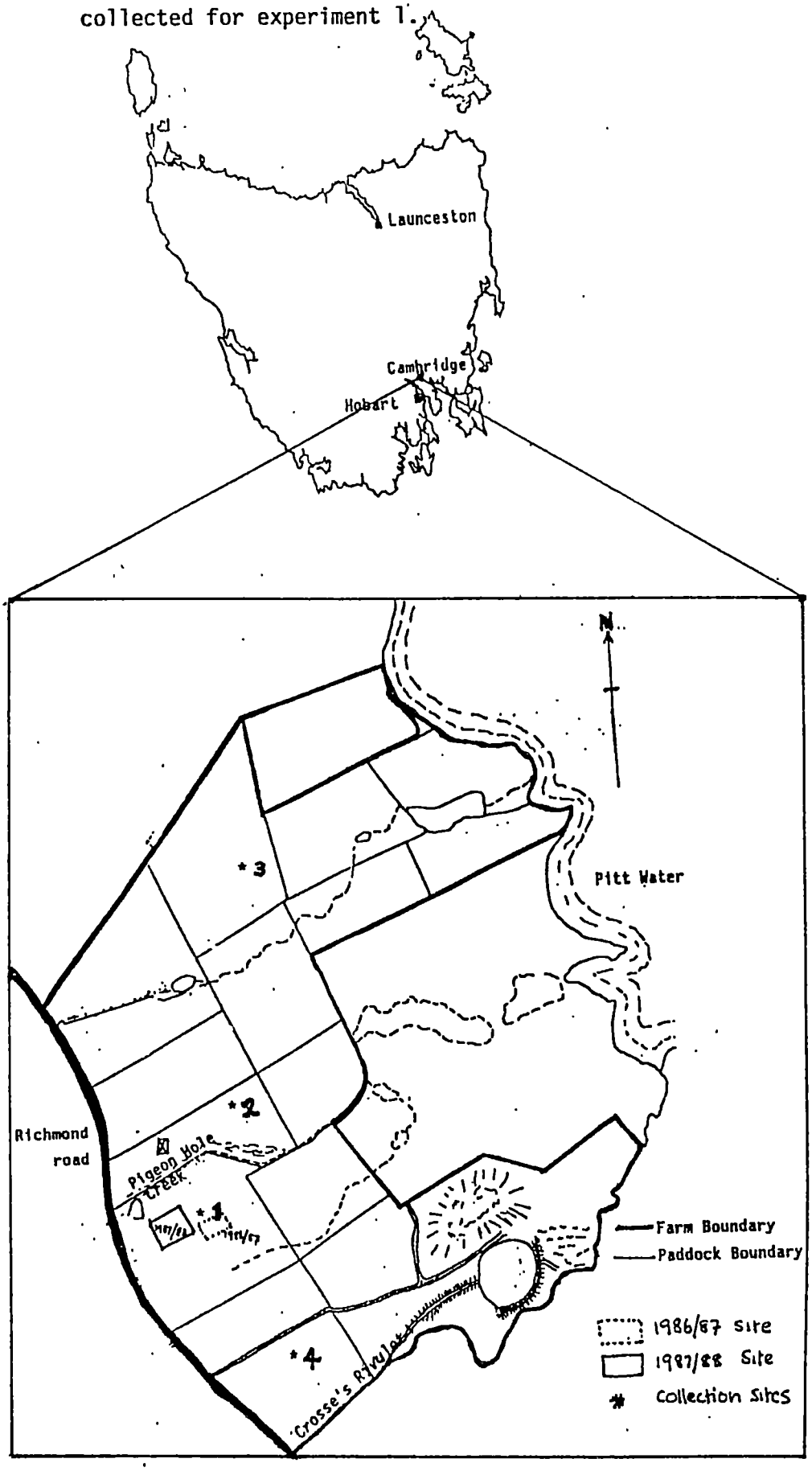


A- Soil with higher pH : supports a larger nitrifying population.
B- Soil with lower pH : supports a smaller nitrifying population.

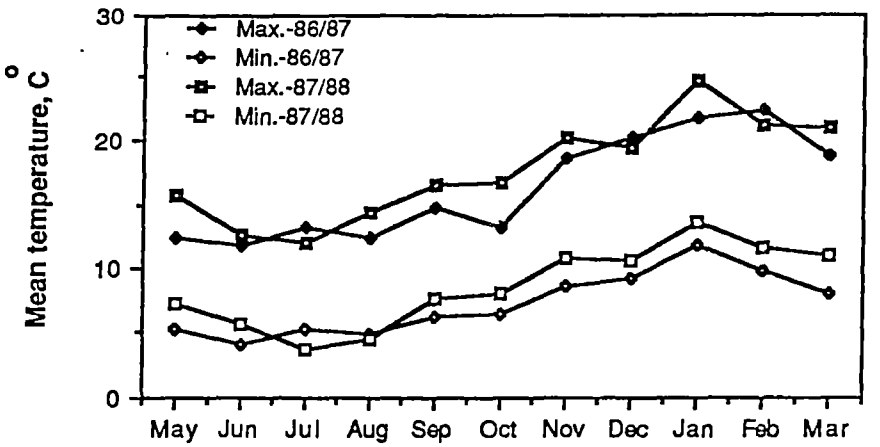
Note:
1. Greater decrease in % nitrification rate in A compared to B, immediately after nitrapyrin application because higher number of nitrifiers were inactivated per equal volume of soil. This means a higher initial inhibition of nitrification at higher pH.
2. At stage 1, nitrification in A is recovering quickly, because of more favourable pH conditions whereas nitrification in B is just begining to recover at a very slow rate.

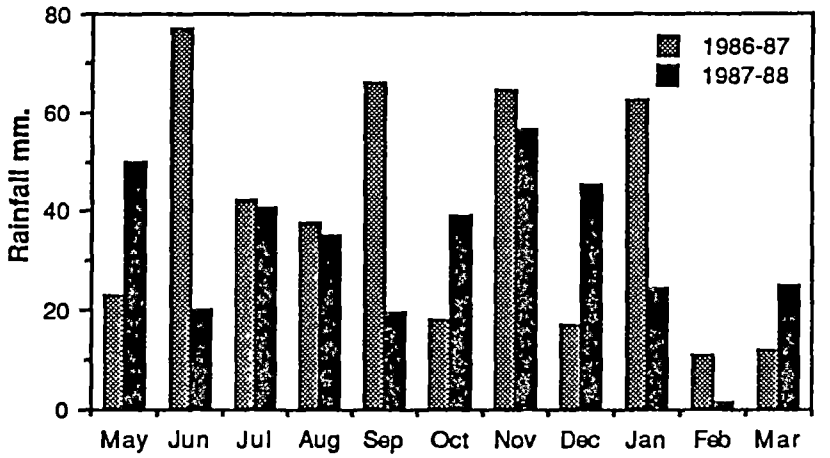
3.At stage 2, nitrification in A has recovered fully, but in soil B nitrification has still not reached the original level resulting in a longer period of delay in full recovery of nitrification.
(modified after Hendrickson and Keeney, 1979)

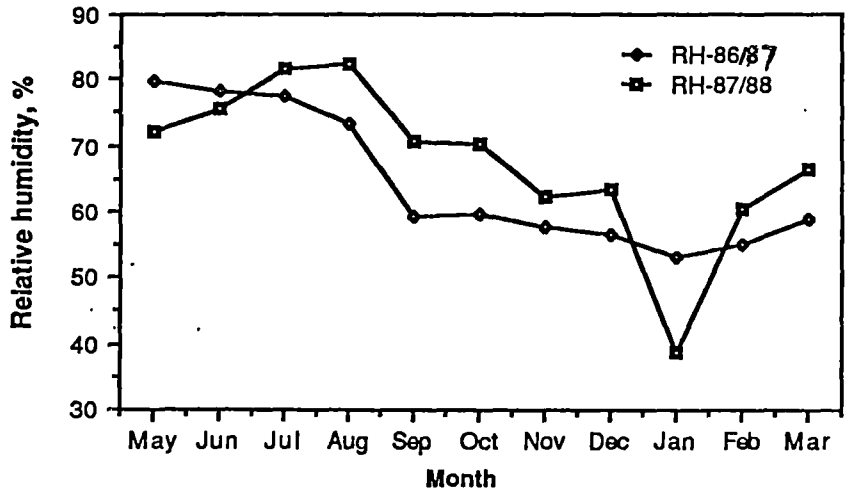
Appendix 5: The University of Tasmania Farm, Richmond Rd. Cambridge showing field experiment sites and locations of soil collected for experiment 1.



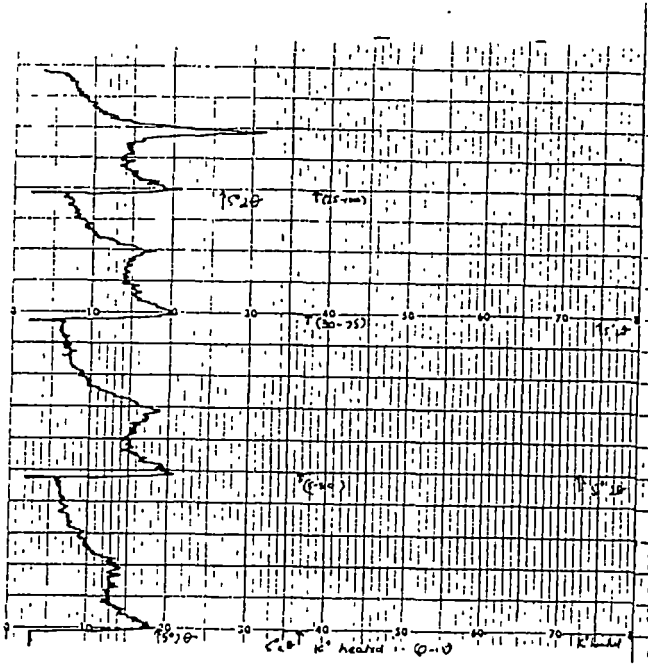
Appendix 6: Climatic data during 1986/87 and 1987/88 seasons



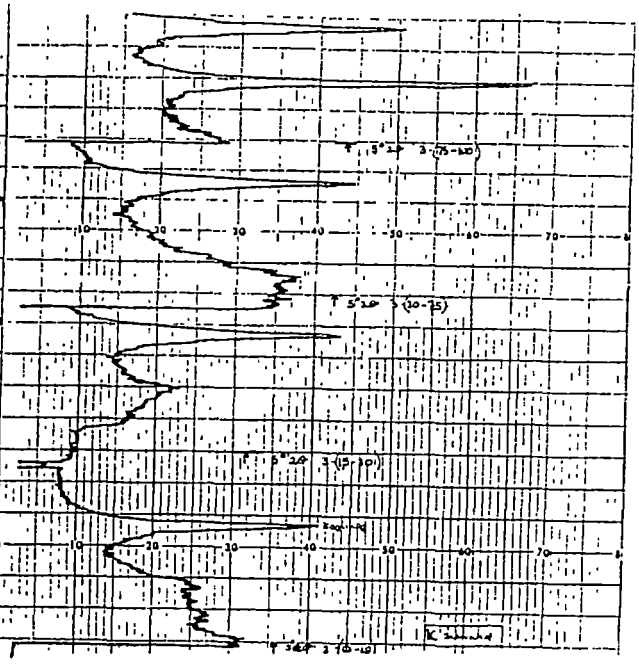




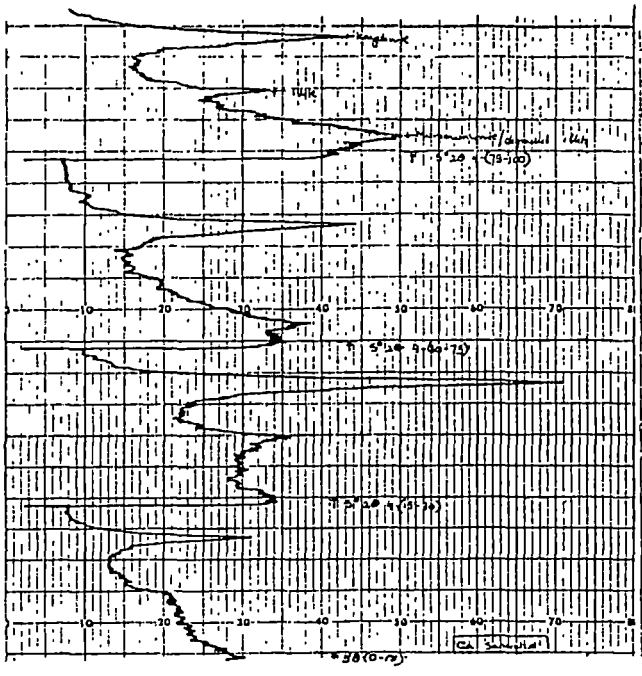
Appendix 7: X ray diffractogramsused to identify clay minerals in soil.



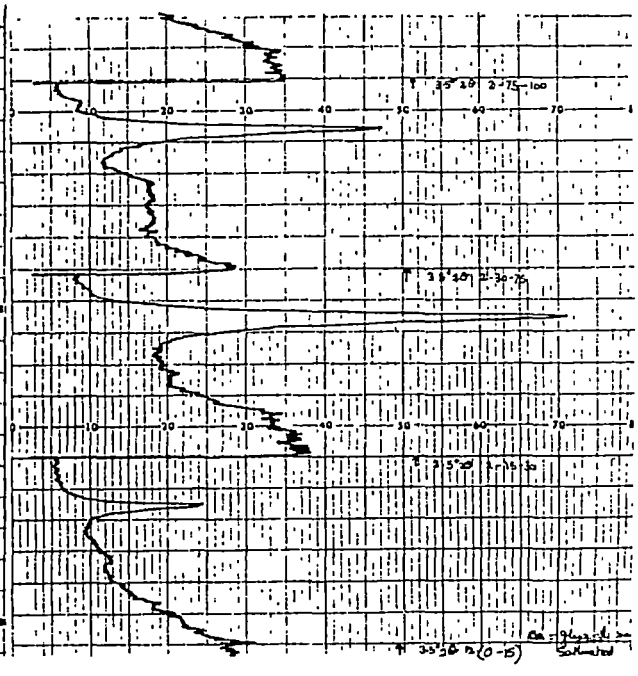
K^+ Heated



K Saturated



Ca Saturated



Ca/ Glycerol Saturated

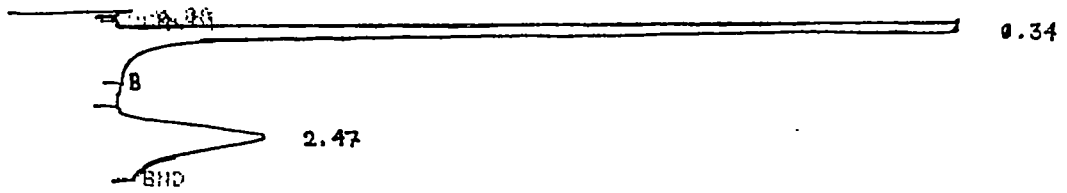
Appendix 8. Gas chromatographs used in detection of carbon dioxide and nitrous oxide.

SENSITIVITIES 400 250

INST 2 METH 4 FILE 102

RUN 1

SENSITIVITIES 400 250



INST 2 METH 4 FILE 102

RUN 1 1 : 47.2 11 / 13 / 86

SENSITIVITIES 400 250

TIME	AREA	BC	RRT	RF	C	NAME
0.21	1.7386	T	0.617	1.000	0.1168	AIR:
0.34	1364.0499		1.000	1.000	88.5708	AIR:
			4.787	0.250		CARBON DIOXIDE:
2.47	188.5491		7.264	0.924	11.3124	NITROUS OXIDE:

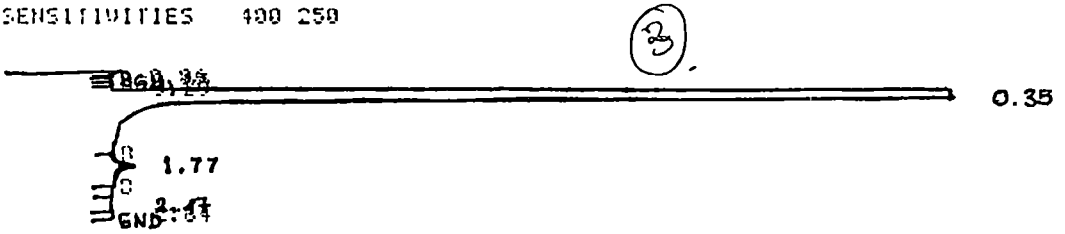
(a). A typical gas chromatogram of Nitrous oxide (conc.10%)
Oven temperature: 150 C; Inj. temp:25 C; Flow rate : 45ml/min.

(B)

INST 2 METH 4 FILE 85

RUN 2

SENSITIVITIES 400 250



INST 2 METH 4 FILE 85

RUN 2 1 : 51.7 11 / 13 / 86

SENSITIVITIES 400 250

TIME	AREA	BC	RRT	RF	C	NAME
0.22	1.8695	T	0.628	1.000	0.1493	AIR:
0.29	0.0981	TS	0.828	1.000	0.0078	AIR:
0.35	1248.8704		1.000	1.000	99.7669	AIR:
1.77	3.5326		5.057	0.250	0.0706	CARBON DIOXIDE:
2.47	0.0682	T	6.805	0.924	0.0050	NITROUS OXIDE:

(b) . A typical gas chromatogram of carbon dioxide (Conc. 0.1%)
Oven temp.: 150 C; inj. temp. 25 C; Flow rate 45ml/min.

Carrier gas: Helium; Packing of column: Microsieve: 5A;
Column details: 6ft. x 1/8" SS.

Appendix 9: Urease activity[1] and urea-N[2] remaining in four soil types

Soil type	Urease activity control(-U)	Urease activity with urea(+U)	mean (S)	% urea-N remaining
1	37.07 (4.28)	75.08 (4.96)	56.07	14.32
2	32.97 (4.03)	72.95(10.69)	52.96	18.69
3	47.41 (3.44)	99.25 (8.07)	73.33	4.58
4	46.27 (6.33)	107.75(11.58)	77.00	6.83
mean (U)	40.93	88.76		
Lsd p=0.05				
	urea(U)	10.94		
	soil(S)	8.29		
	U x S	NS		

1. Urease activity as ammonium-Nμg/g soil

2. as ammonium-N produced,μg/ g soil produced

figures given in paranthesis are standard errors

Appendix 10: Ammonium N and Nitrate N in four soil types

	Soil type								LSD(0.05)
DAF	1	1(+Ur)	2	2(+Ur)	3	3(+Ur)	4	4(+Ur)	(LxNI)
Ammonium N									
0	29.254	22.820	19.421	16.512	28.427	26.224	28.386	20.898	NS
5	32.102	66.344	25.293	30.848	26.295	78.166	23.604	87.898	14.24
10	24.360	74.829	19.449	54.515	22.969	48.661	23.760	60.010	11.62
20	31.990	56.235	22.049	41.232	28.312	44.022	24.189	44.567	13.74
40	29.356	32.314	28.176	35.235	22.992	41.015	21.684	40.646	8.51
60	28.255	20.482	24.221	28.565	24.361	36.031	20.321	31.651	NS
Nitrate N									
0	79.66	78.46	66.99	69.48	21.65	18.22	41.73	41.93	NS
5	83.57	86.0	62.98	93.32	22.11	32.15	46.15	51.26	13.84
10	82.64	98.33	64.12	141.2	30.26	55.55	48.65	61.16	12.88
20	89.24	134.89	72.59	133.62	24.53	58.89	46.95	74.89	13.08
40	86.95	158.24	68.94	130.9	26.36	59.8	47.89	86.12	13.94
60	87.18	148.08	71.00	131.6	28.22	57.25	45.78	72.33	15.47

(For comparison of the means over four soil types and over all urea treatments)

LSD (0.05)	Soil(NH_4^+)	Urea (NH_4^+)	Soil (NO_3^-)	Urea(NO_3^-)
0	5.13	NS	10.53	NS
5	12.97	9.17	11.72	5.21
10	9.01	5.19	7.68	7.32
20	5.82	4.82	8.8	6.84
40	NS	NS	9.53	7.19
60	Ns	NS	9.77	8.49

+ UR: urea added ; -

Appendix 11: Net CO_2 production in four soils following addition of a C source (1% dextrose broth). CO_2µmol/ g soil.

	Hours ofincubation.							
	2		4		8		24	
Soil	-Ur	+Ur	-Ur	+Ur	-Ur	+Ur	-Ur	+Ur
1	0.18	0.2	0.17	0.42	0.73	1.06	1.17	1.98
2	nd	0.14	0.26	0.48	0.82	0.85	1.32	1.86
3	0.13	0.83	0.19	1.97	0.95	2.99	1.33	4.11
4	0.12	0.53	0.18	1.09	0.89	2.34	1.18	2.44
LSD (p=0.05)								
Ur x Soil		NS		0.09		0.33		0.15
Ur		0.03		0.05		0.20		0.09
Soil		NS		.07		0.21		0.25

(nd- not detectable)

Appendix 12: Change in ammonium and nirate-N in soil type 2 .

Fertilizer type & rate.

DAS	Ur1	Ur 2	CN1	CN2	AN1	AN2	AS1	AS2
ammonium-N.								
0	29.62	22.18	28.45	21.49	25.33	21.2	25.61	31.75
5	63.42	102.88	31.25	28.2	48.1	73.2	59.62	119.25
25	16.34	15.18	17.96	17.14	18.00	10.05	16.79	17.22
50	10.48	12.42	9.54	14.79	11.35	9.32	9.95	11.64
80	14.55	16.91	12.58	17.39	13.37	16.57	16.63	15.04
120	18.74	18.68	15.83	20.04	16.28	18.23	17.05	16.46

Table of means LSD (p=.05)

low	high	Ur	CN	AN	As	(type)	(rate)	(tx r)
27.25	24.16	25.9	24.97	23.27	28.68	ns	ns	ns
50.6	80.88	83.15	29.72	60.65	89.44	20.39	14.42	28.83
17.27	14.9	15.76	17.55	14.02	17.00	ns	ns	ns
10.33	12.04	11.45	12.16	10.33	10.79	ns	ns	ns
14.28	16.48	15.73	14.99	14.97	15.84	ns	ns	ns
16.98	18.35	18.71	17.93	17.25	16.76	ns	ns	ns

nitrate-N

DAS	Ur1	Ur 2	CN1	CN2	AN1	AN2	AS1	AS2
0	33.55	29.48	37.58	30.06	31.13	26.81	31.86	27.8
5	48.55	44.42	76.53	131.46	49.18	86.09	57.95	44.13
25	49.18	64.13	78.37	73.66	54.85	79.6	57.95	55.1
50	30.46	49.9	53.37	66.41	42.54	61.02	48.99	45.92
80	24.74	32.95	31.27	41.99	28.44	46.83	29.22	37.74
120	16.67	20.15	26.1	28.91	28.08	33.97	18.51	23.64

Table of means LSD (p=0.05)

low	high	Ur	CN	AN	As	(type)	(rate)	(tx r)
33.53	28.54	31.51	33.82	28.97	29.83	ns	ns	ns
58.05	76.53	46.48	104.00	67.63	51.04	20.223	14.31	28.61
60.09	68.12	56.66	76.02	67.23	56.53	16.07	ns	ns
43.84	55.81	40.18	59.89	51.78	47.45	10.44	7.38	ns
28.42	39.88	28.85	36.63	37.64	33.48	4.27	3.02	ns
22.34	26.67	18.41	27.51	31.02	21.07	5.26	5.97	ns

Ur: Urea, CN: Calcium nitrate, AN: Ammonium nitrate, AS Ammonium nitrate.

1: Low rate of application, i.e 50 kg N/ha.

2: High rate of application, i.e 120 kg N/ha.

Appendix 13: CO_2 production (ug/g soil) at 25 °C at various time intervals

Treatment	Hrs of incubation 2	5	8	24	72	120
LoNIo	0.84	1.73	2.81	5.03	46.59	48.14
LoNIi	1.11	1.78	2.53	4.41	54.79	58.12
LoNIii	1.62	2.11	2.35	3.58	37.97	55.48
LiNIo	2.23	1.83	2.85	6.25	48.27	61.04
LiNIi	1.64	2.49	2.75	6.58	91.37	114.16
LiNIii	2.25	2.59	3.43	5.63	108.48	113.66
LiiNIo	2.18	2.94	4.82	7.78	63.22	84.33
LiiNIi	2.63	3.66	4.95	8.31	99.16	124.08
LiiNIii	3.23	4.38	4.00	8.42	128.00	160.5
mean values						
Lo	1.19	2.04	2.68	1.75	1.79	2.36
Li	1.87	2.30	3.66	2.167	2.638	3.03
Lii	2.56	3.00	4.59	3.49	3.41	3.26
NIo	4.34	6.15	8.17	6.35	6.43	5.87
NIi	46.45	82.71	96.98	52.88	81.77	91.49
NIii	53.91	102.95	122.97	64.5	98.78	116.55
LSD at p= 0.05						
pH	0.155	0.118	0.676	0.686	5.885	12.97
NI	0.276	0.335	ns	ns	9.630	12.54
pH x NI	0.405	ns	ns	ns	10.753	20.38

Appendix 14. Urea recovered (urea-N ug/depth) from leached soil columns

	air-dry soil					wet soil	
Leaching run,	1L-i	1L-ii	1L-iii	2	3	1	2
(i)	1	2	3	4	5	1	2
Depth (cm)							
0-1.4	15.62	9.6	3.35	0	0	3.11	0
1.4-2.8	12.56	5.6	0	0	0	8.92	0
2.8-4.2	32.46	14.2	5.13	0	0	14.89	2.05
4.2-5.6	71.45	10.9	15.58	2.11	0	11.84	0
5.6-7.0	83.22	40.08	9.85	0	0	14.3	8.94
7.0-8.4	42.12	55.98	12.39	0	0	21.3	6.81
8.4-9.8	-	59.88	22.05	5.51	2.65	29.81	9.52
9.8-11.2	-	39.586	29.56	0	1.1	21.84	10.21
11.2-12.6	-	-	28.42	12.6	0	24.89	10.15
12.6-14.0	-	-	38.54	22.1	1.25	26.97	19.42
14.0-15.8	-	-	26.92	18.2	8.62	18.88	15.37
15.8-17.2	-	-	18,99	24.56	19.42	16.53	14.81
leachate	-	-	-	11.81	14.31	2.57	15.54
Duration of leaching hrs. (D)	8	13	18	72	144	18	144
Total urea :N recovered at each sampling							
ug/column (T)	257.47	236.12	210.81	96.98	46.09	216.25	112.82
% recovery	85.9	78.7	70.26	32.33	15.36	72.01	37.61
Rate of urea hydrolysis (urea N ug/hr):							
incremental	1.75	4.34	5.06	2.11	1.06	4.65	1.43
cumulative	1.75	4.91	4.96	2.81	1.76	4.65	1.3

(Total urea -N added= 300 g/column)

Incremental rate of hydrolysis = $\frac{T(i)- T (i-1)}{D(i)-D(i-1)}$

In the case of leaching run 1 (i), the Ti is same as the total urea N added ie. 300 g ureaN/column

Cumulative rate of hydrolysis = $\frac{300-Ti}{Di}$

(—) indicate samples that were not analysed since soil at these depths was not wet at the time of sampling.

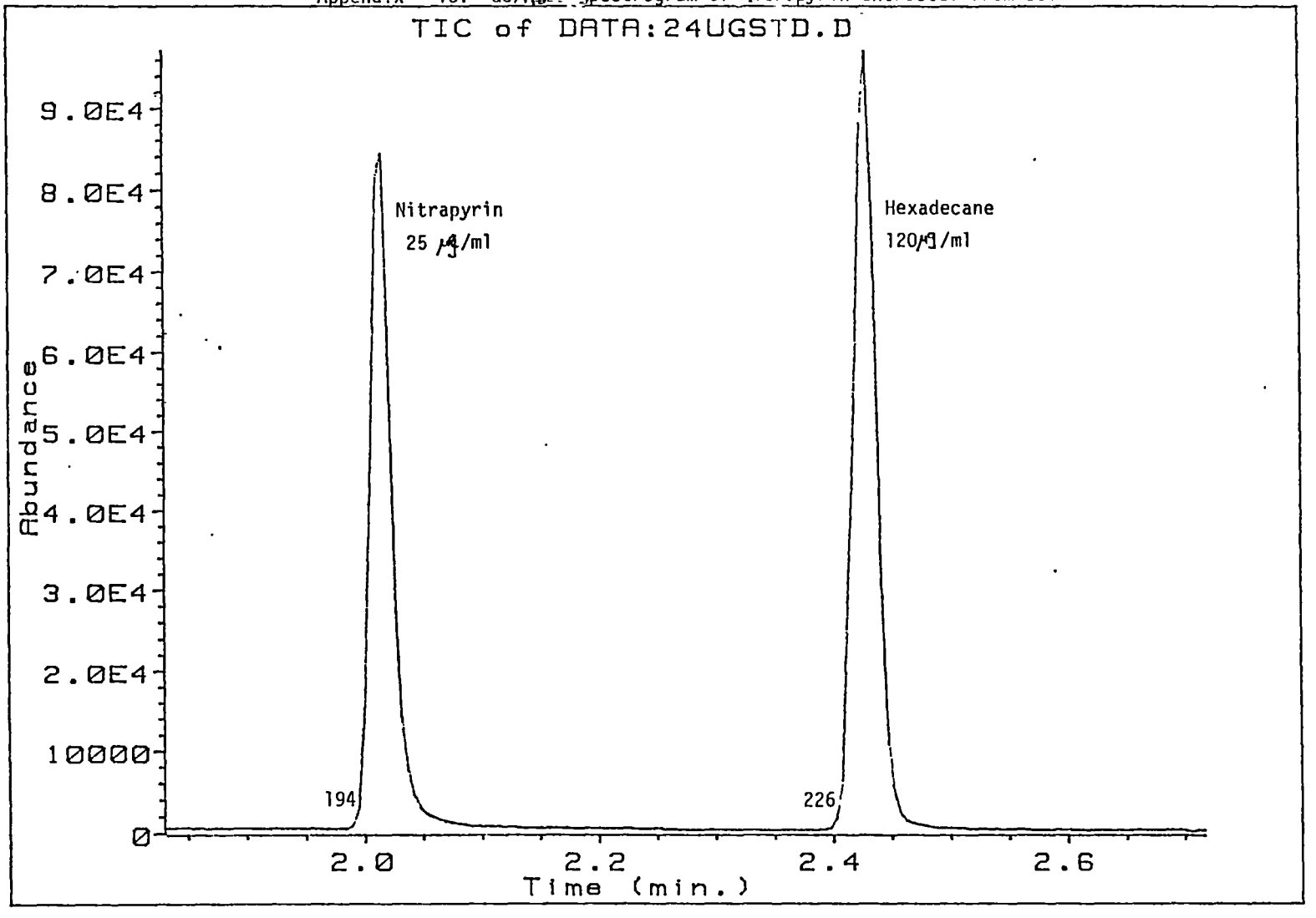
Appendix 15: Persistence of nitrapyrin (ng/g soil) in soil at different temperatures

Treatment	Number of DAYS 1	4	7	28	34
F0T10-1	34951.95	14207.70	4821.81	1222.47	829.92
F1T10-1	17580.70	6414.38	1986.58	174.69	227.47
F0T25-1	29743.39	3549.08	2783.661	846.95	641.50
F1T25-1	10491.08	1667.42	529.73	93.00	43.62

F= air flow (0= still air, 1= . air flow 2ml/sec)

T= temperature (10°C and 25°C)

Appendix 16: GC/MS spectrogram of nitrapyrin extracted from soil



Column type: HP5 , 25m. ; at 9.5psi., 200C isothermal. Injected volume = 1.0 µl Solvent: Redistilled Hexane.

Appendix 17: Mean top dry matter production (g/0.25m2)

treatment	20DAS	45DAS	100DAS	140DAS
Lime (L):(averaged over all N/NI treatments				
0	7.53	43.65	419.69	538.13
i	7.94	53.61	473.47	630.17
ii	7.85	50.05	496.34	599.73
N/NI (T) (averaged over all lime treatments)				
1	7.86	51.77	353.02	414.03
2	7.03	46.33	442.03	515.67
3	7.03	56.63	508.85	496.1
4	8.45	43.63	413.8	517.63
5	7.57	58.54	482.5	670.2
6	9.07	55.67	509.36	664.06
7	7.76	49.23	501.21	666.38
8	7.29	37.33	409.47	564.25
9	7.92	42.77	548.26	795.81

L x T	Lo	Li	Lii	Lo	Li	Lii	Lo	Li	Lii	Lo	Li	Lii
0	(6.3,	7.6,	9.7)	(44.4,	54.80,	56.1)	(375.5,	325.2,	358.4)	(429.5,	372.0,	440.6)
1	(6.8,	6.8,	7.5)	(51.6,	51.8,	35.6)	(421.0,	421.8,	483.2)	(499.3,	461.6,	586.1)
2	(8.3,	7.1,	7.7)	(43.2,	76.3,	5.04)	(471.8,	527.7,	527.0)	(586.2,	546.4,	555.7)
3	(7.3,	9.1,	8.9)	(38.4,	48.1,	44.4)	(355.6,	395.7,	490.1)	(533.0,	490.1,	529.8)
4	(8.3,	7.2,	7.3)	(50.8,	58.4,	66.4)	(411.4,	529.9,	506.3)	(553.1,	734.1,	723.30)
5	(8.2,	10.1,	8.9)	(46.9,	60.9,	59.3)	(487.9,	536.3,	504.2)	(551.3,	754.3,	686.6)
6	(7.8,	8.1,	7.4)	(42.1,	47.2,	58.3)	(426.3,	506.0,	571.3)	(521.2,	782.1,	695.8)
7	(7.2,	7.0,	7.7)	(35.2,	38.3,	38.6)	(381.3,	438.9,	408.2)	(516.1,	598.4,	578.2)
8	(7.6,	8.6,	7.5)	(40.3,	46.7,	41.3)	(446.8,	579.7,	618.3)	(653.5,	932.5,	801.5)

Lsd at p= 0.05				
(T)	NS	13.56	39.93	71.93
Lime(L)	0.25	5.05	36.53	25.03
L x T	1.78	18.36	113.09	24.59

*(three figures within parenthesis represent corresponding data for lime treatments 0,i, and ii respectively)

Appendix 18: Regression equations used to estimate the rate of top dry matter production

T	Lo	Li	Lii
0	-83.48 +3.91x r=0.97	-95.32 + 4.08x r=0.99	-82.04+3.91x r=0.98
1	-101.61+4.54x r=0.98	-90.01 + 4.27x r=0.96	-133.27+5.39x r=0.97
2	-129.61+5.33x r=0.98	-98.33 + 5.08x r=0.96	-103.05+4.31x r=0.99
3	-122.42+4.59x r=0.99	-113.43+4.72x r=0.99	-110.17+4.96x r=0.96
4	-117.85+4.90x r=0.97	-164.63+6.52x r=0.99	-157.36+6.34x r=0.99
5	-116.08+5.11x r=0.98	-167.05+6.66x r=0.99	-149.59+6.09x r=0.99
6	-112.42+4.74x r=0.99	-185.42+6.84x r=0.99	-150.99+6.35x r=0.98
7	-116.82+4.61x r=0.99	-137.95+5.36x r=0.99	-132.45+5.12x r=0.99
8	-152.81+5.77x r=0.98	-229.25+8.16x r=0.99	-190.00+7.30x r=0.98

x= time

Appendix 19: Means of leaf area index LAI and % light intercepted

treatment	LAI or L 30DAS	50DAS	70DAS	120DAS	% LI 120DAS
Lime treatments (L)-averaged over all N/NI treatments					
0	0.35	1.30	2.68	6.90	49.39
1	0.38	1.38	3.09	7.66	56.83
2	0.39	1.48	2.88	7.66	56.94
N/NI (T)-averaged over all lime treatments					
T0	0.38	1.00	2.99	6.15	37.67
T1	0.38	1.14	4.97	6.14	53.00
T2	0.38	1.14	2.62	7.72	52.83
T3	0.33	1.14	2.21	7.10	54.67
T4	0.41	1.87	3.46	7.75	60.50
T5	0.41	1.91	2.51	7.47	59.17
T6	0.41	1.88	2.61	7.34	60.83
T7	0.33	1.04	2.23	6.49	48.5
T8	0.31	1.37	2.39	8.53	62.33

L x T interaction *

	30DAS	50DAS	70 DAS	120 DAS	120DAS (LI)
T0	(0.39,0.35,0.41)	(1.0,0.94,1.09)	(2.86,2.98,3.12)	(5.63, 6.62, 6.19)	(35.0, 38.5 39.5)
T1	(34, .42,0.4)	(1.19,1.11,1.1)	(3.64, 3.94, 5.33)	(7.84, 8.42, 8.16)	(43.0, 50.5, 65.5)
T2	(0.31,0.41,0.43)	(0.96,1.15,1.31)	(3.14, 2.35, 2.38)	(7.48, 7.51, 8.16)	(44.5, 59.5, 54.5)
T3	(0.32,0.29,0.39)	(1.16,1.09,1.16)	(2.04, 2.26, 2.34)	(6.11, 7.13, 8.06)	(53.0, 56.5,54.5)
T4	(0.32,0.29,0.39)	(1.62,1.99, 1.99)	(3.15, 4.25, 2.99)	(6.69, 8.95, 7.13)	(52.6, 65.5, 59.5)
T5	(0.39,0.44,0.41)	(1.75,2.0, 1.99)	(2.42, 2.59, 2.52)	(7.13, 7.47, 7.81)	(53.5, 65.5, 63.0)
T6	(0.39,0.43,0.41)	(1.81,1.95, 1.88)	(2.67, 2.66, 2.51)	(6.99, 7.12, 7.91)	(58.0, 62.0, 62.5)
T7	(0.40,0.39,0.43)	(1.1,0.99,1.02)	(2.04, 2.20, 2.43)	(6.43, 6.91, 6. 13)	(46.0, 49.5, 50.0)
T8	(0.29, .37, .34)	(1.09,1.23,1.8)	(2.16, 2.64, 2.36)	(7.82, 8.84, 8.93)	(59.0, 64.5, 63.5)

Lsd(p0.05)

(T)	NS	0.236	0.623	0.59
Lime(L)	0.12	0.05	0.36	0.39
L x T	NS	0.266	0.85	NS

*(three figures within parenthesis represent corresponding data for lime treatments Lo, Li, andLii)

Appendix 20 : Total plant N content (in terms of grain-N and other plant parts-N) and the nitrogen use efficiency of crop (NUE).

Treat	Grain N kg/ha	straw N kg/ha	RE/ grain	RE/straw	YE/grain	YE/straw
Lime (values averaged over all N/NI treatments)-(L)						
0	94.6	33.21	26.81	13.24	22.14	10.27
1	131.2	43.4	38.03	26.28	45.25	31.91
2	130.36	41.54	33.25	23.98	42.68	26.14
N/NI (values averaged over all L treatments)-(T)						
T0	56.15	27.17	-	-	-	-
T1	107.76	35.32	31.3	21.71	31.26	14.74
T2	128.2	32.54	28.17	16.48	55.27	14.55
T3	106.5	28.63	27.8	8.32	51.73	16.31
T4	137.16	41.47	31.59	17.7	32.7	25.52
T5	140.0	44.03	30.89	19.57	36.41	24.28
T6	153.13	51.02	29.51	26.55	38.79	23.65
T7	90.74	38.17	32.92	27.41	11.5	26.5
T8	148.85	56.08	42.4	31.6	35.39	36.65
L x T interaction						
T0 Lo	50.78	24.5	-	-	-	-
Li	56.39	23.2	--	--	-	
Lii	61.27	33.9	--	-	-	-
T1 Lo	76.55	35.1	27.5	21.3	25.8	4.11
Li	128.7	32.1	35.8	15.3	38.1	13.9
Lii	118.1	38.7	30.6	28.5	29.8	26.3
T2 Lo	83.3	28.1	25.0	7.3	32.8	8.5
Li	146.3	38.6	30.9	23.2	69.6	23.3
Lii	155.1	33.9	28.6	18.9	64.8	11.9
T3 Lo	83.7	26.3	24.8	3.7	27.6	8.22
Li	122.2	28.2	32.8	7.5	71.8	20.9
Lii	113.7	31.4	25.8	13.8	55.7	19.9
T4 Lo	109.9	27.5	24.3	5.1	22.6	7.8
Li	154.9	49.8	39.1	25.4	40.4	34.4
Lii	146.6	47.1	31.4	22.7	35.1	34.4
T5 Lo	117.4	33.8	21.6	9.4	25.6	6.95
Li	151.3	51.6	35.5	27.2	41.7	43.4
Lii	151.3	46.7	35.5	22.2	41.9	22.6
T6 Lo	135.9	49.3	26.6	24.9	35.1	8.9
Li	161.9	62.5	36.2	38.0	43.2	35.5
Lii	161.6	41.2	25.8	16.7	38.1	26.5
T7 Lo	67.0	33.5	28.4	18.0	-11.6	15.3
Li	99.2	39.6	46.7	30.2	10.6	33.6
Lii	106.1	41.5	44.6	34.0	35.5	30.7
T8 -Lo	126.9	40.8	36.2	16.4	19.1	22.3
Li	159.9	68.0	47.2	43.5	46.6	50.5
Lii	159.9	59.4	43.8	34.9	40.3	37.2
Lsd (p≤0.05)						
N/NI	40.56	15.92	6.4	NS	17.31	5.57
L	7.84	3.76	3.52	4.39	3.46	5.88
N/NI x L	NS	18.4	NS	23.68	19.07	NS

RE- recovery efficiency (Bock, 1984).
YE- Yield efficiency (Bock, 1984).

Appendix 20b: Yield components of barley (cv. Triumph) .

Treatment	Ears/m2			Grains/Ear			Yield mt/ha			Harvest Index			Straw Yield (mt/ha)		
	Lo	Li	Lii	Lo	Li	Lii	Lo	Li	Lii	Lo	Li	Lii	Lo	Li	Lii
mean values															
TOLo	731	784	726	19	20	23	4.43	4.38	4.88	51.19	51.58	53.18	4.29	4.12	4.32
T1	798	812	712	24	24	26	5.72	5.34	6.17	55.74	51.93	53.5	4.51	4.94	5.36
T2	752	820	792	24	26	24	6.07	7.91	7.67	56.74	58.44	62.35	4.64	5.66	4.62
T3	766	813	798	24	28	26	5.91	7.97	7.22	55.59	59.89	57.63	4.72	5.34	5.3
T4	781	968	956	24	24	26	6.67	8.47	7.94	54.27	52.33	50.60	5.63	7.74	7.73
T5	810	1040	820	24	23	26	6.99	8.35	8.62	58.22	48.86	56.81	5.00	8.74	6.57
T6	800	980	810	23	24	24	7.94	8.75	8.28	60.8	52.75	54.09	5.20	7.85	6.95
T7	788	820	812	23	20	24	3.78	4.96	6.20	42.6	45.41	51.73	5.06	5.98	5.78
T8	898	1100	948	24	26	28	6.30	9.09	8.49	51.27	51.21	51.42	6.03	8.68	8.02
L x T Lsd (p≤0.05)															
T	31.83			NS			1.18			NS			0.96		
L	31.93			1.05			0.25			NS			0.43		
L x T	84.44			NS			1.33			NS			1.42		

(for details of T and L refer Table 9.1)

Appendix 21: Ammonium-N recovered (2xug/g soil)from the field plots at different sampling times.

Treatment		before sowing	Days after sowing (DAS) 6	13	42	83
Lime- average over all T treatments						
Lo		31.11	82.12	69.4	23.38	6.87
Li		29.14	74.7	56.55	25.69	6.81
L2		30.72	70.53	52.6	20.94	6.96
N/NI - average over all L treatments						
T0		32.35	28.67	11.98	5.29	3.34
T1		29.20	70.49	47.58	22.94	4.80
T2		34.66	68.36	62.28	19.91	8.28
T3		32.00	67.36	65.62	19.2	8.06
T4		27.54	116.51	80.19	17.63	8.57
T5		30.30	119.52	93.56	21.86	6.79
T6		28.43	117.21	93.06	18.09	8.10
T7		29.72	28.45	13.46	35.53	7.66
T8		28.75	65.22	67.93	49.58	6.35
T x L interaction						
T0	Lo	34.39	27.54	15.85	6.09	2.00
	Li	34.38	26.85	10.36	4.73	3.52
	Lii	28.27	31.63	9.73	5.04	4.49
T1	Lo	30.86	77.82	56.37	22.71	3.51
	Li	25.9	72.16	42.06	21.95	5.96
	Lii	30.84	61.5	44.3	19.70	4.92
T2	Lo	35.17	74.31	76.92	19.19	7.88
	Li	29.73	69.42	58.28	20.83	9.52
	Lii	39.08	61.36	51.66	18.90	7.44
T3	Lo	34.12	71.58	72.65	18.90	9.13
	Li	30.46	69.72	60.89	21.61	5.52
	Lii	31.41	61.59	63.3	17.09	9.53
T4	Lo	24.4	123.63	85.41	17.49	9.29
	Li	27.81	115.19	81.59	20.29	7.53
	Lii	30.40	110.72	73.57	15.12	8.88
T5	Lo	34.19	130.35	118.21	21.55	6.25
	Li	30.31	118.58	84.12	25.06	7.00
	Lii	26.40	109.64	78.38	18.97	7.13
T6	Lo	24.67	136.00	105.63	18.00	7.02
	Li	27.47	111.15	91.93	20.81	8.95
	Lii	33.17	104.49	81.62	15.44	8.35
T7	Lo	32.50	28.98	14.60	37.55	10.55
	Li	27.59	28.9	14.59	37.1	6.32
	Lii	29.07	27.49	11.18	31.93	6.10
T8	Lo	29.74	68.9	78.93	48.41	6.24
	Li	28.63	60.37	65.19	60.48	6.99
	Lii	27.88	66.38	59.68	39.85	5.83
Lsd (0.05)						
L		NS	7.08	2.62	2.65	NS
T		NS	13.54	21.44	9.18	2.6
T x L		NS	NS	22.38	NS	NS

Appendix 22:Nitrate N/nitrite-N levels in soil (2 x ug/g soil)

Treatment		Before sowing	Days after sowing (DAS) 6	13	42	83
Lime- average over all T treatments						
Lo		21.31	28.24	29.11	42.20	17.93
Li		24.87	36.60	29.26	38.19	14.76
L2		25.51	41.20	30.44	35.95	14.71
N/NI - average over all L treatments						
To		26.21	20.84	20.28	16.17	15.74
T1		22.77	42.82	34.52	47.85	14.33
T2		23.86	37.24	38.89	36.34	11.59
T3		21.49	31.45	32.41	37.82	13.13
T4		22.63	45.87	35.75	36.24	14.04
T5		24.9	45.28	29.91	37.79	14.23
T6		23.71	37.19	29.74	34.84	15.48
T7		22.56	25.32	12.80	53.47	20.06
T8		26.96	32.12	32.15	48.20	23.59
L x T interaction						
To	Lo	26.75	19.12	18.97	16.12	15.21
	Li	27.2	22.21	22.05	15.30	13.58
	Lii	24.67	21.18	19.82	17.99	18.43
T1	Lo	22.79	39.67	32.52	56.84	16.10
	Li	22.55	35.41	31.97	44.91	14.30
	Lii	22.97	53.39	39.07	41.78	12.59
T2	Lo	18.98	28.6	34.42	43.23	15.32
	Li	29.21	41.62	38.78	34.81	10.34
	Lii	23.38	41.50	43.47	30.98	9.11
T3	Lo	18.29	18.86	29.43	43.66	15.57
	Li	22.00	35.07	31.1	35.83	11.48
	Lii	24.18	40.42	36.69	33.98	12.34
T4	Lo	19.20	31.23	32.64	39.37	17.38
	Li	22.79	47.06	36.35	37.73	14.99
	Lii	25.92	59.33	38.25	31.62	9.75
T5	Lo	18.60	30.66	31.69	43.57	16.63
	Li	25.97	52.79	29.98	36.29	13.41
	Lii	30.13	52.4	28.06	33.5	12.66
T6	Lo	22.11	27.36	35.24	40.92	19.20
	Li	22.41	39.92	28.02	33.25	11.00
	Lii	26.59	44.29	25.96	30.34	16.23
T7	Lo	21.29	28.44	14.00	44.17	23.59
	Li	23.43	24.42	12.81	58.72	17.68
	Lii	22.95	23.12	11.57	57.52	18.91
T8	Lo	23.81	30.23	33.11	51.92	22.40
	Li	28.23	30.91	32.27	46.87	26.04
	Lii	28.83	35.23	31.07	45.82	22.34
Lsd (p=0.05)						
L		2.05	2.78	NS	2.27	2.55
T		NS	7.27	3.18	8.24	4.34
T x L		NS	NS	NS	9.94	NS

Appendix 23: Total mineral-N in soil (2 x ug/g soil)

Treatment		Before sowing	Days after sowing (DAS) 6	13	42	83
Lime- average over all T treatments						
Lo		52.43	110.36	98.51	65.58	24.91
Li		54.00	111.3	85.51	63.88	21.57
L2		56.24	111.74	83.04	56.88	21.67
(T) N/NI - averaged over all L treatments						
T0		58.55	49.51	32.26	21.76	19.07
T1		51.97	113.31	82.10	70.79	19.13
T2		58.51	105.6	101.17	56.25	19.87
T3		53.49	99.08	98.02	57.02	21.19
T4		50.17	162.08	115.94	53.87	22.60
T5		55.2	164.8	123.47	59.64	21.03
T6		52.14	154.4	122.8	52.92	23.59
T7		52.28	53.78	26.25	89.00	27.71
T8		55.7	97.34	100.08	97.79	29.94
L xT interaction						
T0	Lo	61.14	46.66	34.81	22.21	17.21
	Li	61.58	49.06	32.41	20.03	17.09
	Lii	52.98	52.81	29.55	23.04	22.91
T1	Lo	53.65	117.49	88.88	79.56	19.60
	Li	48.44	107.56	74.03	66.87	20.26
	Lii	53.82	114.89	83.38	65.94	17.52
T2	Lo	54.16	102.91	111.34	62.92	23.20
	Li	58.94	111.04	97.05	54.00	19.86
	Lii	62.45	102.86	95.12	51.81	16.55
T3	Lo	52.41	90.43	102.07	62.55	24.71
	Li	52.47	104.79	92.00	57.44	17.00
	Lii	55.59	102.0	100.00	51.06	21.87
T4	Lo	43.59	154.86	118.05	56.86	26.67
	Li	50.61	162.24	117.94	58.02	22.52
	Lii	56.32	170.04	111.82	46.73	18.63
T5	Lo	52.78	162.00	149.9	65.12	22.87
	Li	56.28	171.37	114.08	61.35	20.42
	Lii	56.53	162.03	106.44	52.46	19.79
T6	Lo	46.78	163.35	140.87	58.93	26.23
	Li	49.88	151.07	119.95	54.06	19.95
	Lii	59.76	148.78	107.58	45.78	24.58
T7	Lo	53.80	57.41	28.61	81.72	34.14
	Li	51.02	53.31	27.4	95.82	24.00
	Lii	52.02	50.61	22.76	89.46	25.00
T8	Lo	53.56	99.13	112.04	100.33	28.64
	Li	56.85	91.28	97.45	107.35	33.02
	Lii	56.7	101.61	90.75	85.67	28.17
Lsd (p=0.05)						
L		NS	NS	5.23	3.47	2.48
T		NS	14.19	21.22	14.46	5.67
L xT		NS	NS	24.79	16.99	NS

ADDENDUM

THE INTERACTION OF NITRAPYRIN WITH CU^{2+} ON IN VITRO NITRITE PRODUCTION AND THE BACTERICIDAL ACTIVITY OF NITRAPYRIN.

ABSTRACT

Nitrite production in vitro was effectively inhibited by nitrapyrin. The inhibition of nitrapyrin was bactericidal rather than bacteriostatic. Addition of 2μg Cu^{2+} ml^{-1} did not significantly affect nitrification in liquid culture. However, when Copper concentration was increased to 7ug $Cu^{2+}ml^{-1}$ nitrification was inhibited even in the absence of nitrapyrin.

INTRODUCTION

Despite the considerable research undertaken on nitrification inhibitors only a few studies have dealt with their mode of action. This applies even to nitrapyrin (NI) which was the first commercial nitrification inhibitor to be developed (by Dow Chemical Co. USA in 1967) following publication of its inhibitory action by Goring in (1962).

Campbell and Aleem (1965) reported that nitrapyrin specifically inhibited the oxidation of ammonia to hydroxylamine and also observed that addition of Cu^{2+}at 38.4μg ml^{-1} to growth medium resulted in a 45-75% reversal of nitrapyrin-mediated inhibition of Nitrosomonas europea. They therefore concluded that nitrapyrin inhibits nitrification by chelating with the copper component of the enzyme, ammonia monooxygenase, responsible for ammonia oxidation. This proposed mechanism of inhibition was supported by Hooper and Terry (1973) although Powell and Prosse

(1986) found that in the case of Nitrosomonas europea.Cu^{2+}(0.046 μg ml^{-1}) enhanced rather than reversed inhibition of nitrification by nitrapyrin. The later workers used either $CuSO_4$ or $CuCl_2$ while Campbell and Aleem (1965) did not specify their copper source. The contrast in results was attributed by Powell and Prosser (1986) to a difference in response of different strains of bacteria to heavy metals, as shown by Babich and Stotzky (1980) and to differences in experimental systems/techniques employed by the two groups of workers.

If, as suggested by Campbell and Aleem (1965) the enzyme rather than the organism was affected by the inhibitor, then nitrification should recover as the nitrapyrin concentration drops below the bacteriostatic level necessary for effective inhibition of ammonium oxidation. However, Rodgers and Ashworth (1982) observed a bactericidal inhibition in liquid culture and the same effect had been observed previously by Hooper and Terry (1973) in soil. Of some relevance to the topic was the demonstration by Gorin (1962) and Laskowski and Bidlack (1977) of the recovery of nitrification in soils treated with nitrapyrin, indicating at least the survival of a proportion of the nitrifying population.

The present work was undertaken to assess whether the mode of action of nitrapyrin was bactericidal or bacteriostatic on a soil-derived nitrifying population in liquid culture, and also to investigate the effect of Cu^{2+} (supplied as $CuSO_4$)on nitrite production.

MATERIALS AND METHODS

Ammonium oxidizing bacteria were isolated from an actively nitrifying fresh soil inoculated into the nitrification medium proposed by Soriano and Walker (1968) containing 1% bromothymol blue as pH indicator. To obtain active nitrifiers five serial enrichments (at 30°C were made with 10-14 days incubation at each stage) with

dilution to 10^{-8} and selection of the highest dilution showing nitrite formation for each enrichment.

The bactericidal/bacteriostatic action of nitrapyrin was assessed as described in Figure 1. Active inoculum and nitrapyrin-treated inoculum were derived from the same original stock culture medium. The latter inoculum however was treated with nitrapyrin at a concentration of 0.05 μg a.i. ml^{-1}as soon as spot tests indicated nitrite production (seven days before use).

To test the effect of Cu^{2+}, tubes containing 7ml of nitrification medium (containing 1% bromothymol blue) were amended with 1ml of $CuSO_4$ solutions to give (Cu=0, 0.25, 0.5, 1.0 and 7.0 μg ml^{-1}) and 1ml of aqueous nitrapyrin solution (NI=0, 0.1, 0.25, 0.5μg a.i. ml^{-1}) with six replicates of each mixture. The Cu=0 tubes were supplemented with 1.0ml of anhydrous Na_2SO_4 (in equal concentration to $CuSO_4$) while sterile, distiled water was used to make up the NI=0 treatments. Each tube was inoculated with 1.0ml of an active nitrite producing culture (one week old culture).

Nitrite production was measured photometrically using a Skalaar Auto-analyzer following treatment with sulphonilic acid and alpha napthylamine hydrochloride. Tests for ammonium-N and nitrate-N (not reported here) were also made using the same instrument.

RESULTS AND DISCUSSION

The scanning electron micrographs of the culture (plate 1) showed predominantly rod-shaped organisms (about 95% of the bacteria found) growing attached to the glass surface. Most probably these were the autotrophic ammonium oxidizers since sodium carbonate was the sole carbon source added. Nitrapyrin (which is effective only on autotrophic nitrifiers) at a conc. of 1 μg ml^{-1} effectively

inhibited nitrite formation and no growth occurred following streak inoculation of dextrose agar plates and incubation for fourteen days at 30°C. Tests for ammonium and nitrate confirmed that ammonium oxidation resulted in nitrite accumulation and that no significant nitrate formation occurred in the tubes during the period of assay.

Figure 1 shows nitrite production following five and twelve days of incubation of treated nitrification medium. Nitrite in the active inoculum at the time of incorporation was detectable by spot test using modified Griess-Illsovay reagent with about 5μg of nitrite being introduced to each tube. Treatments that received inoculum+nitrapyrin also had traces (0.1 - 0.2μg ml^{-1}) of nitrite due to nitrification prior to nitrapyrin addition.

Nitrite levels in the control (treatment 1) increased during the experimental period confirming that nitrifiers continued to function under the conditions of the experiment. Addition of nitrapyrin (0.5μg ml^{-1} final concentration) resulted in effective inhibition of ammonium oxidation (treatment 2)

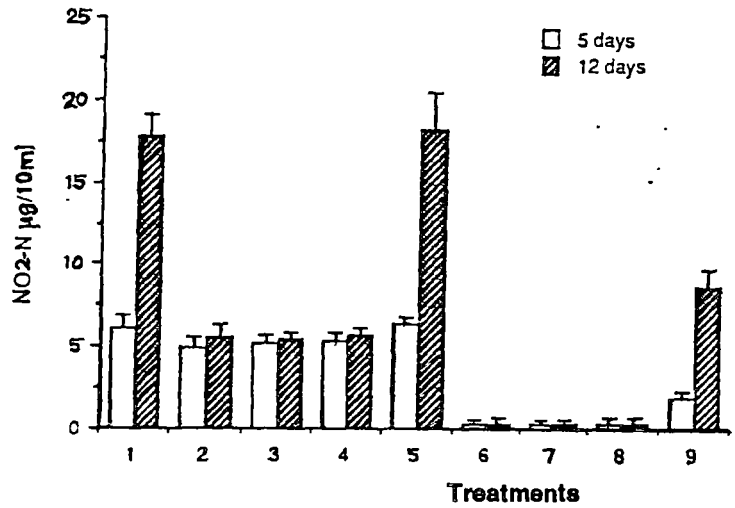
Ammonium oxidation was effectively inhibited in fresh inoculum at nitrapyrin concentrations of ~0.5μg ml^{-1},~0.05μg ml^{-1} and ~0.005 μg ml^{-1} (treatments 2, 3 and 4 respectively), but not at a concentration of approximately 0.0005 μg ml^{-1} (treatment 5). (concentrations were likely to be lower since part of the nitrapyrin added had been used to inhibit activity of the stock culture). In treatment 9 nitrite levels increased similarly to those in the control (treatment 1) suggesting an absence of inhibitory action. On the other hand, in the absence of active inoculum (treatments 6, 7 and 8) the nitrite levels did not rise indicating that nitrite production in treatment 5 was due to the active inoculum alone.

If nitrapyrin was bacteriostatic and only blocked the action of nitrification enzymes treatment 8 at 0.0005μg NI ml^{-1} should have produced nitrite as in treatment 9 which had a similar dilution of nitrification population but no nitrapyrin.

A bactericidal mode of action therefore seems to be more likely for nitrification inhibition mediated by nitrapyrin.

Addition of Copper as $CuSO_4$ did not have any effect on nitrapyrin inhibition since all NI applications, with and without Cu effectively inhibited nitrite production. Hence Figure 2 shows nitrite levels at only one level of nitrapyrin (ie.0.1μg NI ml-1) with no Cu for simplicity. However, when Cu was added to the treatments without NI (NIo), the increasing Cu concentration caused a decrease in nitrite production to the point where 7μg ml^{-1} (not shown but similar to the 0.1μg NI ml^{-1} treatment) completely inhibited the process. Although Cu levels used in this experiment were different to those used by Campbell and Aleem (1965) (38.4μg ml-1 $Cu2^{+}$) or by Prosser and Powell (1986) of (0.046 μg $ml^{-1}Cu^{2+}$) it was apparent that concentrations of Cu^{2+} in the order of 1μg ml^{-1} caused a marked inhibition of nitrification in the absence of nitrapyrin. Hence, the enhanced inhibition of nitrification by nitrapyrin following addition of Cu observed by Powell and Prosser (1986) may have been due to the combined inhibitory action of added Cu and nitrapyrin rather than to interaction of Cu with nitrapyrin . It is also possible that the present result differs from that of Powell and Prosser (1986) because the nitrifier strains responded differently to Cu supplied as $CuSO_4$ The control containing Na_2SO_4 but no $CuSO_4$ had no inhibitory action.

Figure 1: Effect of nitrapyrin and presence of active inoculum on recovery of NO_2^- production in vitro .after 5 days and 12 days of incubation at 30°C



McCartney bottles containing 8ml of nutrient medium/pH indicator were treated in three replications To 8 ml of nitrification medium following was added:

Treatment 1: 1ml of fresh inoculum + 1ml of water.

2: 1ml of fresh inoculum +1ml of aqueous nitrapyrin solution, to give 0.5 µg ml^{-1} nitrapyrin.

3: 1ml of fresh inoculum +1ml of inoculum incubated 7 days with 0.05µg ml^{-1} nitrapyrin.

4: 1ml of fresh inoculum +1ml from treatment 3, to give 0.005µg ml-1 nitrapyrin.

5: 1ml of fresh inoculum+1ml of treatment 5, to give 0.0005µg ml^{-1} nitrapyrin 1ml of

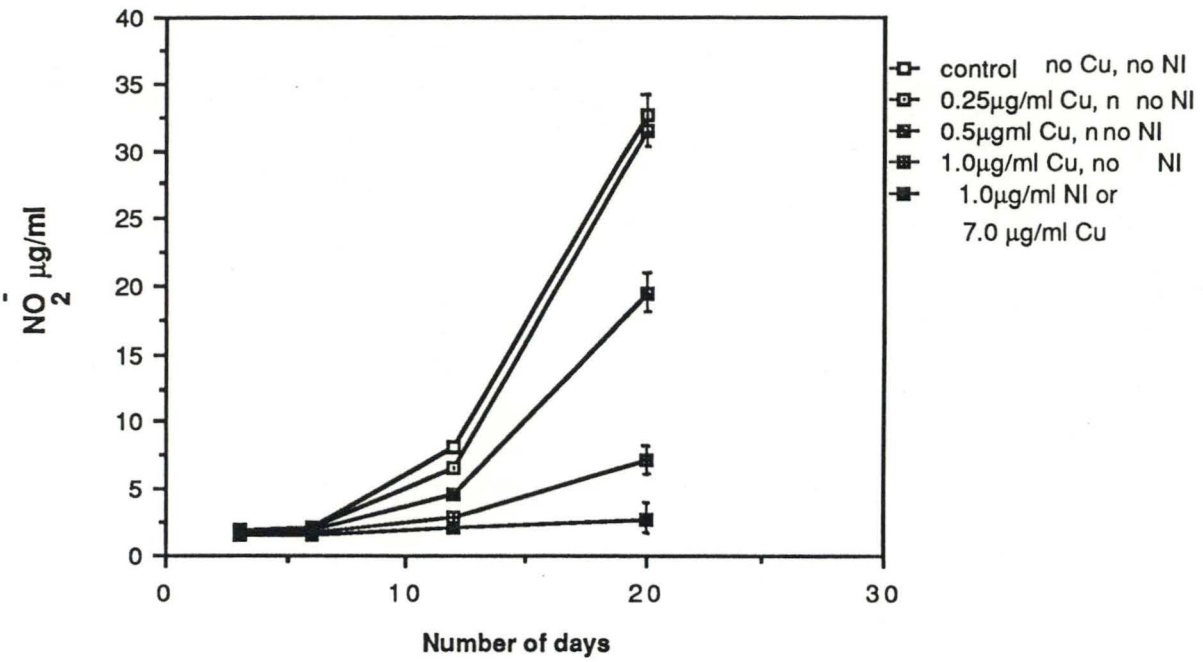
6: 1ml of water+1ml of inoculum incubated incubated 7 days with 0.05µg ml^{-1} nitrapyrin

7: 1ml of water + 1ml of treatment 3, to give .005µg ml^{-1}nitrapyrin.

8: 1ml of water+ 1ml of treatment 7, to give 0.0005µg ml^{-1} nitrapyrin.

9: 1ml of water+ 1ml of inoculum free of nitrapyrin, previously stored at 7- 8°C for 7 days with dilution to 10^{-2} prior to addition.
(fresh inoculum was not treated with nitrapyrin nor cool stored)
All cultures were incubated at 30°C up to 12 days.

Figure 2: Effect of Cu on nitrite production in-vitro



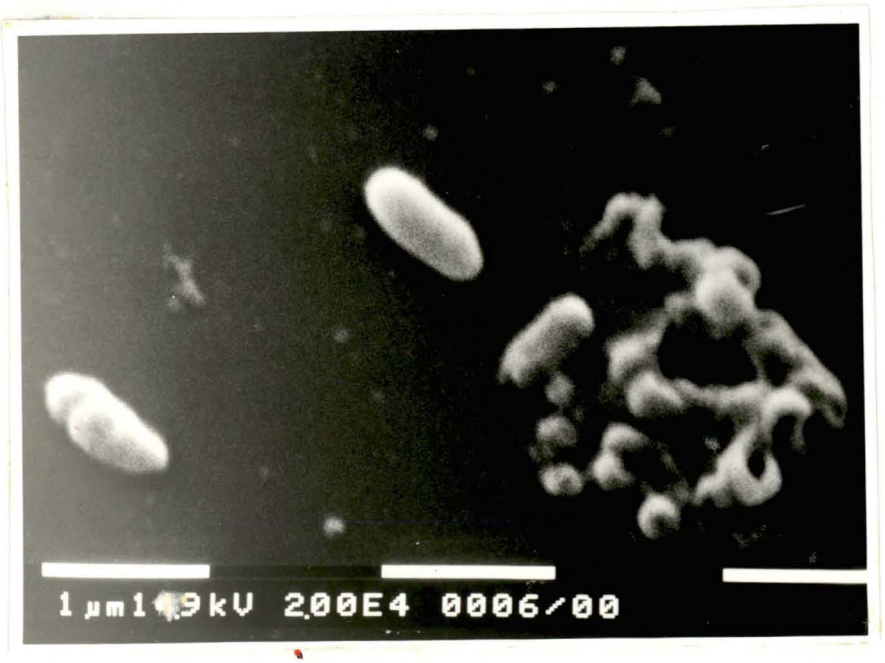


Plate i. Scanning electron micrograph of organisms growing on the surface of a cover slide submerged and incubated with culture solution.

REFERENCES

H. Babich and G. Stotzky. Environmnetal factors that influence the toxicity of heavy metal and gaseous pollutants to microorganisms. CRC Crit. Rev. Microbiol., 8, 99 (1980).

N.E.R.Campbell and M.I.H.Aleem. The effect of 2-chloro,6-(trichloromethyl) pyridine on the chemoautotrophic metabolism of nitrifying bacteria. Antonie van Leeuwenhoek, 31, 124 (1965).

C.A.I.Goring. Control of nitrification by 2-chloro, 6-(trichloromethyl) pyridine. Soil Sci., 93, 211 (1962).

A.B.Hooper and K.R.Terry. Specific inhibors of ammonia oxidation by Nitrosomonas. J. Bacteriol. 115, 480 (1973).

D.A. Laskowski and H.D.Bidlack. Nitrification recovery in soil after inhibition by nitrapyrin. Down to Earth, 33, 12 (1977).

S.J. Powell and J.I. Prosser. The effect of copper on inhibition of autotrophic ammonium oxidation by nitrapyrin. Curr. Mikrobiol.14,177(1986).

G.A.Rodgers and J.Ashworth. Bacteriostatic action of nitrification inhibitors. Can. J. Microbiol., 28, 1093(1982).

S.Soriano and N. Walker. Isolation of ammonia oxidizing autotrophic bacteia. J. Appl. Bacteriol. 31, 493 (1968).