Transformation and loss of excretal nitrogen under winter management systems

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Abstract

Excreta from cattle animals contain large amounts of nutrients, particularly nitrogen, which could lead to substantial gaseous losses of ammonia and nitrous oxide to the atmosphere, as well as nitrogen leaching. These losses are greatest during wet conditions in winter. However, the situation could be improved through moving cows off grazing paddocks to a stand-off pad or housing system. Therefore, it is necessary to quantify ammonia emissions and evaluate denitrification potential (which leads to emission of N_2O) through various winter management systems in order to determine methods and technologies for efficient and effective mitigation of gaseous emissions.

To understand the mechanism of nitrogen transformation and of reduction on gaseous emission from excreta in various winter management systems, a series of incubation studies and a field study were carried out investigating the suitability of several natural materials with absorbent properties, as media to reduce gaseous emission of ammonia and nitrous oxide.

The incubation studies were undertaken using cow excreta that consisted of a 1:1 (v:w) mixture of fresh urine and dung collected from a dairy farm. A lab incubation study was conducted using excreta, and excreta amended with soil and sawdust treatments. A further lab incubation study was carried out using different levels of natural materials. The field study consisted of two stand-off pads in which crushed pine bark or sawdust were used as bedding materials.

In the incubation study, ammonification was rapid in the case of excreta, compared to excreta amended with addition of natural materials. Whereas nitrification was very slow in the all treatments, only a small amount of nitrate ions could be detected till the end of incubation study.

In the incubation study, both soil and sawdust appeared to significantly reduce ammonia emission. In comparison to excreta, amendment with soil (excreta: soil=1:2, w:w) and sawdust (excreta: sawdust=1:2, w:v) reduced ammonia loss by 32.9% and 19.5%,

respectively. Excreta amended with a combination of soil and sawdust (1:1:1, w:w:v) was most effective, reducing ammonia emission by 34% under aerobic conditions.

Nitrate concentration was found to be the crucial limiting factor affecting the denitrification rate in the incubation studies. When KNO_3 was added to the excreta, the denitrification rate was 43.8μg N_2O-N/g excreta/hour. However, the denitrification rate of the excreta amended with both glucose-C and KNO_3 was 114.4μg N_2O-N/g excreta/hour. Denitrification potential followed: excreta> excreta with sawdust> excreta with soil.

On a field-scale stand-off pad, the carbon-rich natural materials pine bark and sawdust were shown to retain nitrogen effectively. After nine months of use, the bark retained 78% of the deposited excreta-N, while the sawdust pad retained 51%.

Therefore, it can be concluded that reduction of nitrogen losses can be achieved by using stand-off pad or housing systems (herd homes) which incorporate the use of a carbon rich natural material or soil in winter.

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Chapter 1 Introduction

1.1 The issue

Over the last few decades there has been concern in New Zealand at the extent of environmental contamination associated with pastoral farming; particularly the adverse effects on water and atmospheric quality (Bolan *et al.*, 2004a; Parliamentary Commissioner for the Environment Report, 2005). From 1994 to 2002 the number of dairy cows in New Zealand increased by 34% from 3.8 to 5.1 million; (Statistics New Zealand, 2004). Inevitably this large increase in cows has increased the impact of dairy farming on the environment. Feeding this large number of dairy cows has required high rates of fertiliser application, particularly of nitrogenous formulations. As well, the widely adopted policy of irrigating dairy farm effluent to land has meant that all manure nitrogen is returned to pasture. If nitrogen is applied to pasture in excess of plant uptake, the consequences can include nitrate leaching to surface and ground water, volatilisation of ammonia and emission of the greenhouse gas nitrous oxide (Muck and Richards, 1983; Buijsman *et al.*, 1987; Oosthoek *et al.*, 1991; Asman, 1992; Haynes and Williams, 1993; Bolan *et al.*, 2004a).

Under wet conditions high rates of nitrogen loss mainly through nitrate leaching have been reported from dairy farms; these conditions also enhance the emission of nitrous oxide (Luo *et al.*, 2000; De klein *et al.*, 2001). As these conditions are more frequent in colder months, attention is being given to mitigation through winter management systems (Luo *et al.*, 2000; Bolan *et al.*, 2004a). A successful winter management system will maintain satisfactory animal condition and pasture production while preventing adverse environmental effects. Desirable environmental outcomes under winter management systems include maintenance of soil structure and prevention of nutrient leaching and of emissions of ammonia and nitrous oxide. As adverse environment effects are not permitted under the Resource Management Act (1991) the Ministry for the Environment has issued regulations and guidelines for effluent management (MFE, 1995) and these apply to the manures and effluents collected from winter management systems. Guidelines for winter management pads have been

produced in consultations with farmers (Dexcel and Environment Waikato, 2005) that include a requirement for an appropriate effluent collection and retention systems. Although winter management systems are designed to improve environmental outcomes there could be some adverse effects from them, particularly those associated with undesirable gaseous losses. For example, de Klein and Ledgard (2001) suggested that the total NH_3 losses in the nil grazing system (housing shed) were higher than that in the conventional grazing system, with total nitrogen losses being 10-35% higher than under conventional grazing system. It has been reported that about 70% of the excreted nitrogen is transformed to ammonia gas over 9 months storage of manure in herd home bunkers (Longhurst *et al.*, 2006). Therefore it is necessary to understand nitrogen transformation processes and develop optimum mitigation technologies to reduce gaseous losses from winter management systems.

1.2 Winter management systems

Four winter management systems are used commonly on New Zealand dairy farms (Dexcel, 2005; Longhurst *et al.*, 2006): feed pads, stand-off pads, herd homes (wintering barns) and sacrificial paddocks. In the first three systems cows are physically removed from wet pasture for varying periods each day during the winter periods. In the sacrificial paddock system cows are generally left on the same paddock(s) throughout a wet period.

- Feed pads usually consist of a hard surface constructed from permanent materials, requiring an initial capital outlay. When conditions are wet, cows are held on the pad for 1 to 2 hours and are given supplementary feed. The principal purpose is to ensure the efficient consumption of feed without losses due to trampling of feed into wet soil. Urine and dung must be removed from the pad to an appropriate holding facility.

- Stand-off pads are used by cows for up to 20 hours a day which means that they need to be constructed from materials that do not adversely affect hooves and legs. A range of pad materials are in use including sawdust, bark, woodchips, either separately or mixed; sometimes with the addition of suitable proportions of lime or soft rock chip. Pads need to be large enough, usually 8

to 10 m^2 per cow, to accommodate cows for extended periods. Although the initial capital cost is relatively low, pad materials require frequent replacement, at least once a year; and some on-going maintenance to ensure that the surface is not covered with a deep layer of manure. Due to the length of time that cows spend on the pad a properly designed manure collection and storage system is an integral requirement of pad design.

- Herd homes (wintering barns) are a sophisticated design that provide shelter and incorporates a feeding platform and stand-off facility; together with a bunker to contain manure. The structure is made from permanent materials so that the initial cost is high (about $1000 per cow) (Pow T, personal communication). As cows can be maintained in a herd home for 24 hr a day they must be large enough to allow for cows to lie down. The platform needs specialised construction so that hooves and legs are not stressed and the design needs to ensure good airflow while maintaining shelter from adverse weather. The bunker system must be large enough to contain all the manure collected during wet periods.

- Sacrificial paddocks are a traditional wintering system that may have the feature of convenience but that has some undesirable environmental features. Cows remain on the same paddock(s) continuously throughout the wet period and this leads to pasture loss, soil damage and high rates of nutrient leaching and runoff. This sacrificed paddock is usually located close to the feed storage facility and milking yard. The condition of cows may deteriorate from the prolonged holding on wet soil (Longhurst R.D, personal communication).

1.3 Research objective

The overall objective of the study was to investigate nitrogen losses and nitrogen transformation from cow excreta. The research was aimed at selecting natural amendments with strong nutrient retention properties that are suitable for slowing down the transformations, thereby reducing gaseous emissions.

1.4 Layout of thesis

This thesis is comprised of seven chapters.

- Chapter 1 gives an overview of environmental issues and winter management systems.
- Chapter 2 reviews the scientific literature relating to dairying particularly under wet conditions and winter management systems; with emphasis on nitrogen cycle processes including mineralisation, ammonia volatilisation and denitrification potential.
- Chapter 3 describes the experimental design, materials and analytical methods used in the study.
- Chapter 4 reports the findings of laboratory scale investigations carried out to understand nitrogen transformations and ammonia volatilisation in excreta.
- Chapter 5 reports the findings of laboratory scale investigations of nitrification rates and denitrification potential in excreta amended with various natural materials.
- Chapter 6 reports the results of a field-scale investigation of emissions of ammonia and nitrous oxide from stand-off pads constructed from natural materials and of nitrogen transformations in pad leachate.
- Chapter 7 draws conclusions from the study findings and discusses their implications.

Chapter 2 Literature review

Pastoral farming is the major type of New Zealand agriculture. Pastoral animals convert the nutrients they ingest to the milk and/or meat products that are major export earners for the country. Only a small proportion of the nutrients that are ingested, including nitrogen, are converted to products with most excreted to pasture as urine and dung (Bolan *et al.*, 2004a). Excreted nitrogen can cause environmental problems including contamination of aquatic systems as a result of nitrate leaching and atmospheric pollution from ammonia and greenhouse gas (e.g., nitrous oxide) emissions. In New Zealand rainfall rates are high in winter, soils can become very wet, and under these conditions high rates of nitrogen leaching and emissions of gaseous nitrogen have been demonstrated (Luo *et al.*, 2000; De Klein *et al.*, 2001a). It has been suggested that better winter management on pastoral farms would be advantageous to the environment.

2.1 Nitrogen cycling under pastoral farming

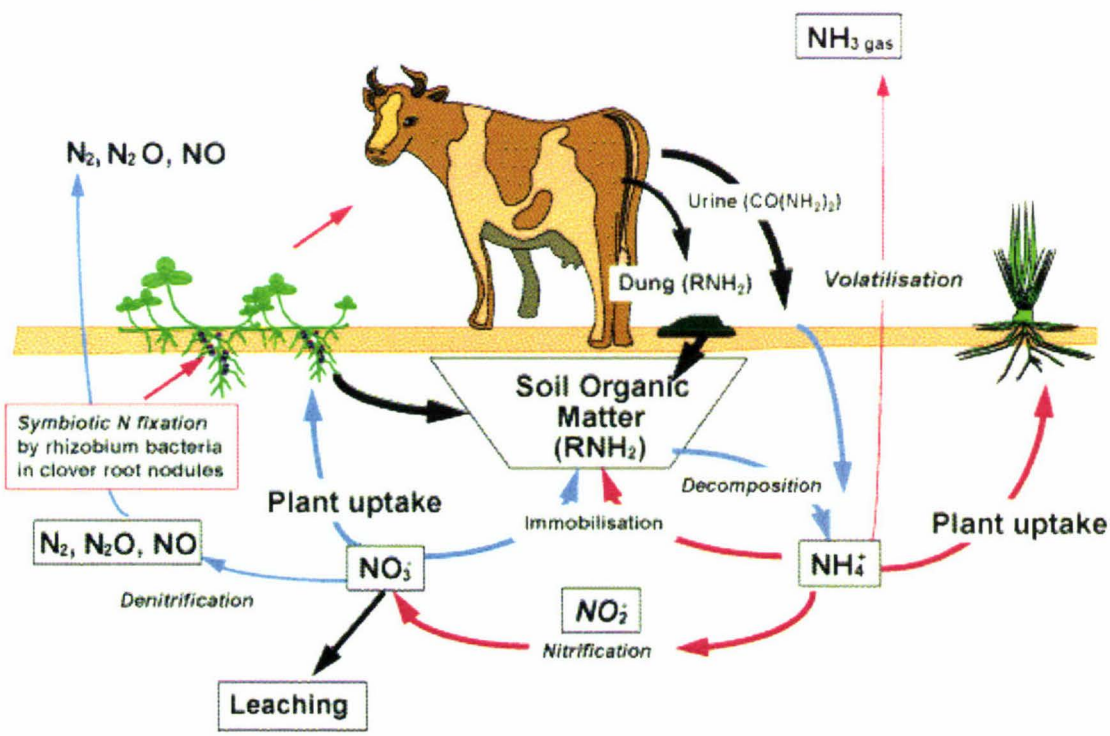

Fig. 2.1 Dynamics of nitrogen transformation in legume-based pastures (Bolan, 2004a).

The transformation of nitrogen in a legume-based pasture is shown in Fig. 2.1. Nitrogen undergoes a number of transformations that collectively constitute the nitrogen cycle. The nitrogen cycle involves the conversion of nitrogen from one form to another, mainly by enzymatic reactions that include nitrogen-fixation, amino acid formation, and mineralisation of organic nitrogen, nitrification and denitrification.

It has been reported that under pastoral farming in New Zealand the top 15cm of soil contains between 2,000 and 120,000 kg of nitrogen per hectare (Zhao, 2000). Soil-nitrogen occurs in organic and inorganic forms. Organic forms (proteins and amino acids) are found in plant roots, soil microorganisms and humus. Inorganic forms; ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-), are found in the aqueous soil solution and also (as ammonium-NH_4^+) within the structure of clay minerals absorbed to negatively charged exchange sites (Cameron and Haynes, 1986; Mclaren and Cameron, 1996). About 90% of the total nitrogen in most soils is in the form of organic nitrogen (Stevenson, 1986). A number of researchers have identified factors affecting N transformations in soil and these include pH, temperature, moisture and aeration state (Griffin and Honeycutt, 2002; Thomsen *et al.*, 2003).

2.1.1 Transformations of Nitrogen

The reactions that transform nitrogen from one form to another are biotic and abiotic. Biotic transformations include nitrogen mineralisation, nitrification, mineralisation and denitrification processes. Abiotic transformations include ammonium fixation, nitrate leaching and ammonia volatilisation. Bolan *et al.* (2004a) have comprehensively reviewed nitrogen transformation in pastoral systems.

2.1.2 Mineralisation

Mineralisation is the biological conversion of plant-unavailable organic forms of nitrogen (e.g., proteins and amino acids) to plant-available inorganic forms (e.g. $NH_4^+$and NO_3^-) by soil microorganisms (i.e. aminisation or ammonification reactions) (Bolan *et al.*, 2004a).

2.1.3 Immobilisation

Immobilisation, the reverse of mineralisation, occurs when soil microorganisms convert plant-available inorganic N (NH_4^+ and NO_3^-) to plant unavailable organic-N as a

consequence of their metabolism of inorganic nitrogen. The addition of carbon-rich materials (such as silage) to soil increases the rate of immobilisation. It has been found that when the soil C: N ratio is greater than 30, mineralisation predominates, and however, when the ratio is 20 or less, mineralisation exceeds immobilisation (Bolan *et al.*, 2004a).

2.1.4 Nitrification

Nitrification is the transformation of ammonium ions to nitrate ions, which also is a biological conversion. Nitrification is a two stage process in which NH_4^+ ions are first oxidised to nitrite ions (NO_2^-), after which, in step 2, nitrate is converted to NO_3^- ions. Stage 1 is carried out by *Nitrosomonas* bacteria and stage 2 by *Nitrobacter* bacteria. Stage 2 occurs immediately after stage 1.

$NH_4^+ + 1.5\ O_2$ (fast) $\rightarrow NO_2 + H_2O + 2H^+$ Stage 1

$NO_2^- + 0.5\ O_2$ (very fast) $\rightarrow NO_3^-$ Stage 2

Soil chemical and physical properties, such as temperature, moisture content, pH, aeration state, as well as C/N ratio, influence nitrification rates.

2.1.5 Ammonium fixation

Ammonium fixation can be described as a process in which either ammonium ions are retained on the surface of soil particles due to a cation exchange effect or temporally fixed in the interlayers of 2:1 phyllosilicate clay minerals. NH_4^+ ions can be replaced by other cations of a similar size (Mclaren and Cameron, 1996).

2.1.6 Denitrification

Denitrification is a biological process which can lead to loss of valuable nitrogen from the soil-plant system. The process is carried out under anoxic or anaerobic conditions. Bacteria convert nitrate ions to N_2O or N_2 gases (Firestone, 1982; Stevenson, 1986).

$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$

(5+) (3+) (2+) (1+) (0)

2.2 Inputs of nitrogen to pastoral farmland

2.2.1 Atmospheric deposition

Nitrogen deposition adds nitrogen onto the surface of soils. There are two mechanisms: wet and dry deposition. Dry deposition occurs through gravitational influence and molecular diffusion of dust particles. In contrast, wet deposition occurs due to inputs of rain or snow precipitation. Although these depositions are very important on a global scale, Ledgard (1992) showed that the amount of nitrogen returned to pastoral soil by atmospheric is less than 15 kg ha $^{-1}$ yr $^{-1}$ and 0 to 3 kg ha^{-1} yr^{-1} by rainfall in New Zealand, and this process may fail to fulfil the nitrogen requirements of intensive animal production.

2.2.2 Biological N_2-fixation

Biological fixation of atmospheric N is usually carried out in legume-based pasture through the root nodule bacterium, *Rhizobium,* in association with clover (Davidson and Robson, 1986; Haynes, 1986; Bolan *et al.*, 2004a). Gallon and Chaplin (1987), Ledgard and Steele (1992) and Bolan *et al.* (2004a) have reported details of physical and biochemical processes of biological N_2-fixation. These workers found factors that affected N_2 fixation included nutrient supply, temperature and moisture, fertiliser application and legume species. Excessive amounts of N-fertiliser application lead to a decline in N-fixation rates (Ledgard *et al.*, 1996).

2.2.3 Nitrogen fertiliser

Sustaining high production of pasture on many grassland soils relies on fertiliser inputs, especially in less-fertile soils (Bollin and Arrhenius, 1977; Bolan *et al.*, 2004a). Mineral N fertiliser applications in spring are likely to be beneficial to pastures in New Zealand as pastures need N-nutrient replenishment due to the large amount of N removed when grass is harvested (e.g. to make silage). Late-winter is the favoured time for calving, so a small amount of N-fertiliser may be applied to meet pasture requirements at this time. However, mineralisation rates will be low when conditions are cold, impeding pasture growth. Many reports have quoted favourable yield responses to N-fertiliser application to the grass-based intensive pasture production when applications coincide with plant growth requirements (Whitehead, 1995; McLaren and Cameron, 1996).

2.2.4 Effluent and manure application

Effluent irrigation and manure application are widely used in New Zealand and some

European countries to increase pasture yield and maintain soil properties, such as cation exchange capacity, water holding capacity as well as organic matter content and soil nutrient availability. Bolan and Adriano (2004c) reported that in the US, levels of N and P nutrients sourced from manure by-products were about 83% and 143% respectively of that of commercial fertilisers. In general, farm effluents have a large amount of nutrients. For example, in New Zealand, effluent from dairy and piggery farms which contain large amount of N and P nutrient could satisfy the requirements of 40,500 ha of corn for N and 62,500 ha of pasture for P respectively (Bolan and Wang, 2004b). In global terms, application of these effluents could provide sufficient N for 6.7 million ha of corn and P for 10.5 million ha of pasture (Bolan and Wong, 2004b). In order to minimise contamination of effluent to surface water and conform to the Resource Management Act (1991), land disposal has been widely adopted in New Zealand (Selvarajah, 1999). There are some limitations to land disposal, such as requirements for pond storage facilities, and management of application when soil and climate conditions are unfavourable. Bolan and Wang (2004b) found that if bark and zeolite materials were added into two-pond systems, they were more effective in reducing N, P, and K from effluent. Reduction of nutrients could mean that the effluent would not need to be applied to such a large land area.

2.3 Losses of nitrogen from pasture farmland

The fate of nitrogen in pastoral agriculture includes the conversion of pasture-N into animal tissues and products. However there will also be direct losses to the environment due to excretion of nitrogen by animals. The latter can lead to environmental problems including ammonia volatilisation, denitrification and nitrate leaching to surface water and ground water. Two of these processes, ammonia volatilisation and denitrification, produce gaseous nitrogen emissions.

2.3.1 Ammonia volatilisation

The main sources of ammonia in pastoral soils are N-fertiliser, protein, amino acids and amides, urea and hippuric acid (from animal urine).

(1) Release of ammonia from breakdown of urea

I. $(NH_2)_2CO+2H_2O \rightarrow (NH_4)_2CO_3$

II. $(NH_4)_2CO_3 \rightarrow NH_4^+ + CO_3^{2-}$

III. $CO_3^{2-}+H_2O \rightarrow HCO_3^-+OH^-$

IV. $NH_4^+ + OH^- \rightarrow NH_3\uparrow + H_2O$ Equation (a)

Urea, which accounts for about 70-90% of urine-N, is hydrolysed by the enzyme urease to NH_4^+ ions, which will cause a high pH around hydrolysis sites.

(2) Release of ammonia from breakdown of hippuric acid

I. $C_5H_4N_4O_3+H_2O+1/2O_2 \rightarrow C_4H_6N_4O_3+CO_2$

II. $C_4H_6O_3+2H_2O \rightarrow 2CO(NH_2)_2+CHOCOOH$

III. $(NH_2)_2CO+2H_2O \rightarrow (NH_4)_2CO_3$

IV. $(NH_4)_2CO_3 \rightarrow NH_4^+ + CO_3^{2-}$

V. $CO_3^{2-}+H_2O \rightarrow HCO_3^-+OH^-$

VI. $NH_4^+ + OH^- \rightarrow NH_3\uparrow + H_2O$ Equation (b)

(3) Release of ammonia from the decomposition of protein

I. Proteins→ amino acids

II. $RCHNH_2COOH+O_2 \rightarrow RCOOH+CO_2+NH_3\uparrow$

Or $RCHNH_2COOH+H_2O \rightarrow RCH_2OH+CO_2+NH_3\uparrow$ Equation (c)

Generally, protein and ammonium ions excreted directly by animals are derived from faeces. Mineralisation of protein in faeces is a multi-step process. First step, protein macromolecules are hydrolysed to peptides and amino acids through proteolytic and deaminative bacterial activity. Then, the products of this hydrolysis, the peptides and amino acids, are further degraded by deamination to NH_4^+ ions. Urine and dung mineralisation, as well as N-fertiliser application result in release of NH_4^+ ions, which can be transformed to gaseous NH_3 emissions. As NH_4^+ ions accumulate in soil, there is an associated increase in soil pH.

$NH_4^+ + OH^- \rightarrow NH_3\uparrow + H_2O$ Equation (d)

The equilibrium between NH_4^+ ions and NH_3 depends on many factors, among of which the most important are soil pH and temperature (Haynes and Sherlock, 1986). Other affecting factors include soil moisture, soil texture, soil cation exchange capacity, pressure equilibrium between the interfaces of soil and atmosphere, C/N ratio, and wind velocity (Bolan, *et al.*, 2004a).

A positive correlation between the rate of ammonia volatilisation and soil temperature at a 3cm depth within 3 days of urine deposition was reported by Lockyer and Whitehead (1990). Muck and Richards (1980) found urea could be hydrolysed and converted to NH_4^+ ions within a few hours, depending on pH, moisture and temperature. It has been reported that the optimum pH for urease enzyme activity (the enzyme that catalyses urea hydrolysis) is pH 8.6 (Vlek *et al.*, 1980; Sherlock and Goh, 1984). Hadas *et al.* (1983) illustrated that an increase in the incubation temperature from 14 °C to 35 °C was conducive to organic N mineralisation, under aerobic conditions. As the concentration of NH_3 increases from 0.1% to 1%, 10%, and 50%, the pH increases from 6 to 7, 8, and 9 respectively (Freney *et al.*, 1983). De Bode (1991) and Sommer (1992) reported N losses were greater from a mixture of dung and urine in summer than that in winter. Although it is considered that hydrolysis of urea from the urine of grazing animals would usually be completed within a few days, the process would occur very slowly under very low temperature (Fenn and Hossner, 1985).

Soil cation exchange capacity (CEC) also has an influence on NH_3 emission because NH_4^+ ions (the product of decomposition), can be absorbed by negatively charged cation exchange sites, delaying volatilisation. Some researchers have found an inverse relationship between the amount of NH_4^+ ions and NH_3 losses. It has been demonstrated that the stronger the absorption capacity of soil sites, the lower the rate of NH_4^+ ion conversion to NH_3 gas (Selvarajah *et al.*, 1989; Whitehead and Raistrick, 1993).

The influence of soil moisture on NH_3 losses has been elucidated by some workers. For example, Birch (1964) and Cabrera (1993) showed that when air-dried soil was rewetted, mineralisation of nitrogen increased owing to resumption of microbial activity. Standford and Epstein (1974) concluded there was a linear relationship between soil nitrogen mineralisation and soil water content, over the range of 0.33-15 bars, although this varied with soil types.

Soil moisture and temperature are two major abiotic factors that affect microbial activity in soils. Water shortage in soils can limit nutrient movement (including NH_4^+) and air fluidity, with a consequent decrease in microbial activity.

The addition of some substances with high C: N ratios can cause immobilisation of soil N by the microorganisms, thereby decreasing the amount of plant-available soil N. Barbarika *et al.* (1985) showed that increasing C to N ratios had a negative affect on N mineralisation. If C-rich substances, such as sawdust, wood chips and bark are added to soil, the effect can be a reduction in N mineralisation, resulting in a reduction in NH_3 losses (Luo *et al.*, 2004). Research has shown that when the ratio of C/N is greater than 30, nitrogen immobilisation predominates rather than mineralisation in soil (Bolan *et al.*, 2004a). In the C: N ratio range of 20-30, mineralisation and mineralisation are about equal. In contrast, Wagner and Wolf (1999) believed ratios of C/N less than 20 probably lead to net mineralisation.

2.3.2 Denitrification

2.3.2.1 Definition of denitrification

Denitrification can lead to loss of valuable soil nitrogen by conversion to atmospheric nitrogen gas as well as emission of the undesirable green house gas N_2O. There have been many comprehensive literature reviews published on denitrification, including Fillery (1983), Tiedje (1988), Nieder *et al.* (1989), Eichner (1990) and Luo *et al.* (1999a, 1999b, 2000).

Denitrification is an agriculturally important process that transforms NO_3^- ions to gaseous products. It can occur in both aquatic and terrestrial ecosystems. Although it can lead to considerable N losses from agricultural systems, it can also reduce levels of nitrate-nitrogen (NO_3^-), which can be leached to ground or surface water. Recently a number of reviews have discussed the environmental implications of denitrification that included depletion of stratospheric ozone and possible deleterious effects on global warming and climate change (Wang *et al.*, 1976; Knowles, 1982). Many studies at both laboratory and field scale have identified correlations between adverse environmental factors and denitrification (Knowles, 1982; Rolston *et al.*, 1984; Davidson and Swank, 1986; Aulakh *et al.*, 1991a; Luo *et al.*, 2000). Kuenen *et al.* (1994) found nitrification and denitrification could occur simultaneously with production of a range of NO_x gases. Dalal *et al.* (2003) reported three pathways of N_2O production in soils:

(1) Nitrification, during which process nitrite was used as an electron acceptor, finally reduced to N_2O.

(2) Denitrification, i.e. dissimilatory nitrate reduction.

(3) Assimilatory nitrate reduction. Assimilatory nitrate reduction is likely to be of minor importance as it is easily inhibited by low concentrations of ammonium-nitrogen and soluble organic nitrogen in soils.

2.3.2.2 Denitrification in grazed pasture

Luo *et al.* (1999a) and De Klein *et al.* (2001a) have demonstrated high rates of N loss due to denitrification in grazed pasture compared to those in ungrazed pasture. As the excreta of animals contain readily degradable carbon and nitrogen, higher rates of denitrification are expected under grazing (Saggar *et al.*, 2002). Pugging by animals causing soil compaction enhances anaerobic conditions and facilitates denitrification (Oenema *et al.*, 1997). In particular urine patches in grazed pasture are active sites of denitrification due to carbon availability from soil organic matter and the high pH resulting from hydrolysis of urea to ammonia. It has been estimated that about 20-40% of urine-N undergoes denitrification (Fraser *et al.*, 1994; Clough *et al.*, 1996; Bhandral *et al.*, 2003). Ledgard *et al.* (1999) and Luo *et al.* (2000) determined the total New Zealand N losses via denitrification in non-fertilised grazed ryegrass/clover pasture at 5 kg N ha^{-1}.

2.3.2 3 Factors affecting denitrification

Generally, denitrification rates are favoured by the availability of soluble carbon, moderate soil temperature, high soil moisture and anaerobic conditions, neutral pH as well as an adequate concentration of NO_3^--N.

Carbon availability

Studies show the rate of denitrification depends on soil organic carbon content, especially the soluble and readily decomposable carbon that are used as substrates for denitrifying bacterial growth (Burford and Bremner, 1975; Payne, 1981; Reddy *et al.*, 1982; Myrold and Tiedje, 1985; Drury *et al.*, 1991; Iqbal, 1992). Amendment of soil by addition of organic materials, such as plant residues or animal excreta, can stimulate the denitrification process (Paul and Beauchamp, 1989; Aulakh *et al.*, 1991a; Dorland and Beauchamp, 1991). The addition of soluble-C compounds (i.e. degradable carbon), such as glucose, stimulate the rate faster than complex C compounds containing cellulose or lignin that require degradation prior to utilisation as a substrate for

denitrification. It has been demonstrated that an increase in readily available carbon reduces the ratio of N_2O/N_2 (Arah and Smith, 1990; Weier *et al.*, 1993; Dendooven *et al.*, 1994). It has also been shown that incorporation of plant materials enhances the rate of denitrification (Aulakh *et al.*, 1991a).

Soil temperature

As soil temperature affects microorganism activities, it will also influence denitrification rates. Denitrification occurs over a very wide temperature range (0-75°C), However, there may be different optimal temperatures in various regions (Knowles, 1982; Malhi *et al.*, 1990). Some workers reported that addition of organic manure and mineral N-fertiliser to soil enhanced denitrification at low temperatures, but the others concluded denitrification rates were reduced by low soil temperatures (Thompson *et al.*, 1987; Jarvis *et al.*, 1991a, b; De Klein and van Logtestijn, 1994; Schwartz *et al.*, 1994). In New Zealand, although the soil temperature is usually 10°C or less during winter period, quite high denitrification rates have been reported. These high rates have been attributed to the high rate of precipitations, which raised soil moisture content and low level of evaporation (Luo *et al.*, 2000). Keeney *et al.* (1979) concluded from a soil incubation study under controlled conditions that a decrease of temperature increased N_2O emission rate during the denitrification process, thereby increasing the ratio of N_2O/N_2. Conversely, Maag and Vinther (1996) concluded the ratio of N_2O/N_2 from denitrification decreased as the temperature declined.

Soil moisture and aeration

Soil moisture content affects oxygen diffusion rate and the aerobic status of the soil and therefore affects denitrification (a process that occurs in the absence of oxygen). Some field studies have shown high denitrification rates correlated with soil aerobic status and increased when there was high moisture (Ledgard *et al.*, 1999; Luo *et al.*, 2000). As there is considerable evidence for a positive correlation between soil water content and denitrification rate (Parsons *et al.*, 1991; Weier *et al.*, 1993), the soil water threshold, i.e. "water-filled porosity" is measured to determine the relationship between denitrification rates and soil water content. If the water content is above the threshold, there will be a positive correlation. However, below the critical water threshold, there appears to be no relationship between them (De Klein and van Logtestijn, 1994). Addition of organic material amendments and manure to aerobic soil can be followed

by an increase in denitrification rate as aerobic bacteria decompose these materials, synchronically consuming oxygen and leading to anaerobic conditions (Guenzi *et al.*, 1978; Aulakh and Rennie, 1985). Bakken *et al.* (1987) found that soil compaction by heavy agricultural vehicles increased denitrification rates since it caused oxygen reduction. Similarly, Luo *et al.* (1999b) found low oxygen levels caused by animal treading grazed wet pasture could enhance denitrification rates.

Soil pH

The favorable pH for denitrifying bacteria is within the range pH 6-8, moreover, the optimum rate for both nitrification, which is a necessary pre cursor for denitrification, and denitrification are in the range of pH 7-8 (Focht and Verstraete, 1977; Haynes, 1986). However, Knowles (1982) found denitrification could occur over a broader pH range although the rate decreased under acid condition (Bryan, 1981), moreover, it has been found to occur at pH values as low as 3.3 in naturally acid soils (Weier and Gilliam, 1986). Some reports have postulated an indirect influence of low pH as a result of low absorbable carbon availability so that rates of denitirfication were generally low in acid soils (Koskinen and Keeney, 1982; Fillery, 1983). Barton *et al.* (1999) showed that in comparison with ammonium-based fertiliser (DAP), application of urea fertiliser was accompanied by high rates of N loss from denitrification due to an increase of pH from the initial ammonification process (urea-ammonium) that increased nitrate concentration and also soil carbon availability. It also has been observed that the ratio of N_2O/N_2 was negatively correlated in an exponential relationship with soil pH in both laboratory and field studies that examined a wide range of soil types (Rochester, 2003). Christensen (1985) and Parkin *et al.* (1985) concluded soil acidity affected the ratio of N_2O:N_2 and that the proportion of N_2O increased as the soil pH decreased.

Nitrate concentration

An adequate supply of nitrate-N for denitrifying bacteria is an essential precursor to biological denitrification. Generally, without any other factors being limited, denitrification rates increase consistently with increasing NO_3^- content in soil (Ledgard *et al.*, 1999; Saggar *et al.*, 2002; Dalal *et al.*, 2003). The availability of NO_3^- as a substrate for denitrifying bacteria depends on the rate of nitrification (ammonium→ nitrite→ nitrate) and the rate of other processes that decrease NO_3^-, including NO_3^- leaching and plant intake. Luo *et al.* (1999b) showed that when the soil

moisture of grazed pasture was low, the rate of diffusion of NO_3^- would decrease and limit denitrification. It has been observed that high nitrate concentrations inhibited N_2O reduction to N_2 so that relatively low NO_3^- concentrations promoted N_2 formation, conversely, high NO_3^- concentrations was conducive to N_2O production (Bremner and Blackmer , 1978; Arah and Smith, 1990).

2.3.2 4 Methods for measuring denitrification

Over the last few decades, direct gas measurement methods have been developed (Smith, 1987; Tieje *et al.*, 1989; Moiser, 1990; Myrold, 1991; Luo *et al.*, 1999a). The methods include:

- The use of acetylene (C_2H_2) to inhibit N_2O reduction to N_2 in order that all denitrification N loss will be measured in the form of N_2O; this method is used in conjunction with gas chromatography with a N_2O detection system. This is the one of most widely used methods in denitrification studies.
- A micrometeorological approach is a conceptually ideal method to measure trace gases emissions over large ecologically uniform areas, and the technique can reduce the problems of spatial variability that limit the accuracy of other technologies when sampling. The shortcoming of this method is that the techniques have not been extensively used to measure N_2O flux, as analytical methods to quantify sensitively and respond rapidly to N_2O are not yet available.
- The use of labelled isotopes, such as ^{15}N-labelled fertiliser, is relatively common as a technique to measure denitrification. The ^{15}N-labelled gases are then determined by mass spectrometry that allows quantification of the N_2 that is solely produced from denitrification.

2.3.2.5 Denitrification enzyme activity

As the measurement of denitrification rate at the field scale is limited by the temporal and spatial variability, accurate measurement is quite difficult. In order to overcome this deficiency, laboratory experiments are often used to indicate the potential for denitrification in the test soil.

This can be done by the denitrification enzyme activity (DEA) measurement that is carried out (Tiedje, 1982) under anaerobic conditions without of any limitation of NO_3^--

N and C availability. In practice it has been found that the results from this procedure are strongly correlated with actual annual rates of denitrification (Groffman and Tiedje, 1989). However, more work is required to allow DEA values from various sites to be compared.

2.3.3 Leaching of nitrate-nitrogen

Recently, nitrate leaching and water pollution have received considerable attention due to intensification by the dairy industry (Spalding and Exner, 1993; Cameron *et al.*, 1997). Nitrate leaching is a public concern due to its effect on the quality of drinking water for human and animals. The World Health Organization has a specified drinking water standard of less than 11.3 mg NO_3^--N L^{-1} as higher concentrations may cause methemoglobiemia in human infants and miscarriage in cattle (Golden and Leifert, 1999). Due to its high solubility, nitrate is readily leached when water drains through the soil (McLaren and Cameron, 1996) and workers, including Cameron and Haynes (1986); Follett *et al.* (1991) and Di *et al.* (2002), have described factors affecting this leaching and have developed management strategies to reduce it.

As drainage volume and concentration of NO_3^- are two major factors that affect the extent of NO_3^--N leaching, removing cows from pasture land during wet autumn-winter periods should minimise NO_3^- leaching. Ledgard *et al.* (1996) found that under rainfall resulting in drainage of 550-620 mm, with N-fertiliser (Urea) added at rates of 0, 200, 400 kg N/ha/yr to a free-fraining soil of volcanic material (Umbric Dystrochrept), the NO_3^--N concentration in the leachates they collected was 12, 18 and 37 mg/L, respectively. Silva *et al.* (1999) found when urine (1000 kg N/ha) was applied in combination with dairy shed effluent (200 kg N/ha) and urea (200kg N/ha), the mineral N leaching loss was up to 14% of the total N applied. A number of workers including Di *et al.* (1998), Carey *et al.* (1997), Mclaren *et al.* (1999) and Silva *et al.* (1999) showed that the potential for NO_3^- leaching depended on the form in which nitrogen is applied with a descending order of leaching: pig slurry>urea>dairy shed effluent>sewage sludge. These findings were attributed to the high proportions of mineralised N in pig slurry that promoted leaching losses, compared with predominantly highly recalcitrant organic-N compounds in sewage sludge.

The presence of organic substrates and nutrients promoted soil microbial biomass N and extracellular enzyme activities (protease, deaminase and urease) after the application of dairy shed effluent. The nitrification rate was significantly correlated to biomass N and the enzyme activities (Zaman *et al.*, 1999).

2.4 Winter management systems

2.4.1 Types of winter management systems

The objective of winter management systems on pastoral farms is to protect soil quality and maintain pasture production under wet conditions. Ledgard *et al.* (1996) reported that a consequence of cattle grazing under very wet conditions was a 20 to 80% decrease in pasture production depending on soil types, and that these consequences could last for 4 to 8 months. De Klein (2001a) reported that when cows were kept off wet paddocks there was an increase of 20% or more in pasture yield. In practical terms it may be advantageous to allow some grazing under wet conditions to meet at least some of the animals feed requirements. For example, Ward and Greenwood (2002) and Fisher *et al.* (2003) found that feed demand could be meet by 3 to 5 hours of grazing. In studies of different winter grazing periods: 2, 4 or 12 hours it was found that pasture losses were 19, 28 or 40% respectively, demonstrating the effectiveness of reducing grazing time (Fisher *et al.*, 2003). Decreasing grazing of pasture under wet conditions has been shown to be accompanied by increases in milk quantity and quality (Hedley and Kolver, 2006). De Klein and Ledgard (2001a) studied winter managements systems in which excreta were collected and stored for an extended period before application of the effluent to farmland. During storage they observed significant transformation of nitrogen into gaseous form or leaching. Effluent irrigation to land, rather than direct discharge to waterways, is favoured by environmental authorities as it reduces contamination of surface water and from a farm point of view all the nitrogen excreted by animals is returned to the soil.

Current New Zealand winter management systems are of four types: sacrificial paddocks, feed pads, stand-off pads and herd homes or wintering barns (Table 2.1). Each of these systems has advantages and disadvantages that farmers need to consider

before making a decision on which to use. These include capital and operational costs, environmental impacts and regulatory requirements.

2.4.2 Advantages and disadvantages of four winter management systems

2.4.2.1 Sacrificial paddock

Sacrificial paddocks are the traditional winter management system in which cows are held on the same paddock throughout the wet season rather than being moved under normal paddock rotation. This system does not require capital expenditure and there are no associated labour costs as cows remain on the same paddock 24/7 including being fed supplementary feedstuffs in same areas. After the wet period the cows are removed to allow recovery of the sacrificial paddock. Problems associated with this system include the treading and pugging of wet soil that adversely affects soil structure and reduces subsequent pasture growth. As well, cows are susceptible to mastitis and lameness from lying on wet compacted ground. Environmental issues include contamination of ground and surface water by the high rates of leaching and overland flow. In addition these paddocks generate substantial gaseous emissions of nitrous oxide (Luo *et al.*, 1999b; De Klein *et al.*, 2001b). Overall, problems resulting from utilisation of this system exceed the benefits.

2.4.2.2. Feed pad

A feed pad is a type of winter management system in which an area, usually in close proximity to the dairy shed is covered with concrete or some other permanent material. Its principal purpose is to provide an area where supplements are fed to cows so as to avoid losses that occur if animals trample feed into wet ground. The hard surface is durable and easy for farmers to clean. However, there could be a risk of injury due to slipping and prolonged standing could damage hooves and legs. Normally cows remain for only 1-2 hours before or after milking. There is a capital cost associated with building feed pads and labour and other costs associated with removing manure from the surface. This is usually done by scraping and washing and a storage pit and/or other facilities are required to hold the manure/effluent prior to disposal.

The advantages include reduction of feed wastes compared to paddock feeding, as the trough or feed lanes are mounted above ground. A concrete surface is easily scraped by

a tractor with a large scraper blade and a final hosing down produces a relatively small amount of liquid waste (effluent). However, to protect the environment, the manure storage facility and tank (or pond) receiving liquid wastes needs to be large enough to contain the effluent until conditions are suitable for land disposal. Such land disposal allows return of excreted nutrients to the farm.

2.4.2.3 Stand-off pads

Stand-off pads are usually constructed from a suitable free-draining material; such as carbon-rich materials like sawdust, bark or wood chips or soft metal (rock) or lime chips. They allow cows to remain on the stand-off pad for about 20 hours per day and minimise the damage to soil and pasture. Because of the prolonged time spent on pads the area must allow 8-10 m^2 for each cow. In addition cows may require supplementary feeding while on the pad and this needs to be taken into account when designing these pads. Some workers are currently evaluating various treatment systems to improve the quality of this leachate prior to application to land. The accumulated pad leachate can be applied to pasture when conditions are suitable to allow return of nutrients to the farm. A stand-off pad system requires initial capital investment and there are associated labour costs in moving cows on and off the pad to allow time for pasture grazing. There may be some difficulty in obtaining suitable materials in some locations and these also need to be replaced regularly.

Table 2.1 Summary of characteristics of winter management systems.

System	Features
Sacrificial paddock	
Traditional winter management system on which keep cows stay 24/7, with supplements feed in same area.	Cows treading and pugging damages wet soil reducing spring pasture growth. Non point-source contamination of surface and groundwater. Cows are susceptible mastitis and lameness from lying on wet ground.
Feed pad	
A surface of permanent (hard) material, near the milking shed, on which cows stand for 1 – 2 hr per day before or after milking to receive supplementary feed	Moderate capital cost. Manure easily removed by scraping and washing. Effluent storage facility required which could be the farm pond.
Stand-off pad	
A permanent structure that contains soft-draining material on which cows are kept for up to 20 hours a day. Typically sized at 8-10m^2 per cow.	The design requires a proper drainage system and substantial effluent storage capacity. Design needs to incorporate an ability to supply supplements to cows on these pads. Suitable soft materials need to be obtained and replaced annually. Soil and pasture benefit from very reduced grazing and the associated environmental damage is avoided.
Herd home	
This kind of system combines a feeding platform, stand-off pad facility, shelter and concrete floor and bunker in which cows can be withheld from pasture 24/7 if required. There are rooms for cows can lie down	The capital cost is high (about $1000/cow). Heed homes prevent environmental damage to wet soil. Cows health and production improves. The bunker must be adequate to contain manure until conditions are suitable for land application. Gaseous emissions, such as ammonia and hydrogen sulphide, need to be addressed.

2.4.2.4 Herd homes

Herd homes or wintering barns are a new type of winter management system to New Zealand that essentially combines the features of both feed pad and stand-off pads and allows cows to be kept off pasture for up to 24/7 if necessary. A herd home is usually

covered (e.g. with a plastic roof), and the "floor" is constructed from specially designed concrete slats that allow cows to stand for prolonged periods without damage to hoof and legs. Below the slatted surface there is a bunker for collection of dung and urine, which may contain 150 - 200mm depth of soil as an absorbent. A herd home is intended to provide a comfortable place for cows to get through a prolonged time in the wet winter. The advantages of herd homes include the size being large enough to allow cows to lie down and the slatted floor that allows dung and urine to fall into an underground bunker from which the manure can easily be removed when required. It is claimed that the favourable conditions of a herd home improve the cow's health and production. The environmental benefits of herd homes are avoidance of pugging wet soil and associated pasture damage. It has been shown that the use of carbon-rich natural materials in bunkers alleviates gaseous loss of ammonia and hydrogen sulfide by absorbing/absorption mechanism. A disadvantage of the system is the high initial capital costs of approximately $1000 per cow but the nature of the materials used in construction means that these structures are very durable.

2.4.3 Environment implications associated with winter management systems

2.4.3.1 Ammonia volatilisation

All winter management systems produce ammonia and hydrogen sulphate emissions, which can cause human beings health problem and accelerate global warming, as well as depletion of the stratospheric ozone layer (O'Neill and Phillips, 1992). Historically, it can be tracked back to the 19th century, when agronomists began to explore the best way to cope with ammonia loss from dairy farms (Sprengel, 1839; Boussingault, 1851; Heiden, 1887; Wagner *et al.*, 1897). They found that ammonia losses were associated with livestock waste management and slurry application into farmland. In the following years, studies were conducted on reducing ammonia losses, by addition of chemicals, acids, salts and formalin. Other studies suggested applying waste to soil and immediately harrowing it was an improved method to inhibit ammonia emission (Blank, 1918; Gerlach, 1919; Iversen, 1934). More recently, with dairy livestock systems rapidly developing and the stocking density of cows increasing there has been more concern about ammonia losses and volatilisation, especially the last two decades. Cattle farming, particularly dairy farming is regarded as the largest husbandry source of NH_3

emission. Total ammonia losses from animal husbandry grew by about 50% between 1950 and 1980 (Apsimon *et al.*, 1987). In the diary farm system, it is estimated that less than 30% total nitrogen feed intake is retained in cows' milk or meat, with 50 to 80% of the reminder excreted in the urine and 20-50% excreted in the faeces. So N intake, N digestion and excretion, as well as N losses and N supplement constitutes whole N recycle system (Whitehead, 1970, 1986; McCrory and Hobbs, 2001).

Excreta is usually stored or composted in the paddock. Due to this storing method, a large amount of N can be lost through ammonia volatilisation, along with lost amounts by nitrate-N leaching into underground water. Ammonia volatilisation mainly derives from the decomposition of cows' urine and dung with other sources being plant residue and decaying dead animal bodies (Isermann, 1994). Haynes and Williams (1993) showed that more than a third of pasture land in intensive high output dairy systems were affected by excreta. Urine-N (containing 70-90% of nitrogen as urea-N) is rapidly hydrolysed to forms which can be taken by plants within 3-5 days. The conversion period is much shorter than dung decomposition. Afzal and Adams (1992) showed the mineralised N content of a urine patch was over 10 times higher than a dung patch and 30 as much as an area containing no excreta. Dewes and Schmitt (1990) found the nitrogen emission during the storage of liquid livestock manures were higher than they were previously thought to be, with 1.4% and 20.5% of the total N lost after 35 days, and 17.1%-53.5% after 180 days. The true nitrogen losses were strongly correlated with the storage time.

Lauer *et al.* (1976); Doehler and Wiechmann (1987) reported that the percentage of ammonium nitrogen in the liquid manure converted to ammonia, then emitted, was about between 10-90%, depending on NH_4^+-N concentration, soil moisture content, wind speed and soil pH. Ammonia volatilisation, offensive odours released and the cost of collecting excreta are the main concerns of farmers to ensure management systems meet Resource Management Act (1991). Consequently, Regional Councils advise covering the excreta and neutralising with lime to prevent gaseous losses.

2.4.3.2 Some strategies to prevent and control ammonia emission

Bussink and Oenema (1998) reviewed ammonia volatilisation from dairy farming systems in temperate areas and found losses of NH_3 occur during slurry application, housing, slurry storage, grazing, and fertiliser application and from crops, in descending order of importance. They elucidated three technical strategies that could be used to prevent NH_3 losses while applying slurry; optimising N intake and N retention, directly incorporating slurry into the soil, diluting or acidifying slurry and covering slurry during storage. Mixing urine with straw was found to reduce NH_3 emissions in animal housing facilities, presumably because of the adsorption capacity of straw (Van Faassen and Van Dijk, 1979; Meyer and Sticher, 1983). Spraying water or acid solution on the slatted floor of animal housing facilities reduced NH_3 losses by 15-30% (Monteny and Verboon, 1994). Covering the slurry, together with addition of oil or straw diminishes ammonia losses and slurry stored in a closed tank can reduce the surface area and substantially decrease NH_3 emission (De Bode, 1991; Sommer, 1992). Studies have also demonstrated the reduction of ammonia emissions after application of slurry onto land by tine or disc injection, incorporation of slurry with soil, prior treatment with acid, dilution with water, band spreading and irrigation immediately after slurry spreading. Reductions in volatilisation ranged from 20-100%, according to the strategies used (Thompson *et al.*, 1987; Pain *et al.*, 1989; Bussink and Bruins, 1992; Stevens *et al.*, 1992; Bussink *et al.*, 1994; Huijamans *et al.*, 1996). *Cai et al.* (1987) demonstrated that covering slurry with a surface film of cetyl alcohol dissolved in ethanol could considerably abate the rate of ammonia emission in a flooded rice field, provided there was no strong wind. As indicated earlier, optimising N intake and N retention can also be used as effective mitigation strategies to reduce not only NH_3 losses also NO_3^- leaching and denitrification; and also increasing N availability (Bussink *et al.*, 1998; Cookson and Cornforth, 2002; Bolan *et al.*, 2004a). For instance, decreasing the N content in grass through improved grassland management and replacement of grass by low-protein concentrates and forages in the diet can achieve a reduction in N intake (Bussink and Oenema, 1998). Decreasing the number of herd-replacement and fattening cattle is an acceptable strategy to increase N retention due to greater nitrogen retention in high-yield milking cows (Coppoolse *et al.*, 1990).

Various amendments applied to alleviate gaseous emissions in excreta storage have been developed (Pain *et al.*, 1987; Ritter, 1989; Zhu *et al.*, 1997), such as oxidizing

agents, disinfectants, urease inhibitors, masking urgent and adsorbents. Questions as to their efficiency and difficulty of use exist. In the oxidizing process, potassium permanganate or hydrogen peroxide could be used as the oxidizing agent to reduce NH_3 emission from livestock slurry (McCrory and Hobbs, 2001). Chlorine, as a disinfectant, has been proved to be effective in decreasing NH_3 emission despite its high expense (Warburton *et al.*, 1980). Some urease inhibitors have been used to inhibit urease enzyme activities by slowing urea hydrolysis which is a main source of NH_4^+ ions. Phenyl phosphorodiamide, cyclohexylphosphoric triamide, and N-(n-butyl) thiophosphoric triamide have been employed in cattle feedlot slurry (Varel *et al.*, 1997). Some researchers have tried to use mineral adsorbents with a high absorption capacity, such as zeolites and clay mineral bentonite. Mumpton and Fishman (1977) showed that several examples where zeolites were used to control odours from both poultry and pig manure. A practical and cost-effective technology has been developed, using addictives of C- rich bedding materials, such as straw, woodchips, sawdust and tree bark, with large capacities to promote mineralisation of N (Mahimairaja *et al.*, 1994).

2.5. Research topics covered by this study

In regarding to the current progress in nitrogen losses in different winter management systems, the following research areas are required:

- Although many research projects have been conducted on studying nitrogen losses from pastoral farmland, very few have been done on understanding ammonia emission and nitrogen mineralisation on winter management systems in New Zealand. Incubation experiments will be conducted to quantify ammonia emission and nitrogen mineralisation rates.
- Meagre information is available on the potential of nitrogen loss through denitrification within winter management systems. Studies will be carried out to quantify the denitrification potentials and evaluate importance of affecting factors.
- Do field evaluation on a stand-off pad to determine the ability of bark and sawdust to reduce ammonia and nitrous oxide emission, as well as nitrate-nitrogen leaching losses.

Chapter 3 Materials and Methods

A series of experiments was carried out to examine the transformations of nitrogen in animal excreta and determine whether the addition of natural materials would reduce ammonia emissions when cow excreta are stored under conditions that simulated either stand-off pad or herd home winter management effluent storage conditions.

3.1 Materials

3.1.1 Cow excreta (dung and urine)

Before each incubation experiment was set up, fresh samples of cow dung and urine were collected from 10 cows in Ruakura dairy farm and stored in the laboratory at 4°C, then immediately used to establish incubation experiments. Dung and urine sub-samples were analysed immediately for physical and chemical properties. (Overall about 16 kg of urine and 11kg of dung were used in each incubation stage). Excreta used in the incubation study consisted of mixture of urine plus dung (1:1 v/w).

3.1.2 Preparation of amendment materials and soil extract inoculum

Two amendment materials were used: sawdust and soil

(a) Sawdust from untreated timber was collected from Ruakura Agriculture Research Centre work-shop and passed through a 2 mm screen to remove particles greater than this size (around 6.6 kg in all was used for all the treatments).

(b) Surface soil (0-10cm) sample was taken from an area of Hamilton clay loam (Soil Survey staff, 1990). The soil was evenly crushed and passed through a 4-mm sieve to remove stones and coarse particles.

(c) Soil extract – a soil extract inoculum was prepared by mixing soil with distilled water (700g soil in 1.4L sterile water) and filtering through a muslin cloth.

The microbial activity of the soil extract inoculum was determined. Serial ten-fold dilutions of the filtered soil extract were prepared. One hundred microlitre volumes of the appropriate dilutions were spread over the surface of duplicate agar plates (Tryptic Soy Agar, TSA, DIFCO Laboratories, Detroit Michigan 48232 USA). All the spread

plates were then incubated aerobically at 25°C for 48 hrs. The colonies of microorganisms (bacteria, fungi and actinomycetes) appearing on TSA agar plates were counted on the duplicate plates of the highest countable dilution (30-300CFU). Results were expressed as the average number of colony forming units (CFU) per gram of soil (Fan *et al.*, 1988).

3.2 Incubation experiments

Incubation experiments were divided into three stages.

- Stage 1: To examine the transformation of nitrogen in animal excreta. This was carried out over a period of 78 days using dung and urine samples representing various winter management systems.
- Stage 2: To examine the influence of different levels amendments on nitrogen mineralisation and losses. This was also carried out over a period of 78 days using dung and urine samples.
- Stage 3: To examine the nitrification and denitrification potentials in animal excreta. This was conducted over a period of 15 days.

First stage incubation study

Determine nitrogen mineralization and nitrogen losses

The incubation experiments were carried out using 1 Litre screw-capped plastic containers (internal diameter 80mm; height 160mm). For aerobic incubation experiments the containers were left uncapped and the container contents stirred frequently once per day to ensure the contents remained aerated. All incubations were carried out in a controlled temperature room at 15 °C. At intervals of 1, 3, 7, 10, 15, 21, 28, 36, 45, 55, 66, 78 days, about 2 grams of material was taken from each treatment bottle and analysed for ammonium and nitrate (as 2M KCl extractable mineral N) and dissolved organic carbon (0.5 M K_2SO_4 extractable carbon).

The treatments used are listed in Table 3.1 and the materials required for each treatment were collected and added to a container in the proportions given in Table 3.1. Each treatment was replicated four times. The incubation experiment was carried out as follows:

Table 3.1 Outline of experiments in the first stage incubation study.

Treatment number	Treatment	Bottle number	Urine	Dung	Sawdust	Soil	Inoculum	Plant Oil
1	Pure urine	1, 2, 3	300g					
2	Urine+dung (1:1)	4, 5, 6	150g	150g				
3	Urine+vegetable oil on surface	7, 8, 9	300g					2cm thick
4	Urine+dung+sawdust (1:1:4) (w:w:v)	10, 11, 12	75g	75g	300ml (48g)			
5	Urine +dung+soil (1:1:4) (w:w:w)	13, 14, 15	75g	75g		300g		
6	Pure urine+ inoculum (50:1)	16, 17, 18	300g				6ml (20%)	
7	Urine+dung+inoculum (25:25:1)	19, 20, 21	150g	150g			6ml (20%)	

1. The properties of the urine and dung used were analysed before the incubation experiments commenced. These properties included total nitrogen, total phosphorus, potassium, total sulphur, organic carbon, pH, and moisture.
2. If required, a soil extract was prepared and analysed for the total bacterial count.
3. All containers were weighed and the weights recorded.
4. All containers were transferred to the 15 °C temperature-controlled room.
5. All chemical properties analyses of the incubated samples were performed at days 1, 3, 7, 10, 15, 21, 28, 36, 45, 55, 66 and 78.
6. All the samples were analysed for mineral N (ammonium and nitrate using 2M KCl extraction), pH; moisture; and K_2SO_4 extractable dissolved organic carbon at each sampling time.
7. Total nitrogen of all samples was analyzed at days 0, 3, 7, 15, 36, 55 and 78.

Second stage incubation study

Investigate the effect of different ratios of excreta to the two amendments on reduction of nitrogen losses

Eight treatments, in which each treatment was replicated four times, were set up in 1 L screw-capped containers in a 15 °C incubation room (Table 3.2). The containers were left uncapped and the container contents stirred frequently once per day to ensure the contents remained aerated. The incubation experiment was conducted for 78 days following the protocols given for Stage 1.

Table 3.2 Outline of experiments in the second stage incubation study.

No	Urine+Dung (g)	Sawdust (ml)	Soil (g)
1	150+150	0	0
2	150+150	0	75
3	150+150	0	150
4	150+150	0	300
5	150+150	300 (48g)	0
6	150+150	300 (48g)	75
7	150+150	300 (48g)	150
8	150+150	300 (48g)	300

Third stage incubation study

Nitrification potential and Denitrification potential experiments

Three treatments, each replicated four times, were set up in 1 L screw-capped containers in a 15°C incubation room (Table 3.3).

Table 3.3 Outline of experiments in the third stage incubation study.

No	Urine+Dung (g)	Sawdust (ml)	Soil (g)
1	150+150	0	0
2	150+150	0	300
3	150+150	300 (48g)	0

After fifteen days of incubation, all treatments were destructively sampled and used for measuring short-term nitrification potential and short-term denitrification enzyme activity.

Short-term Nitrification Assay

The aim of this experiment was to find out the potential of transformation of ammonium N to nitrate N (i.e. nitrification potential) in all the treatments listed in Table 3.3.

Reagents:

1. Sterile 0.5 M phosphate buffer.
2. 0.25 M ammonium sulphate [$(NH_4)_2SO_4$] (sterile).
3. 0.1 M potassium chlorate ($KClO_3$) (sterile).
4. Merthiolate (ethyl mercurithiosalicylic acid, sodium salt) 1% (wt/vol). This reagent was stored at room temperature in a foil-covered bottle and was replaced at monthly intervals.
5. Reagents and instrumentation for quantitative NO_2^- analysis.

Procedure

Excreta samples (20g) were weighed in triplicate to individual 250ml cotton-stoppered conical flasks containing 90ml of phosphate buffer and 0.2ml of $(NH_4)_2SO_4$ solution. Flasks were placed on a rotary shaker at 20°C, and 1.0ml of $KClO_3$ solution added to each flask. Five ml aliquots were sampled after 1hour, 2 hours, 4 hours and 24 hours

and transferred to a vial containing 0.05ml merthiolate to stop the nitrification reaction. The aliquots were analysed for NO_2^- and NO_3^- ions using Skalar autoanalyzer. The rate of substrate oxidation was calculated according to Michaelis-Menten kinetics expression (Ardakani *et al.*, 1973; Suzuki et *al.*, 1974).

Short-term denitrification enzyme assay

Procedure

As denitrification is an anoxic enzyme activity, it requires anaerobic incubation. Acetylene, C_2H_2 (25ml) was added to block N_2O to N_2. When N_2O (in presence of C_2H_2) was the sole gaseous product of denitrification, the amount of N_2O formed by denitrification in the reaction mixtures could be determined using a gas chromatograph (Yoshinari *et al.*, 1977; Ryden *et al.*, 1979; Tiedje, 1982).

The following procedure was used to measure denitrification potential: four replicated samples, each of about 20g, were accurately weighed and placed in a 250ml Erlenmeyer flask fitted with gas-tight suba-seals. To remove oxygen the Erlenmeyer flasks were evacuated (using a vacuum pump) and flushed with pure N_2 gas 4 times and vented so that each flask was at atmospheric pressure. About 25 ml of headspace was replaced with C_2H_2 and this resulted in a C_2H_2 concentration in the flasks of 10% v/v.

Four treatments as summarised below were introduced into the flasks to determine the limiting factor for denitrification.

- control treatments with no addition
- addition of KNO_3 (50 μg N g^{-1} material),
- addition of glucose (300 μg C g^{-1} material),
- addition of glucose and KNO_3,

After incubation at 20°C, a 20ml gas sample was collected with syringes and transferred to gas-tight vials analysis. Gas samples were collected at the following intervals: immediately after addition of the treatments and 15 min, 60 min and 90 min after incubation. The gas samples were analysed for N_2O by Landcare Research, Massey University, Palmerston North using a Shimadzu GC-17A gas chromatograph equipped with a ^{63}Ni electron capture detector (Tiedje, 1982; Saggar *et al.*, 2004). The

experiment was conducted according to Table 3.4. Each treatment was replicated four times.

N_2O-N measurement

The amount of N_2O emitted was used to determine denitrification rate. The equation to calculate the mass of N_2O was:

N_2O-N (g) = 1.83*0.636*10^{-6}* N_2O (µl l^{-1})*[Vol. of headspace (l.)+Vol. of soil solution (l.)*0.632]

The value of N denitrified was plotted against time for each treatment. The denitrification activity was calculated by dividing the slope of the relationship by the mass of dry material.

Table 3.4 Outline of short-term denitrification potential experiments.

No	Urine+dung (g)	Sawdust (ml)	Soil (g)	control	Addition of KNO_3	Addition of glucose	Addition of KNO_3 and glucose
1	150+150	0	0	nil	50 µg N per g of material	300 µg C per g of material	50 µg N per g of material+300 µg C per g of material
2	150+150	0	300				
3	150+150	300	0				

3.3 Chemical Analytical methods

Total Nitrogen

Total nitrogen in all samples was analysed by Kjeldahl digestion, distillation and titration. Briefly about 1.0g fresh sample was added to a 50ml test-tube, together with 0.3g iron powder, a couple of boiling chips and 5ml 25% sulphuric acid. Tubes were placed on a digestion block at 185°C till water evaporated for pre-digestion. After predigestion, 2g of catalyst (ground mixture of 100g potassium sulphate, 10g copper sulphate and 1g selenium powder) was added, the sides of each tube washed down with 5ml concentrated sulphuric acid and tubes returned to the digestion block. The

temperature was gradually increased to 365°C and the digestion continued for 2.5-3 hours until the tube contents were clear. After digestion, the flask contents were cool.

For distillation, the digest was transferred to a distillation flask. 30ml of 5M NaOH was added and the flask connected to a distillation apparatus. A 100ml flask containing 5 ml of boric acid collected the condensate. When approximately 50ml of condensate was collected the flask was transferred to an auto-titrator to determine the concentration of ammonia released by the digestion and the total nitrogen content of the sample was calculated (Goh, 1972; Bergersen, 1980).

Analytical method of NH_4^+-N and NO_3^--N

About 2g sample was weighed in a centrifuge tube and 30ml 2M KCl added. The tube was stoppered and shaken on an end-over-end shaker for 1 hour at 20°C. Following extraction, the sample-KCl suspensions were centrifuged at 20000 rpm for 10 minutes, and the supernatant filtered through Whatman No. 42 filter paper. These KCl extracts were analysed for NH_4^+-N and NO_3^--N colourimetrically using a Skalar autoanalyzer.

Potassium sulphate extractable C

About 2g samples were weighed into 50ml polypropylene centrifuge tubes and extracted with 25 ml 0.5M K_2SO_4 for 1 hour on end-over-end shaker at 20°C. Following extraction, the sample-K_2SO_4 suspensions were centrifuged at 20000 rpm for 10 minutes, and the supernatant filtered through Whatman No. 42 filter paper. The amount of carbon in the extract was measured on a Shimadzu Total organic Carbon analyzer model 5000A. Aliquots of 40µl of each extract were injected into the detection chamber for the analysis of extractable total carbon. Each sample was analysed with three injections.

Organic carbon

Sample of solid materials were dried at 105°C and ground in a mortar and pestle. The residue material was analysed by infra-red spectroscopy on a TOC-5000A carbon analyzer with a SSM-5000A solid sample module (Shimadzu Corporation, Kyoto, Japan).

pH

About 2g solid sample was weighed into a 20ml beaker and 10ml distilled water added. The sample was left for half hour, occasionally stirred with a glass rod. The pH was determined using an Orion Model 370 pH meter fitted with an Orion Model 9202 electrode (Orion Research Inc, Beverly, USA). The sample was analysed in triplicate. The pH of liquid samples was measured in the original samples (i.e. without addition of distilled water).

Moisture of sample

Solid samples were weighed into a pre-weighed ceramic dish or aluminium foil cup that was then placed in the oven and dried at 105°C overnight. Percentage of moisture content was calculated on a wet weight basis.

Calculation of gas emission

Ammonia emission was not quantified in the incubation experiments. Kulasegarampillai *et al.* (2005) have noticed that significant amount of ammonia emission occurred from stored manure and sewage biosolids under aerobic and anaerobic conditions. It was assumed that the decrease in total nitrogen in the excreta sample with incubation time resulted from the loss of nitrogen through ammonia (NH_3) volatilization. The amount of NH_3 losses was calculated by subtracting total nitrogen at each sampling date from the total nitrogen value at the start of the incubation (see Chapter 4 and Chapter 5).

3.4 Materials and methods for the field study (stand-off pad)

Field-scale stand-off pad study

Two stand-off pads were constructed in May 2005 at Dexcel's Scott Farm, in the Waikato region in New Zealand. This farm is associated with the Resource Efficient Dairying (RED) farmlet systems trial (Clark, 2003; Ledgard *et al.*, 2006). The pads, each 20 m long, 7.5 m wide and 0.9 m deep for setting bedding materials and with its own separate drainage system, contained either crushed pine bark (particle size 3-12 mm) or sawdust. A range of chemical characteristics for both bark and sawdust are summarised in Table 6.1. Each pad was overlaid with about 0.1 m depth of coarse bark

on top of 0.9m depth bark or sawdust (particle size 12-25 mm). Pads were used for holding 21 non-lactating cows for about 18 hr a day (following 6 hr grazing pasture) during the winter period (31 May to early August) in 2005. Monitoring of the pad performance has been regularly carried out with regard to N retention in pad materials and for N losses into drainage water and to the atmosphere by denitrification.

Nitrogen in Drainage water and natural materials

All drainage water was collected and the volumes recorded in field studies. Samples of the drainage were stored frozen until analysis. Drainage water was analysed for total Kjedahl N (TKN), NH_4^+-N and NO_3^--N according to standard methods (APHA, 1995). The bark and sawdust materials were also sampled from the field stand-off pad in April 2006, and analysed for the same species of N.

Nitrogen loss from field-scale stand-off pad by denitrification

The rate of denitrification for the field-scale stand-off pads was measured using the acetylene (C_2H_2)-inhibition technique (Tiedje, 1989), using the incubation system as described previously for soil denitrification measurements (Luo *et al.*, 1998). At each sampling date, about 200g of materials from several depths (0-10, 30-40 and 50-60 cm) in the stand-off pads were collected, and transferred into 1-L glass jars. The jars were closed. Sixty ml of air was withdrawn and the same amount of C_2H_2 was injected into each jar using a syringe. The jars were incubated for 24 hours after addition of C_2H_2 on the ground in a shaded place close to the stand-off pads. At 1 and 24 hours after the addition of C_2H_2, a sample of the headspace gases was collected in a 12 ml evacuated test tube. A gas chromatograph equipped with a ^{63}Ni electron capture detector was used to measure the concentrations of N_2O in the samples. The quantity of N denitrified (measured N_2O) was divided by the mass of dry materials, which gave the daily denitrification rate. Total daily N losses from the stand-off pads were estimated by considering the total amounts of materials in the stand-off pads.

Chapter 4 Nitrogen transformation in animal excreta

The study was divided into two parts. In part 1, four treatments were used to conduct a nitrogen mineralisation and ammonia emission study in the first stage. These treatments included:

- pure urine (300 ml/bottle)
- urine+dung (150ml urine+150g dung/bottle)
- urine+dung+sawdust (75ml urine+75g dung+300ml sawdust)
- urine+dung+soil (75ml urine+75g dung+300g soil)

In part 2, eight treatments were used to measure the effect of different levels of natural material amendments on ammonia emission from animal excreta in the second stage. These treatments included:

- urine+dung (150ml urine+150 mg dung)
- urine+dung+75g soil
- urine+dung+150g soil
- urine+dung+300g soil
- urine+dung+300ml sawdust
- urine+dung+300ml sawdust+75g soil
- urine+dung+300ml sawdust+150g soil
- urine+dung+300ml sawdust+300g soil

4.1 Results of part 1

4.1.1 Properties of excreta and natural materials (used in first stage incubation study)

Urine

The properties of urine were analysed before all experiments were set up. The values for urine were determined with four replications: the total nitrogen content of urine ranged from 0.825% to 0.864% (Table 4.1). Overall only 0.38% of the total nitrogen content was in inorganic forms and the remaining nitrogen (> 99%) was in organic

forms. The mean percentage values of total P, K, and S contents in the urine were <0.001%, 1.373% and 0.128%, respectively.

Table 4.1 Characteristics of urine in wet basis (n=4).

	minimum	maximum	average
Total N (%)	0.835	0.864	0.850
Total P (%)	<0.001	<0.001	<0.001
Potassium (%)	1.289	1.401	1.373
pH	8.96	9.07	9.02
NH_4^+-N (%)	0.0030	0.0037	0.0033
Total S (%)	0.106	0.213	0.128
DOC (%)	2.171	2.294	2.227

Dung

The property of dung was also determined with four replications: the total nitrogen varied from 0.241% to 0.263% (Table 4.2). Only 5.64% of the nitrogen content was in inorganic forms and the remaining (>90%) remained as organic form.

Table 4.2 Characteristics of dung in wet basis (n=4).

	minimum	maximum	average
Total N (%)	0.241	0.263	0.255
Total P (%)	0.067	0.083	0.079
Potassium (%)	0.157	0.172	0.161
pH	8.06	8.36	8.22
NH_4^+-N (%)	0.0130	0.0147	0.0144
Total S (%)	0.027	0.041	0.032
Organic carbon	4.27	4.56	4.40
Moisture	89.0	89.4	89.3
C/N			17.25
DOC (%)	3.607	3.932	3.760

The mean percentage values of total P, K, and S contents in the dung were 0.079%,

0.161% and 0.032%, respectively. The proportion of inorganic nitrogen was greater in dung than in urine but the actual amount of total nitrogen, total potassium and total phosphorus were less than urine.

Natural materials-untreated sawdust and soil

The properties of sawdust and soil were analysed before all experiments were set up.

Table 4.3 Characteristics of untreated sawdust (n=4)
(Determined on a wet weight basis).

	minimum	maximum	average
Total N (%)	0.047	0.063	0.057
Total P (%)	<0.001	<0.001	<0.001
Potassium (%)	0.027	0.039	0.035
pH	4.20	4.25	4.22
NH_4^+-N (%)	Trace	Trace	Trace
Total S (%)	0.0074	0.0085	0.008
Organic carbon	27.53	31.10	28.94
Moisture	40.4	45.3	43.1
C/N	506.4	508.8	507.7
DOC (%)	0.018	0.021	0.020

Table 4.4 Characteristics of soil (n=4)
(Determined on a wet weight basis).

	minimum	maximum	average
Total N (%)	0.289	0.311	0.301
Total P (%)	0.072	0.089	0.084
Potassium (%)	0.088	1.008	0.094
pH	4.86	4.98	4.97
NH_4^+-N (%)	<0.001%	<0.001%	<0.001%
Total S (%)	0.045	0.053	0.049
Organic carbon	2.99	3.11	3.05
Moisture	22.4	25	23.2
C/N	9.4	11.8	10.1
DOC (%)	0.0031	0.0036	0.0034

The chemical and physical characteristics of natural materials (soil and untreated sawdust) used in this experiment are summarised in Table 4.3 and Table 4.4. The properties of these materials were assessed for their potential to either enhance or reduce the loss of nitrogen especially through ammonia emission. The moisture contents of both the sawdust (43%) and the test soil (23%) were sufficient to promote reducing gaseous emission (Bohn, 1992). The pH of soil and sawdust were all initially acidic, conditions that would minimise the loss of gaseous ammonia (Molloy and Tunney, 1983; Pain *et al.*, 1990).

4.1.2 Nitrogen mineralisation among different treatments

Changes in ammonium ion concentration

The concentrations of mineral nitrogen (NH_4^+ and NO_3^-) in the animal excreta were measured regularly over the 78 day period of incubation and all results are summarised in Table 4.5. In order to calculate the mass balance of nitrogen in various treatments the concentration of nitrogen was expressed per bottle.

In the urine treatment the NH_4^+-N concentration increased with time and reached a maximum (0.910g/bottle) at 10 days (Table 4.5), and remained at more than 0.5 g/bottle NH_4^+-N for 45 days indicating rapid urea hydrolysis due to urease enzyme activity in the urine (Sherlock and Goh, 1984; Bolan *et al.*, 2004a).

In the urine+dung treatment there was a relatively high concentration of NH_4^+ ions at the onset of incubation and the concentration had peaked by day 3 (0.489g/bottle). After day 3, the concentration of NH_4^+ started to decline and was barely detectable at the end of incubation on day 78.

In the urine+dung+sawdust treatment the NH_4^+-N concentration reached a maximum at day 3 (0.170 g/bottle), the lowest concentration of any of the 4 treatments. After day 3 NH_4^+-N concentration decreased and reached to a barely detectable level by day 78.

The urine+dung+soil treatment had an initial NH_4^+-N concentration of 0.473 g/bottle and increased to a maximum by day 3 (0.524 g/bottle). The concentration decreased very slowly over the remaining incubation period and reached a value of 0.180 g/bottle by day 78. This slow decline in the concentration of NH_4^+ ions in this treatment

compared to other treatments can be attributed to the cation exchange capacity of the soil, resulting in the retention of NH_4^+ ions onto cation exchange sites (Selvarajah *et al.*, 1989).

Table 4.5 Concentration (g N/bottle) of NH_4^+ and NO_3^- among the 4 different treatments over 78 days aerobic incubation at 15°C
(Number in brackets are standard deviation of the mean, n=4).

	Time of incubation (day)							
	1	3	7	10	15	21	28	36
sample	NH_4^+	NH_4^+	NH_4^+	NH_4^+	NH_4^+	NH_4^+	NH_4^+	NH_4^+
urine	0.010 (0.001)	0.070 (0.0007)	0.901 (0.036)	0.910 (0.012)	0.807 (0.043)	0.784 (0.035)	0.747 (0.022)	0.711 (0.007)
urine+dung	0.399 (0.026)	0.489 (0.023)	0.422 (0.001)	0.244 (0.028)	0.116 (0.003)	0.061 (0.01)	0.023 (0.007)	0.007 (0.0002)
urine+dung+ sawdust	0.084 (0.003)	0.170 (0.0085)	0.150 (0.008)	0.124 (0.01)	0.056 (0.007)	0.032 (0.005)	0.014 (0.001)	0.018 (0.0016)
urine+dung+ soil	0.473 (0.026)	0.524 (0.0081)	0.373 (0.012)	0.363 (0.023)	0.336 (0.011)	0.351 (0.024)	0.293 (0.01)	0.288 (0.0036)
LSD (0.05)	0.0286	0.0197	0.029	0.031	0.035	0.034	0.020	0.0019

	Time of incubation (day)							
	45		55		66		78	
sample	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
urine	0.533 (0.023)	nd	0.480 (0.023)	nd	0.037 (0.008)	nd	0.038 (0.007)	nd
urine+dung	0.004 (0.0004)	nd	0.001 (0.0005)	4.0×10^{-4}	0.001 (0.0002)	2.9×10^{-5}	0.001 (1×10^{-5})	1.7×10^{-5}
urine+dung+ sawdust	0.008 (0.0007)	7.7×10^{-4}	0.005 (0.0007)	2.8×10^{-4}	0.003 (0.0003)	2.1×10^{-4}	0.003 (0.0003)	1.0×10^{-4}
urine+dung+ soil	0.262 (0.013)	6.1×10^{-3}	0.201 (0.013)	3.2×10^{-3}	0.196 (0.01)	5.9×10^{-3}	0.180 (0.013)	7.1×10^{-3}
LSD (0.05)	0.021		0.013		0.011		0.011	

nd=not dectected

There was no detectable NO_3^--N before 45 days in any of the treatments. The appearance NO_3^--N, at extremely low concentration, was observed on day 45 in urine+dung+soil and even lower concentration in urine+dung+sawdust and similarly in urine+dung on day 55, demonstrating the delay in the onset of the nitrification process. The highest concentration of NO_3^--N (7.1×10^{-3} g/per bottle) for urine+dung+soil

treatment by day 78 was a still negligible suggesting that the nitrifier population was very low in the sample materials. The highest NO_3^--N concentration in the urine+dung+soil treatment indicated the presence of some nitrifiers in the soil but still at a very low level.

4.1.3 Overall Nitrogen transformation among the four different treatments

Table 4.6 summarises nitrogen transformation among four treatments during incubation study.

Table 4.6 Changes in nitrogen (total N, inorganic N and organic N) in the 4 treatments after 78 days aerobic incubation at 15°C (results are given on a g/bottle basis) (Numbers in brackets are standard deviation of the mean, n=4).

treatments	Properties	Total N (g/bottle)	Inorganic N* (g/bottle)	Organic N (g/bottle)	pH	Moisture (%)
urine	Onset of	2.403	0.010	2.393	9.02	nd
	incubation	(0.026)	(0.001)	(0.033)	(0.25)	
	After	0.7448	0.038	0.7068	9.31	nd
	incubation	(0.0086)	(0.0122)	(0.0251)	(0.14)	
urine+dung	Onset of	1.661	0.399	1.262	9.08	92.6
	incubation	(0.038)	(0.026)	(0.024)	(0.24)	(0.001)
	After	0.578	0.001	0.577	8.33	13.56
	incubation	(0.014)	$(1*10^{-5})$	(0.017)	(0.06)	(0.01)
urine+dung+sawdust	Onset of	0.854	0.084	0.77	8.39	75.9
	incubation	(0.010)	(0.003)	(0.011)	(0.21)	(0.015)
	After	0.406	0.003	0.403	8.30	12.6
	incubation	(0.010)	(0.0003)	(0.013)	(0.03)	(0.022)
urine+dung+soil	Onset of	1.735	0.473	1.262	8.46	44.9
	incubation	(0.022)	(0.026)	(0.015)	(0.17)	(0.005)
	After	0.976	0.180	0.796	8.21	18.0
	incubation	(0.008)	(0.013)	(0.011)	(0.04)	(0.003)

Inorganic N* was determined at day 1.

Urine

As shown in Table 4.6 there was a loss equivalent to 69% of the initial total nitrogen

in the urine treatment after 78 days of incubation. During incubation the pH increased slightly from 9.02 to 9.31. These data demonstrate substantial conversion of urea nitrogen to inorganic nitrogen and also an overall loss of nitrogen from the liquid contents of the bottle. The loss was presumably due to emission of gaseous ammonia that has been demonstrated to be the predominant mechanism by which nitrogen is lost from urine as only negligible NO_3^- was detected, indicating no N_2O or N_2 emission occurred during the incubation period. A high rate of ammonia volatilisation is driven by the high concentration of ammonium ion formation, the high pH and aerobic conditions (Bolan, *et al.*, 2004a).

Urine+dung

In the urine+dung treatment 65% of the initial total nitrogen was lost over the 78 day incubation period. There was a significant loss of inorganic nitrogen (99.8%) and the organic nitrogen also decreased (54%) but to a lesser extent. The pH of this treatment decreased from 9.08 to 8.33 but was still high enough to allow ammonia volatilisation. During incubation the material in the bottle lost 79% of their initial moisture contents.

Urine+dung+sawdust

In the urine+dung+sawdust treatment 52% of the initial total nitrogen was lost over the 78 day incubation period, 13% less than in the unamended urine+dung. Similarly to the urine+dung treatment, there was a significant decrease in inorganic nitrogen (96%) and a lesser decrease (48%) in organic nitrogen. There was a slight drop in pH but a 63% loss of moisture.

Urine+dung+soil

In the urine+dung+soil treatment only 44% of the initial total nitrogen had been lost after 78 days of incubation. The pH fell slightly from 8.46 to 8.21. The organic nitrogen accounted for 82% of total nitrogen remaining after 78 days compared to about 99% for the urine+dung and urine+dung+sawdust treatments. These results suggest a higher rate of mineralisation in the urine+dung+soil treatment. As well, remaining inorganic nitrogen accounted for 18.4% of the total nitrogen; a much higher proportion than the other treatments. The pH decreased slightly and there was a 28% loss in moisture.

When the four treatments were compared, there was about 1.5 times difference in total nitrogen loss between the treatment (urine) with the highest loss and that (urine+dung+soil) with the lowest loss. Urine and urine+dung had very high rates of total nitrogen loss. In the urine treatment only 29% of the initial organic nitrogen remained in this form compared to 46% for urine+dung, 52% for urine+dung+sawdust and 63% for urine+dung+soil; reflecting the differences in mineralisation rates that would be influenced by the high rate of moisture loss in the amended treatments. The pH decreased by the end of incubation in all the four treatments except for urine with the largest decrease (0.75 pH units) in urine+dung. The urine treatment had the most favourable conditions for ammonia volatilisation as pH was over 9.30. The concentration of NH_4^+ was negligible in the urine+dung and urine+dung+sawdust treatments after 78 days but was relatively high (0.18 g/bottle) in the urine+dung+soil treatment; demonstrating binding of NH_4^+ ions to exchange sites on the soil.

4.1.4 Cumulative mineralised nitrogen

Cumulative Nitrogen mineralisation varied amongst the different treatments. The results are shown in Fig 4.1.

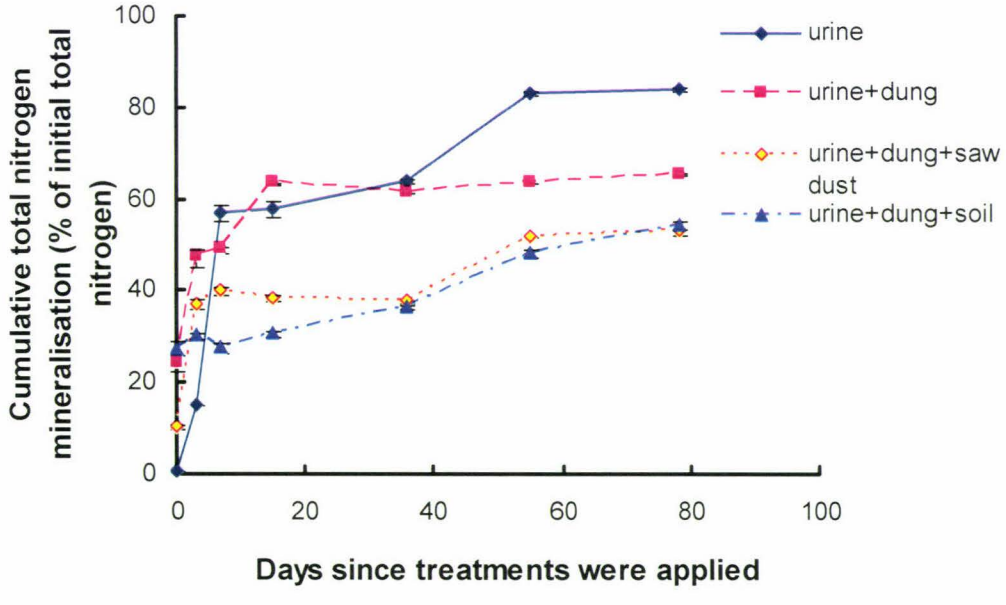

Fig. 4.1 Cumulative nitrogen mineralisation from different treatments in incubation bottles under aerobic condition at 15°C.

The percentage of total nitrogen mineralised to inorganic nitrogen in various treatments was calculated as follows:

% mineralisation of total nitrogen =100 x N_m/ (N_A),

Where N_m=mineral nitrogen (NH_4^+-N + NH_3 losses (calculated)) at different sampling periods (g N /bottle) as amounts of NO_3^--N were negligible detected in all treatments, and N_A=content of initial total nitrogen (g N/bottle).

The mineral nitrogen content increased from 0.01±0.001 g N/bottle at the beginning of incubation to 2.01±0.028 g N/bottle at the end in the urine treatment under aerobic conditions at 15°C (Table 4.7). Net accumulation of mineral nitrogen in the treatments of urine, urine+dung, urine+dung+sawdust, urine+dung+soil treatments at 78 days was 2.002, 1.082, 0.450, 0.938 g N/bottle, respectively (Table 4.7).

Table 4.7 The content of cumulative mineral nitrogen among all treatments during incubation study at 15°C (results are given on a g/bottle basis) (Numbers in brackets are standard deviation of the mean, n=4).

Sampling day	Urine (g/bottle)	Urine+dung (g/bottle)	Urine+dung+sawdust (g/bottle)	Urine+dung+ soil (g/bottle)
Day 0	0.010	0.398	0.084	0.473
	(0.001)	(0.027)	(0.003)	(0.026)
Day 3	0.358	0.780	0.314	0.518
	(0.003)	(0.018)	(0.007)	(0.007)
Day 7	1.373	0.810	0.338	0.474
	(0.033)	(0.007)	(0.008)	(0.011)
Day 15	1.288	1.048	0.325	0.527
	(0.047)	(0.024)	(0.007)	(0.011)
Day 36	1.531	1.014	0.319	0.630
	(0.011)	(0.023)	(0.005)	(0.001)
Day 55	1.995	1.052	0.439	0.832
	(0.013)	(0.024)	(0.005)	(0.01)
Day 78	2.012	1.082	0.450	0.938
	(0.028)	(0.025)	(0.003)	(0.013)

Cumulative mineral nitrogen in urine, urine+dung, urine+dung+sawdust and urine+dung+soil treatments at the end of incubation was 83.74±0.49, 65.18±0.17,

52.74±0.63, 54.12±0.78 %, respectively (Fig. 4.1). Mineralisation of nitrogen was rapid and occurred within the initial three days in all treatments except urine+dung+soil treatment. Cumulative mineralisation of nitrogen among different treatments directly related to initial nitrogen content (R^2=0.92, P=0.01). The urine+dung+soil treatment showed an almost linear pattern after 7 days, with a gradual increase in rate till to the end of incubation. After 78 days aerobic incubation, cumulative nitrogen mineralisation followed: urine+dung+sawdust (52.74%) < urine+dung+soil (54.12%) < urine+dung (65.18%) < urine (83.74%). If the incubation had continued for another few weeks under suitable moisture contents conditions, urine+dung+soil treatment would have gradually mineralised more nitrogen, along with a large amount of NH_4^+ ions absorbed by exchange sites on the soil surface. This could mean that the urine+dung+soil mixture could be used as a potential fertiliser applied to pasture.

4.1.5 Calculated losses of gaseous ammonia in first stage study

An assumption was made that the nitrogen loss as ammonia gas emission (see Chapter 5). Based on this assumption, cumulative ammonia emission rates were calculated as from the difference in total nitrogen between initial total nitrogen and total nitrogen at sampling day:

Cumulative NH_3 emission $=T_{IN}-T_{dx}$ (4-1)

Where T_{IN}=the initial total nitrogen present

T_{dx}=the total nitrogen present at sampling day

Four treatments were selected for measuring ammonia emission. The ratios of components in these treatments were 1:1 (v/w) for urine+dung; 1:1:4 (v/w/v) for urine+dung+sawdust and 1:1:4 (v/w/w) for urine+dung+soil. NH_3 volatilisation loss from different treatments under aerobic incubation was presented in Fig. 4.2. At the end of 78 days incubation the cumulative NH_3 volatilisation losses were significantly less (P<0.05) from the urine+dung+sawdust and urine+dung+soil treatments than those from the urine and urine+dung treatments.

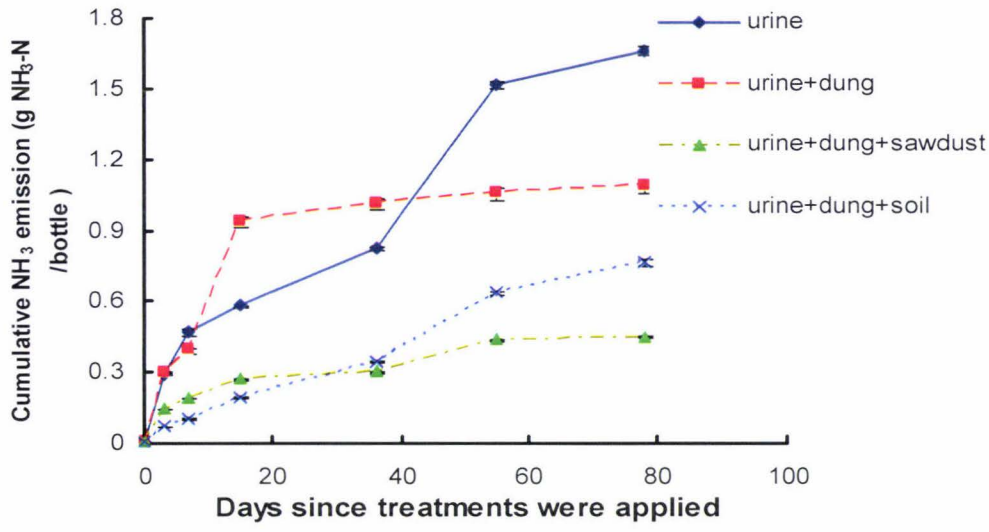

Fig.4.2 Cumulative ammonia (NH_3) losses from different treatments in incubation bottles under aerobic condition at 15°C.

There was no significant difference between the urine+dung+soil and urine+dung+sawdust treatments. Amongst all treatments examined, urine+dung treatment had the highest value of NH_3 emission over day 0-15 (Fig. 4.2). After 15 days the value of NH_3 emission levelled off until the end of incubation. In the urine treatment, the NH_3 volatilisation loss increased from the beginning to end of incubation. However, urine+dung+soil and urine+dung+sawdust treatments showed a gradual rise in NH_3 volatilisation losses.

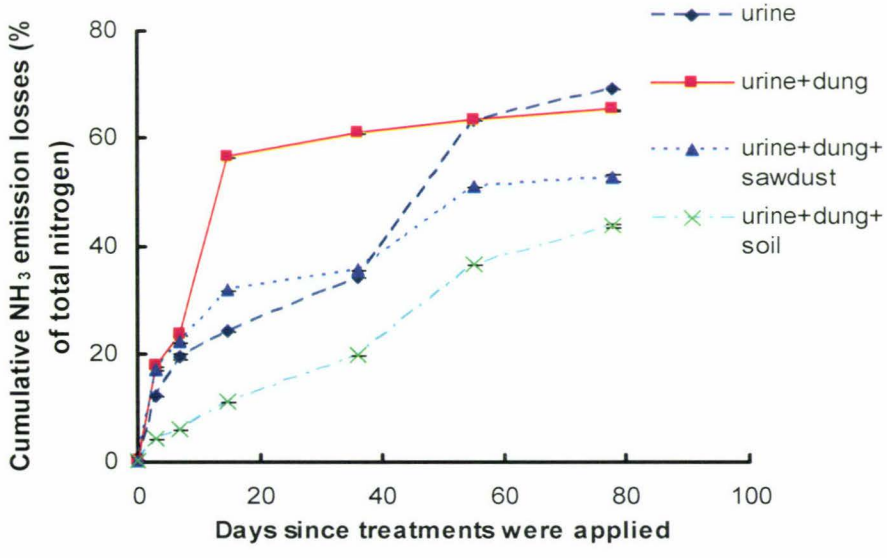

Fig. 4.3 Cumulative ammonia losses from different treatments under incubation condition at 15°C (% of initial total nitrogen).

Fig. 4.3 shows the percentage of total initial nitrogen lost as NH_3 from different treatments under aerobic incubation. At the end of 78 days incubation, significant difference ($P<0.05$) appeared between urine, urine+dung and urine+dung+soil, urine+dung+sawdust treatments. The percentage of total initial nitrogen lost as NH_3 followed: urine (69%) >urine+dung (65.1%) >urine+dung+sawdust (52.41%) >urine+dung+soil (43.71%).

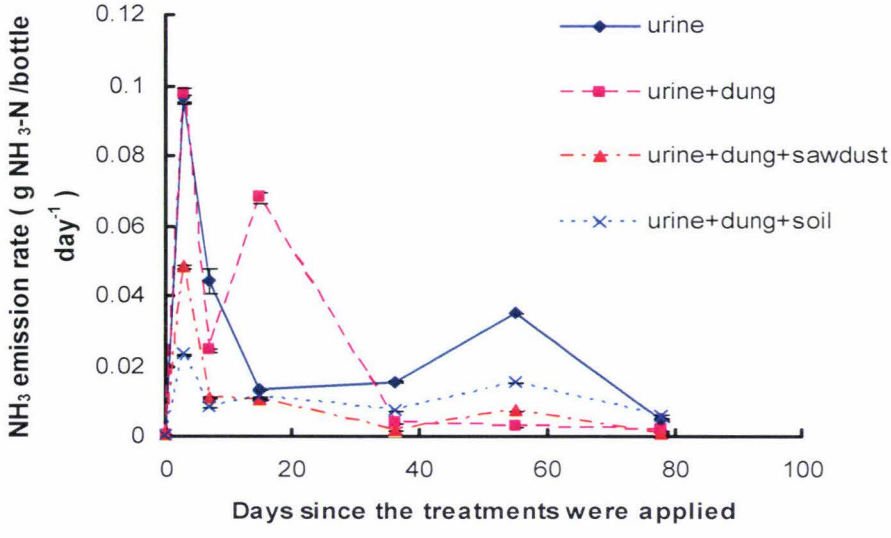

Fig.4.4 Ammonia (NH_3) emission rate of different treatments in incubation bottles under aerobic condition at 15°C.

The rates of NH_3 emission presented significant difference ($P<0.05$) among all the treatments. It demonstrated that the rate of NH_3 emission was affected by the addition of soil and sawdust (Fig. 4.4). The rate of NH_3 emission in all treatments increased to a maximum during the first 3 days, at 0.096±0.001, 0.097±0.002, 0.048±0.0006, and 0.023±0.0003 g NH_3-N/bottle/day for urine, urine+dung, urine+dung+sawdust and urine+dung+soil treatments. For the urine treatment, during 3-7 days and 36-55 days, the rate of was higher than the period of 7-15 days and 55-78days. The rate of NH_3 emission for the urine+dung treatment increased to 0.097±0.002 g NH_3-N/bottle/day at day 3 and then decreased to 0.0013±0.00012 g NH_3-N/bottle/day at the end of incubation. The rate of NH_3 emission for the urine+dung+soil treatment ranged from 0.005±0.0003 g NH_3-N/bottle/day to 0.023±0.0003 g NH_3-N/bottle/day during the period of incubation and was similar to urine+dung+sawdust treatment, except at 3 days after incubation. The decrease in the rate of NH_3 emission in urine+dung+soil and

urine+dung+sawdust treatments was probably related to the soil particle absorption or reduction in nitrogen mineralisation due to the addition of high C/N substance.

4.1.6 Effect of addition of a soil extract inoculum on nitrogen mineralisation

A soil extract inoculum was prepared using sterile water (see Chapter 3). It was added to three treatments that did not contain soil. The objective was to find whether the soil extract inoculum had any effects on nitrogen mineralisation of different treatments.

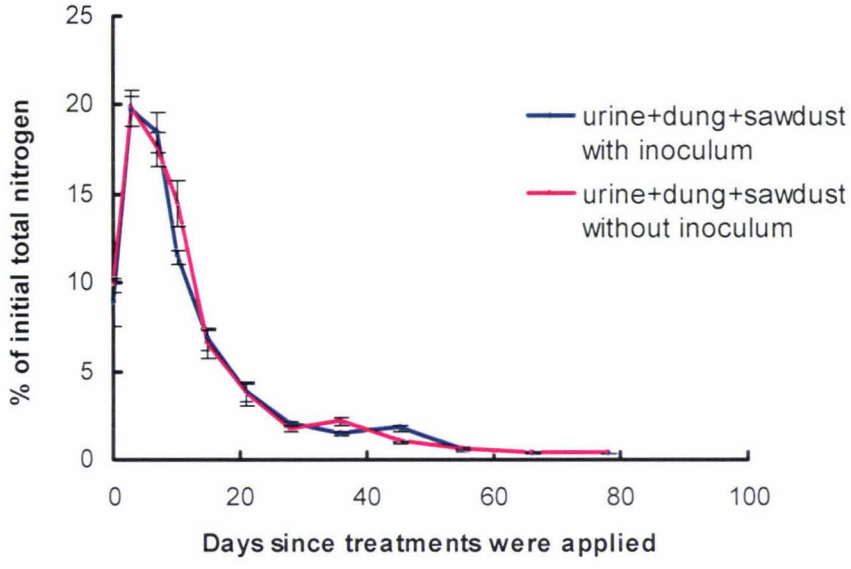

Fig. 4.5 Changes in percentage of NH_4^+ over initial total nitrogen under aerobic condition at 15°C.

In the different treatments with inoculum (soil extract solution) and without inoculum, the results demonstrate that there was a distinct differences ($P<0.05$) in NH_4^+-N content (% of initial total nitrogen) between urine with inoculum and urine without inoculum (Fig. 4.6). However, no significant differences were detected between with and without inoculum addition both in the urine+dung+sawdust and urine+dung treatments (Fig. 4.5 and Fig. 4.7). In the urine treatment, the NH_4^+ ions content reached maximum values at day 7 and day 10, nevertheless, urine with inoculum reached its second highest peak at day 10, and then attained maximum values at day 28. Meanwhile, the peak value of urine with inoculum was 1.5 times that of urine alone, being 57.53% of initial total nitrogen and 37.89% of initial nitrogen, respectively. After day 10 for urine treatment

and day 28 for urine with inoculum treatment, their values of NH_4^+ ions accounting for total nitrogen declined rapidly until the end of incubation.

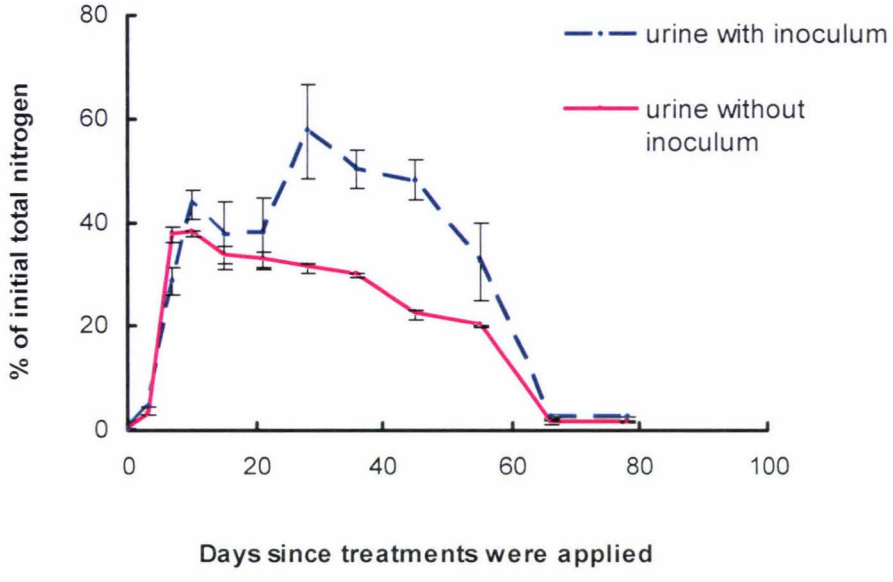

Fig. 4.6 Changes in percentage of NH_4^+ over initial total nitrogen under aerobic condition at 15°C.

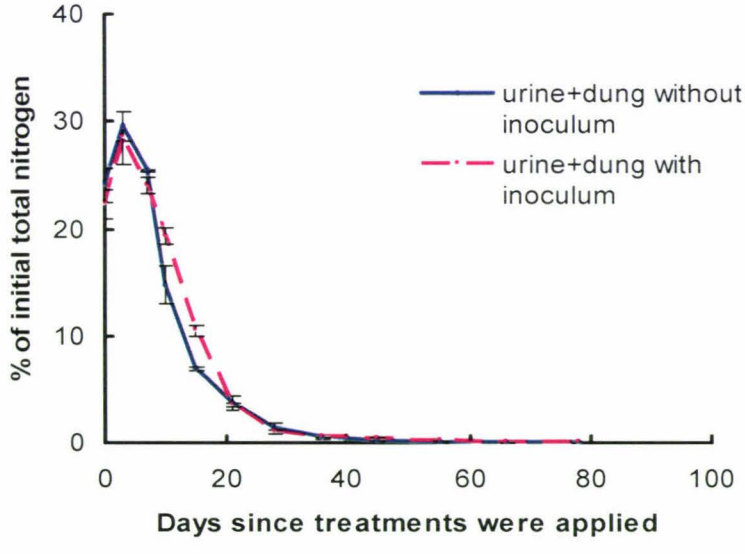

Fig. 4.7 Changes in percentage of NH_4^+ over initial total nitrogen under aerobic condition at 15°C.

4.1.7 Effect of covering urine on the ammonia emission process

Calculated ammonia volatilisation during the incubation

Two additional treatments (urine alone, urine covered with oil) were set up in order to examine the effect of use of plant oil covering urine on reducing ammonia emission.

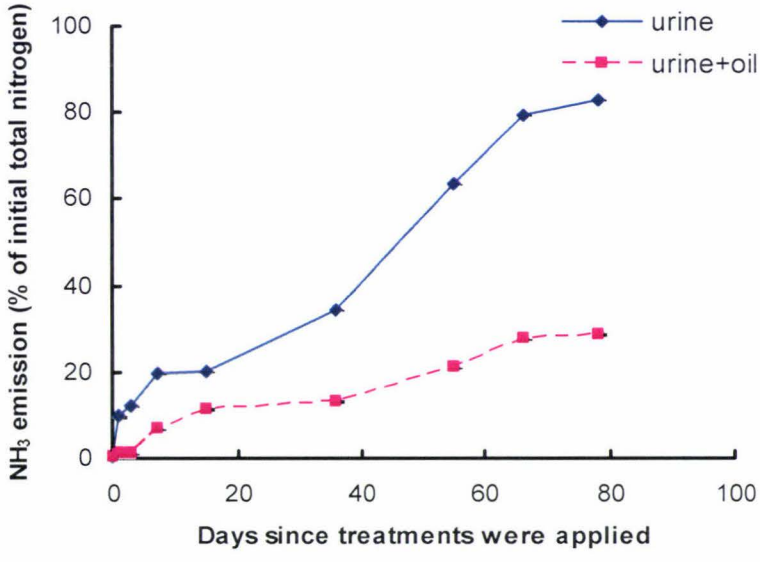

Fig. 4.8 Cumulative ammonia loss from urine in incubation bottles under covering oil vs. no covering oil conditions at 15°C.

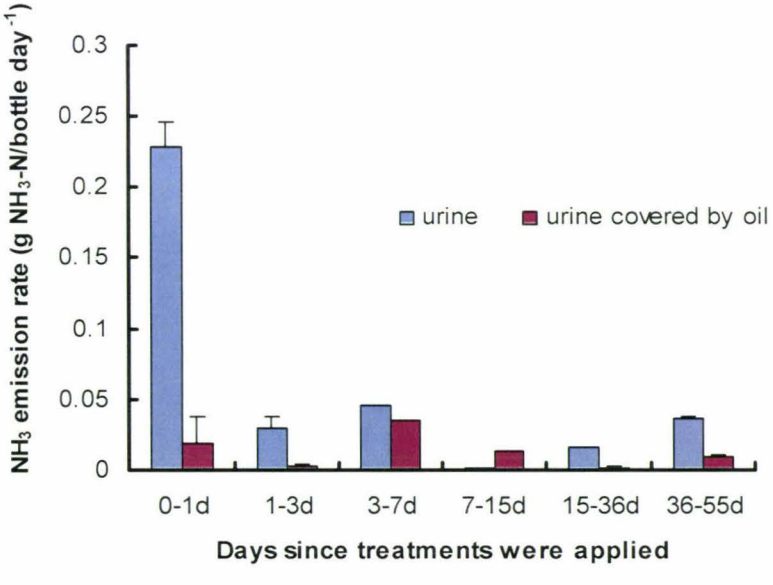

Fig. 4.9 Ammonia emission rate of urine in incubation bottles under covering oil vs. no oil conditions at 15°C.

It was noticed the oil formed a uniform layer at the beginning of incubation, however, at the end, the plant oil being a miscible solution, was mixed with the urine. At the end of 78 days incubation, the cumulative ammonia emission losses were significantly less from urine with oil than that from urine alone treatment (Fig. 4.8. $P<0.05$). Urine with oil achieved 67.5% reduction in ammonia emission compared to urine alone. From day 1, the oil cover decreased ammonia losses. The rate of ammonia emission from urine reached maximum value during day 0-1 (Fig. 4.9), with 0.228 g/bottle/day. The rate of ammonia emission during day 3-7 was similar to day 36-55.

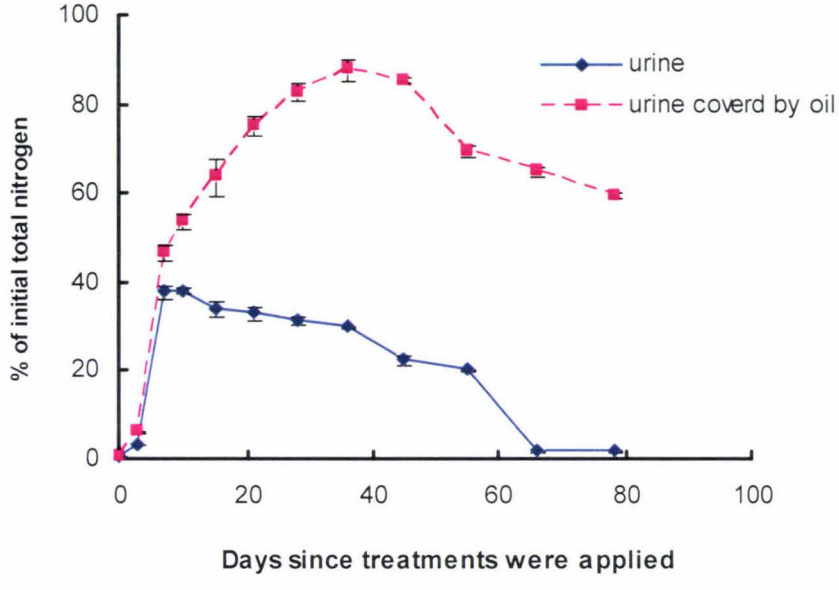

Fig. 4.10 Changes in percentage of NH_4^+ over initial total nitrogen under aerobic condition at 15°C.

At the time of setting up incubation, both treatments showed a very low initial NH_4^+-N content (Fig. 4.10). Within 3 days, a rapid increase in NH_4^+-N was measured, which reached a maximum value (37.89% of initial total nitrogen) at day 7 for urine. From day 7 the NH_4^+-N content of urine treatment declined until at the end of incubation, only 1.6% of initial total nitrogen was presented as NH_4^+ ions. The rest of the ammonium had been converted to ammonia through hydrolysis which resulted in large ammonia emission in the urine treatment. However, the cumulative NH_4^+-N content of the urine with oil treatment reached a maximum value (87.5% of initial total N) at day 36, and declined gradually until the end of incubation.

4.2 Result of part 2 study

4.2.1 Properties of tested materials (urine and dung used in second stage incubation study

The second stage study focused on assessment of different levels of soil or sawdust amendment addition to animal excreta in reducing ammonia losses.

The ratios of tested material for eight treatments used in Part 2 have been described at the beginning of this chapter . The properties of tested materials were analysed before second stage study experiments were set up (Table 4.8). The total nitrogen of all tested materials varied from 0.065% to 0.621%. Only 4.1 % of the nitrogen content was in inorganic form for urine and 34.8% for dung. pH of all tested materials ranged from 4.18 to 8.90.

Table 4.8 Characteristics of tested materials in wet basis (n=4).

Tested materials	Total N (%)	NH_4^+-N (%)	NO_3^--N (%)	Organic carbon	pH
Urine	0.621	0.026	<0.001	nd	8.90
Dung	0.336	0.117	0.001	3.82	8.08
Soil	0.264	Trace	<0.001	2.96	4.80
Sawdust	0.065	Trace	<0.001	39.52	4.18

4.2.2 Effect of different amount of soil amendments on reducing ammonia losses

Analysis of all treatments demonstrated that most of the initial total nitrogen was recovered as organic nitrogen after incubation (Table 4.9). The pH of the soil-amended treatments was also lower than the urine+dung. At the end of the incubation, organic nitrogen portion of total nitrogen was 97% for urine+dung, 98% for urine+dung+75g soil, 97% for urine+dung+150g soil and 92% for urine+dung+300g soil. NH_3 volatilisation (nitrogen losses could be regarded as ammonia emission because no nitrate was detected) varied among the four different treatments. The difference in NH_3 volatilisation between urine+dung and urine+dung+300g soil treatments was statistically significant ($P<0.05$) (Fig. 4.11). NH_3 volatilisation losses for urine+dung+75g soil and urine+dung+150g soil treatments were not significantly

different from that of urine+dung treatment. After 78 days incubation, a 22% reduction of cumulative NH_3 volatilisation was achieved from urine and dung by the addition of 300g soil, while only 4% and 6% reduction were achieved by amending with 75g soil, 150g soil, respectively.

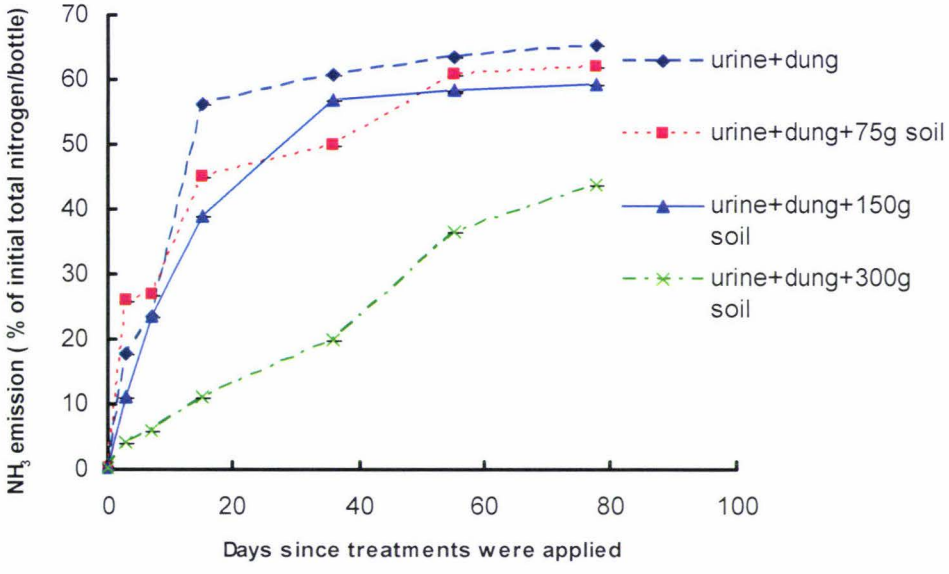

Fig. 4.11 Cumulative ammonia (NH_3) loss from treatments with different rate of addition under aerobic condition at 15°C.

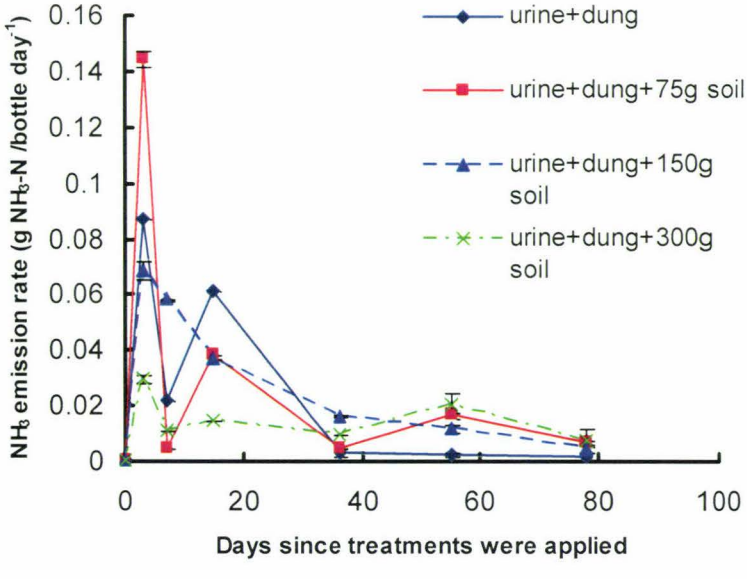

Fig. 4.12 Ammonia (NH_3) emission rate of different treatments in incubation bottles under aerobic condition at 15°C.

NH_3 volatilisation rates (Fig. 4.12) ranged from 0.0011±0.001 to 0.086±4.93x10^{-5} g NH_3-N/bottle/day for urine+dung treatment; 0.006±0.001 to 0.144±0.003g NH_3-N/bottle/day for urine+dung+75g soil treatment; 0.003±0.0014-0.068±0.0012g NH_3-N/bottle/day for urine+dung+150g soil treatment and 0.0071±0.001-0.029±0.004g NH_3-N/bottle/day for urine+dung+300g soil treatment.

Ammonia emission rates for all treatments reached a maximum value by day 3. Urine+dung and urine+dung+75g soil treatment reached their second ammonia emission peaks at day 15. However, the emission rate of urine+dung+300g soil and urine+dung+150g soil treatments gradually decreased after the first 3 days. For urine+dung+300g soil treatment, another peak appeared by day 55 and then gradually declined.

Table 4.9 Effects of soil amendment (Based on g/bottle basis, numbers in brackets are standard deviation of the mean, n=4).

Analytical items	Onset of incubation				The end of incubation after 78 days			
	Treatments				Treatments			
	Urine+dung	Urine+dung amended with 75g soil	Urine+dung amended with 150g soil	Urine+dung amended with 300g soil	Urine+dung	Urine+dung amended with 75g soil	Urine+dung amended with 150g soil	Urine+dung amended with 300g soil
Total nitrogen (g/bottle)	1.482 (0.0002)	1.680 (0.0005)	1.878 (0.0004)	2.274 (0.0027)	0.517 (0.0086)	0.391 (0.0069)	0.506 (0.004)	1.284 (0.0023)
Organic nitrogen (g/bottle)	1.194 (0.0003)	1.1761 (0.003)	1.3084 (0.006)	1.575 (0.004)	0.5017 (0.0002)	0.3838 (0.0003)	0.4947 (0.0005)	1.1884 (0.001)
NH_4^+-N (g/bottle)	0.288 (0.004)	0.5039 (0.006)	0.5696 (0.0081)	0.699 (0.0075)	0.01532 (0.0007)	0.0072 (0.0005)	0.0113 (0.0007)	0.0956 (0.0012)
pH	9.06	8.78	8.58	8.47	8.33	8.26	8.29	8.21

4.2.3 Effect of different amount of sawdust and soil amendments on reducing ammonia losses

Table 4.10 shows that the amount of NH_4^+-N in urine+dung+150g soil +300ml sawdust and urine+dung+300g soil+300ml sawdust treatments was larger than the other treatments, although organic nitrogen was still the predominant portion of total nitrogen. The pH of the urine+dung+soil+sawdust treatment was lower than the urine+dung treatment.

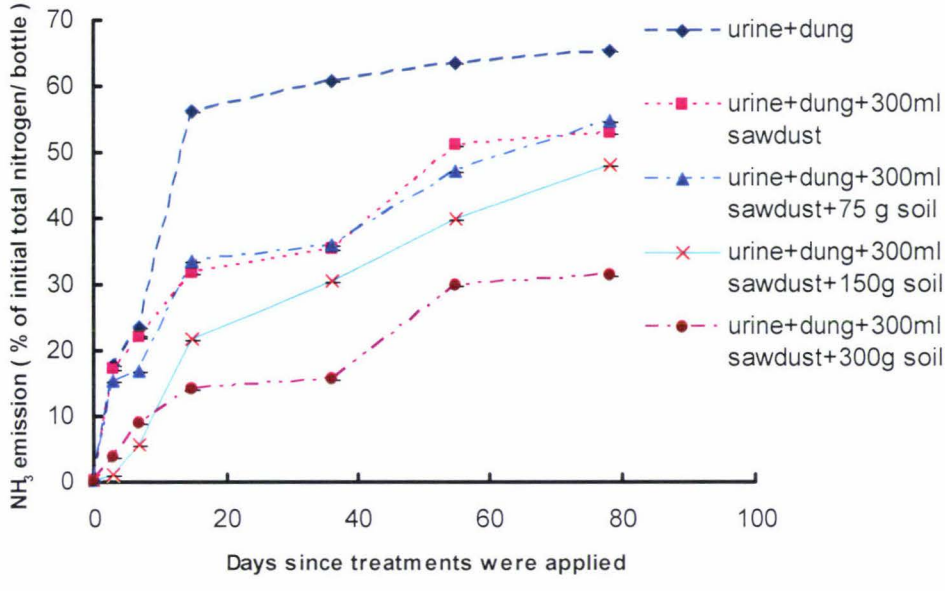

Fig. 4.13 Cumulative ammonia (NH_3) loss from treatments with different rates of addition under aerobic condition at 15°C.

The substantial differences ($P<0.05$) in cumulative ammonia (NH_3) losses (calculated) between urine+dung treatments with and without soil or sawdust are illustrated graphically in Fig. 4.13. Increasing soil additions from 75g to 300g with the same level of sawdust to excreta increased the reduction in NH_3 emission. After 78 days incubation, amendment of all treatments with soil and sawdust reduced the NH_3 losses and followed: urine+dung+300ml sawdust+300g soil (33.7%) <urine+dung+300ml sawdust+150g soil (17.1%) <urine+dung+300ml sawdust (12.7%) <urine+dung+300ml sawdust+75g soil (10.5%). Urine+dung+300ml sawdust+75g soil and urine+dung+300ml sawdust did not show any difference, indicating that a small amount of soil addition is unlikely to cause any significant reduction in ammonia emission.

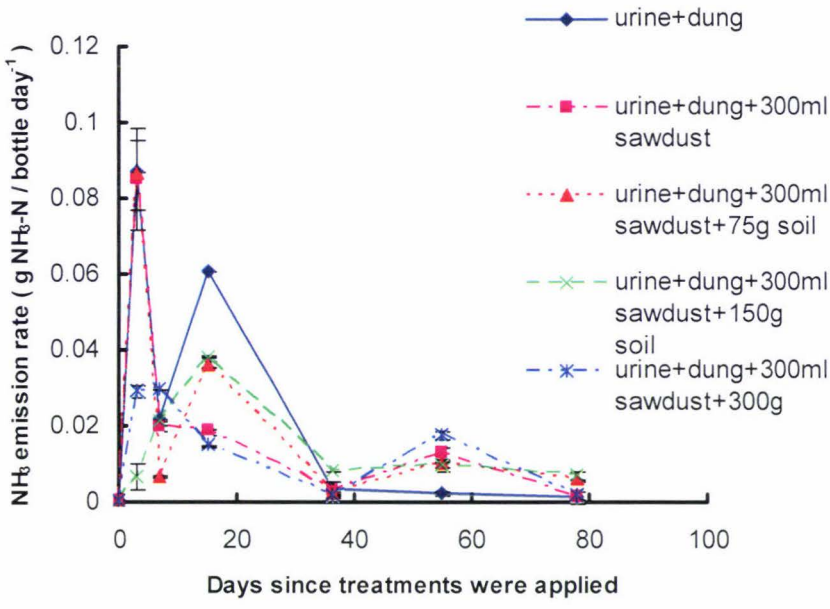

Fig. 4.14 Ammonia (NH_3) emission rate of different treatments in incubation bottles under aerobic condition at 15°C.

Except for the peak of urine+dung+300ml sawdust +150g soil observed at day 15, other treatments showed the highest NH_3 emission rate at day 3. As Fig. 4.14 showed, the peak rates were $0.086\pm4.93\times10^{-5}$, 0.084 ± 0.013, 0.086 ± 0.009, 0.038 ± 0.0002, and 0.0289 ± 0.0013g NH_3-N/bottle/day for urine+dung; urine+dung+300ml sawdust; urine+dung+300ml sawdust+75g soil; urine+dung+300ml sawdust+150g soil and urine+dung+300ml sawdust+300g soil, respectively. At high peak period, the rates of NH_3 volatilisation typically followed the order: urine+dung+300ml sawdust = urine+dung+300ml sawdust±75g soil >urine+dung+300ml sawdust+150g soil >urine+dung+300ml sawdust+300g soil. Both the cumulative NH_3 losses and rate of NH_3 emission for urine+dung+300ml sawdust+300g soil treatment were lowest among all treatments.

Table 4.10 Effects of sawdust and soil amendment (Based on g/bottle basis, numbers in brackets are standard deviation of the mean, n=4).

Analytical items	Onset of incubation				The end of incubation after 78 days			
	Treatments				Treatments			
	Urine+dung amended with 300ml sawdust	Urine+dung amended with 75g soil and 300ml sawdust	Urine+dung amended with 150g soil and 300ml sawdust	Urine+dung amended with 300g soil and 300ml sawdust	Urine+dung amended with 300ml sawdust	Urine+dung amended with 75g soil and 300ml sawdust	Urine+dung amended with 150g soil and 300ml sawdust	Urine+dung amended with 300g soil and 300ml sawdust
Total nitrogen (g/bottle)	1.5103 (0.0055)	1.711 (0.0006)	1.906 (0.0055)	2.304 (0.0052)	0.7139 (0.0087)	0.7780 (0.0049)	0.9922 (0.018)	1.5819 (0.006)
Organic nitrogen (g/bottle)	1.1427 (0.006)	1.2009 (0.006)	1.3201 (0.007)	1.6503 (0.007)	0.701 (0.006)	0.6913 (0.005)	0.8677 (0.015)	1.2717 (0.004)
NH_4^+-N (g/bottle)	0.3676 (0.01)	0.5101 (0.004)	0.5859 (0.009)	0.6537 (0.008)	0.0121 (0.0005)	0.0867 (0.005)	0.1245 (0.01)	0.3102 (0.001)
pH	8.39	8.34	8.28	8.23	8.30	8.27	8.21	8.17

4.3 Disscussion

4.3.1 Nitrogen transformation among four different treatments under aerobic condition

4.3.1.1 Ammonium concentration

The ammonium concentration in the urine increased quickly during the first 3 days and rose to a maximum at day 10. After 10 days the ammonium concentration decreased to only 4.1% of the initial ammonium concentration. Urine+dung showed the same pattern, where a maximum value was reached at day 3 and then declined until the end of incubation when the ammonium concentration was negligible. In these two treatments the initial rapid increase in ammonium concentration was probably due to degradation or hydrolysis of urea or other very readily available nitrogen components such as amino acids and amines (Kessel *et al.*, 1999). On the first day, urine+dung (C/N=3.86) had a higher ammonium concentration than urine and urine+dung+sawdust. This could be attributed to the high pH causing more ammonia loss in the urine sample and the dilution of nitrogen in the urine+dung+sawdust treatment. A high concentration of ammonium ions is a necessary prerequisite for ammonia volatilisation to occur. Other factors affecting it are high pH, high temperature, wind velocity, ratio of NH_4^+ to NH_3 and C/N (Bolan *et al.*, 2004a). Although the ammonium ions concentration of urine+dung+soil was higher than other treatments, it could be hypothesised that ammonia emission losses may be smaller as ammonium ions interacted with the cation exchange complex in the soil or some of NH_4^+ could be fixed in the lattices of 2:1 clay minerals.

4.3.1.2 Ammonia emissions

Urine treatment

The major nitrogen component in urine is urea. It makes up 50-90% of the nitrogen contained in urine. Other components are hippuric acid (1.9-7.7%), allantoic acid (2.2-22.2%), uric acid (0.6-1.9%), creatine (0-6.3%) and creatinine (0-8.1%). Minor components of urine include xanthine and hypoxanthine, free ammonia and amino acids (Doak, 1952; Kreula *et al.*, 1978; Bristow *et al.*, 1992). When urine is excreted, urea is rapidly hydrolysed by the enzyme urease according to (Eq. 4-2):

$$CO(NH_2)_2+2H_2O \rightarrow (NH_4CO_3) \leftrightarrow 2NH_4+CO_3^{2-} \quad (4\text{-}2)$$

Hydrolysis of urea results in the release of OH^- ions, which could increase the pH of the urine. Alkaline condition favours the transformation of NH_4^+ ions to NH_3 emission (Eq. 4-3), leading to a loss of NH_3 from urine (Bolan *et al.*, 2004a).

$$NH_4^+ + OH^- \rightarrow NH_3\uparrow + H_2O \quad (4\text{-}3)$$

By the end of 78 days aerobic incubation the pH of the urine samples increased from 9.02 to 9.31. This may be one of the reasons for the high rate of ammonia volatilisation during the aerobic incubation. Total nitrogen in urine (2.403g/bottle) samples was higher than other treatments. About 13% of total initial nitrogen was mineralised during the first 3 days and 19% at day 7. It also could be also confirmed by highest ammonia emission rate that appearing at day 3. This finding agrees with Whitehead *et al.* (1989) who noticed that NH_3 volatilisation after 8 days amounted to 15% of the nitrogen applied from urea, about 11% from allantion, almost 4% from creatinine and <1% from creatine and hippuric acid. During days 36-55, the second highest ammonia emission losses occurred, which could be the result of slow hydrolysis of hippuric acid. After 78 days aerobic incubation, 69% of initial total nitrogen had been decomposed and lost through ammonia volatilisation. Around 5.524g of NH_3-N was released from 1L cow urine under aerobic condition. The high initial total nitrogen content in urine and high pH from urea hydrolysis may have increased the cumulative amount of NH_3 volatilisation from urine. Among the four treatments urine had the highest ammonia emission losses during incubation.

Urine+dung treatment

Cow faeces contains low amounts of rapidly decomposable nitrogen (Van Faassen and Van Dijk, 1987). However, it does contain large amounts of macromolecules of organic nitrogen compounds (protein). A large proportion of the carbon content is from undigested fibrous material, such as cellulose, hemicellulose and lignin (Bolan *et al.*, 2004a). Urea or NH_4^+ only accounted for a small percent of the nitrogen in fresh dung. The major part of the nitrogen in faeces is organically bound nitrogen. Snel (1990) found that up to 50% of organically bound nitrogen may become mineralised during

storage for six months. Analysis of cow's faeces suggests that the ammonia emission potential is relatively low. However, when urine and dung are mixed together, "priming effects" could stimulate heterotrophic microorganism activity to hydrolyse of macromolecules of organic nitrogen compounds resulting in the production of amines and amino acids. Moreover, amines and amino acids undergo ammonification reaction and are converted to NH_4^+ ions. The process is known as "a positive priming effect", and was denifined by Jenkinson *et al.* (1985): priming is every effect on nitrogen already in the soil by adding nitrogen to the soil. In this treatment, on one hand, urine would provide inorganic nitrogen to microorganisms after urea was hydrolysed; and on the other hand, dung contained a number of microorganisms and carbohydrate materials (cellulose and hemicellulose) that may supply energy to support microorganisms activity. Therefore, the mixture of urine and dung could have a much more intensifying nitrogen mineralisation and further ammonia volatilisation than dung alone. At the onset of the incubation, the ammonium ions concentration of urine+dung treatment was about 5 fold that of urine+dung+sawdust and 40 fold that of urine (Table 4.5). During 78 days incubation, about 48.4g of NH_3-N was released from 1kg of urine+dung sample (dry matter) (Table 4.6 and Table 4.7). About 65.15% of initial total nitrogen was lost through ammonia emission at the end of aerobic incubation (Table 4.6). During the first 15 days, a large amount of ammonia was derived from the hydrolysis of urea and allantion with a small proportion from organic nitrogen compounds. The plot of ammonia emission rate showed a sharp increase and 56.15% NH_3 losses occurred in this stage. After 15 days the NH_3 volatilisation gradually declined and only about 9% NH_3 losses were obtained by day 66 as macromolecular organic nitrogen compound (major components of dung) was slowly decomposed (Fig. 4.2 and Fig. 4.3). Urine+dung had larger ammonia losses, with 13% higher than urine+dung+sawdust treatment and 22% higher than urine+dung+soil treatment.

Urine+dung+sawdust

The ammonium concentration of urine+dung+sawdust was 0.084 g/bottle, which did not have positive priming effects due to C/N>10 (Nicolardot *et al.*, 1986). It could be attributed to addition of enriched-C materials that increased C/N in the samples resulting in low mineralisation. The reduction in ammonia volatilisation from urine+dung amended with C-enriched organic materials such as sawdust was observed in this study. It could be due to the shift in the equilibrium between dissolved and

exchangeable NH_4^+ ions, which were held on the exchange sites of the organic matter in the samples. Consequently, it reduced ammonia volatilisation. Compared to urine+dung, NH_3 emission was reduced in urine+dung+sawdust by 13%, accounting for 52.4% of initial total nitrogen (Table 4.6 and Fig. 4.3). Around 9.37g of NH_3-N was released from 1kg of urine+dung+sawdust at the end of aerobic incubation. A high rate of ammonia emission occurred during the first three days and decreased as incubation experiment proceeded. This may be explained by soluble carbon being released enhancing microorganisms immobilization potential. Reduction of NH_4^+ and addition of acid sawdust also decreases pH in the sample, reducing alkaline conditions. The C/N ratio is generally considered to be a significant factor influencing nitrogen mineralisation of organic nitrogen compound (King, 1984; Barbarika *et al.*, 1985). This study agreed with Mueller *et al.* (1998) and Thomsen and Kjellerup (1997). They found that high carbon bedding materials, such as sawdust and straw, had a strong immobilising effect when present in manures. Highly lignified, mature materials can immobilise nitrogen during the decomposition process. Chadwick *et al.* (2000) concluded that fibrous carbon affected the potential mineralisation of manure organic nitrogen and actively removed the manure NH_4^+ fraction from the manure.

Urine+dung+soil

Quite a high ammonium concentration could be observed at the beginning of the incubation for urine+dung+soil. Dung and soil contain a large amount of proteolytic and deaminative bacteria which initially hydrolyse proteins to peptides and amino acids and finally converted to NH_4^+ ions. The mineralisation is achieved through the activity of both types of bacteria. However, due to the soil's exchange capacity for NH_4^+ ions, the reduction in ammonia volatilisation could be possible. During the 78 days aerobic incubation, only around 3.06g of NH_3-N was released from 1kg of urine+dung+soil. This was much less than that of urine+dung+sawdust (9.37g/1kg) and urine+dung (48.4g/kg). The ammonia losses only amounted to 43.71% of initial total nitrogen. In contrast to urine+dung, a 21.44% reduction of ammonia emission has been accomplished. These results were consistent with Selvarajah *et al.* (1989) who found reduction in ammonia volatilisation was due to cation exchange capacity (CEC) of a number of soils.

4.3.1.3 Comparisons among different treatments

With regard to the results discussed above, it can be concluded that urine+dung+soil had the highest capacity to reduce ammonia emission, followed by urine+dung+sawdust. The amount of ammonia volatilisation losses, as a percentage of initial total nitrogen followed: urine (69%) >urine+dung (65.2%) >urine+dung+sawdust (52.4%) >urine+dung+soil (43.2%). During the 78 days incubation, 48.4, 9.37g and 3.06g of NH_3-N from 1kg dry tested materials were released from urine+dung, urine+dung+sawdust and urine+dung+soil. The benefit from applying carbon-rich amendment materials in reducing ammonia emission could be attributed to direct absorption of NH_4^+ ions and molecular NH_3 (AL-Kanani *et al.*, 1992), acidification of sawdust and enhancement of microbial nitrogen immobilization (Philips *et al.*, 1999).

The benefit from amendment additions in reducing ammonia emission depends on their exchange capacity for NH_4^+ ions and ability to fix NH_4^+. Adsorption of NH_4^+ decreases the quantity of dissolved NH_4^+ ions, an essential prerequisite for ammonia volatilisation, and hence plays an important role in reducing ammonia emission. In addition, due to a buffering effect, the pH of treatment amended with soil maintained between 8.2-8.5 (Table 4.6) that was slightly lower than that in urine+dung. This may be another reason for the reduction in ammonia volatilisation in urine+dung+soil treatment.

4.3.2 Effect of oil-covering of urine

Covering stores of animal excretion with oil or plastic firm has been found by Phillips *et al.* (1999) to reduce ammonia emission by increasing the surface resistance to ammonia volatilisation or by reducing the emitting surface area. At the start of the incubation oil formed a uniform layer, but gradually became miscible with urine. The initial pH (8.63) of urine+oil was lower than that of urine; although the pH increased with incubation, the difference in pH between these two treatments was maintained at the end of the incubation. From day 7 till the end of the incubation, the ammonium concentration of urine+oil was about 60 times as that of urine (Fig. 4.10). Ammonia emission in the urine with oil was reduced by up to 67.5% till the end of incubation (Fig. 4.8). For urine the maximum ammonia emission rate was at day 1, and then declined. However, urine with oil reached its maximum rate of ammonia emission during the first 3 days (0.035 g/bottle/day) (Fig. 4.9). The results from this study support the concept that covering urine leads to a reduction of amount of ammonia emission. The most important factors influencing ammonia emission are concentration of urea in urine,

emitting surface, the pH of the urine, the air velocity and the temperature (Van der Peet-Schwering *et al.*, 1999). In this study, all the factors were the same for the two treatments except for the emitting surface being reduced in the treatment with a surface layer of oil. The oil cover was a physical barrier. It stopped or reduced the air movement over the urine surface and increased the surface's resistance to ammonia volatilisation. The larger and thicker the boundary layer was, the higher the resistance to the volatilisation process. It could be suggested that the reduction of ammonia volatilisation losses achieved by covering oil was mostly owing to a decline in the mass transfer coefficient (Portejoie *et al.*, 2003).

4.3.3 Nitrogen mineralisation with and without inoculum

During the first 7 days, there was no distinct difference in nitrogen mineralisation between urine with and without inoculum. From day 15, there was a significant increase in the concentration of ammonium nitrogen in the urine with inoculum. The maximum value of NH_4^+ ions in urine with inoculum was 1.5 times that of urine. It could be assumed that the bacteria in the inoculum, such as proteolytic, deaminative bacteria and urease enzyme, stimulate urea hydrolysis and decomposition of small macromolecules of organic compounds. Addition of inoculum to urine+dung and urine+dung+sawdust had no distinct influence on nitrogen mineralisation. It can be speculated that 6 ml inoculum added to (150ml urine+150g dung) and (75ml urine+75g dung+300ml sawdust) was not sufficient enough to accelerate the rate of nitrogen mineralisation in these treatments.

4.3.4 Effect of different level natural amendments on ammonia volatilisation

4.3.4.1 Assessment of different rate of soil amendment on reducing ammonia losses

Addition of soil to urine+dung made a significant difference to ammonia volatilisation and effect was more pronounced at high level of soil addition. After 78 days of incubation, a 22% reduction in ammonia emission was achieved by adding 300g soil, while only a 4% and 6% reduction was achieved by adding 75g and 150g soil, respectively. In this incubation study, addition of 300g soil to 150ml urine+150g dung (excreta: soil=1:1) was the optimum treatment to reduce ammonia volatilisation.

4.3.4.2 Assessment of different rate of soil combined with sawdust amendment on reducing ammonia losses

With the quantity of sawdust (300ml) fixed, different amounts of soil were added and the effect it had on ammonia emission during the incubation period was studied. Compared to urine+dung, the reduction of ammonia emission was, in a descending order: 33.7% (300g soil addition)>17.1% (150g soil)>10.5% (75g soil). The reduction of ammonia emission in urine+dung+75g soil+300ml sawdust was slightly less than urine+dung+300ml sawdust. Compared to urine+dung+300g soil, addition of 300ml sawdust reduced ammonia losses by 11%. It was concluded that addition of both sawdust and soil to animal excreta could enhance the reduction in ammonia emission. On the one hand, addition of sawdust increased the C/N ratio in the tested materials, and enhanced nitrogen immobilisation potential; on the other hand, due to the large surface of sawdust, it increased the NH_4^+ absorption capacity.

In general the results of this study show that urine+dung+soil+sawdust (excreta:soil:sawdust=1:1:0.16, w:w:w) is an optimal treatment to reduce ammonia losses from excreta. However, if this treatment concept was adopted, the expense of purchasing and transporting sawdust has to be considered. Field test should be conducted to verify these results as they were obtained under controlled laboratory conditions.

Chapter 5 Measuring denitrification activity in animal excreta

5.1 Introduction

Denitrification requires anaerobic conditions and is also affected by other environmental factors including pH and temperature; as well it is affected by the availability of essential substrates: nitrate ions and available carbon (Firestone, 1982; Tiedje, 1988; Luo *et al.*, 1996). The potential for denitrification can be assessed using a short-term denitrification enzyme activity (DEA) assay (Luo *et al.*, 1996). The laboratory-based assay is an important tool that is used to measure the potential activity that could be reached for a material of interest under optimum conditions. The short-term denitrification enzyme assay is usually conducted under anaerobic conditions with no limiting substrates (i.e. with an excess of NO_3^- and available carbon) and under these conditions the result can be obtained in a few hours (Luo *et al.*, 1996). This assay can be used to compare denitrification rates among different materials. It has also been used to provide a credible estimate of actual field denitrification rates (Tieje *et al.*, 1989; Luo *et al.*, 1998).

In this Chapter a set of experiments were carried out to determine the denitrification potential of the excreta (urine+dung) that consisted of a 1:1 mixture of fresh urine and dung, and mixture of excreta amended by soil and sawdust. A number of prerequisites need to be satisfied to enable denitrification potential demonstrated in these test materials. These included prior determination of the concentration of naturally occurring nitrate (NO_3^-) and available carbon (C) so that any addition of these substrates will ensure they are at optimal level. As well, the incubation time for the assay needs to be calculated; it must be long enough to ensure denitrification is established effectively and at a constant rate but short enough to avoid an increase in the population of denitrifying bacteria that will arise from the products of denitrification being incorporated into new generations of organisms. Until substrates become limiting such an increase in the population will result in an increase in the rate of denitrification and give rise to a value that is greater than the "natural" potential level for that test material.

In this study, experiments were set up to determine the denitrification potential of excreta i.e. a specific mixture of dung+urine and of two amendments, dung+urine+soil and dung+urine+sawdust. Their actual amounts were:

- 150ml urine +150g dung
- 150ml urine+150g dung +300ml sawdust
- 150ml urine+150g dung+300g soil

These treatments were incubated under aerobic conditions for 15 days at 15°C (same conditions as used in the first and second incubation study). Then the aliquots were sampled to determine the chemical properties and further aliquots were transferred to another set of containers that were incubated under anaerobic conditions to determine short-term denitrification enzyme activity (DEA). The DEA experiments were in effect a third stage incubation that was carried out at 20°C. A series of DEA experiments were carried out:

Firstly to determine the time denitrifiers started to establish denitrification. Denitrification activity was determined by measuring the rate of N_2O emission at 0, 15, 60 and 90 min after anaerobic incubation by using the acetylene blockage technique (all Erlenmeyer flasks had 25ml acetylene injected in them to block N_2O conversion to N_2).

Secondly, to assess the denitrification potential for each treatment a series of experiments were carried out in which each of the substrates, glucose and nitrate-N was added separately and also in combination. The amounts added were 300µg glucose g^{-1} test material and 50µg NO_3^--N g^{-1}test material (Luo *et al.*, 1996).

In order to understand the nitrification process of all treatments before assessing denitrification potential for all treatments, the concentration of naturally occurring nitrate (NO_3^-) and nitrite (NO_2^-) was determined in all treatments.

5.2 Results

Nitrification potential activity

The properties of the three treatments used in this study are summarised in Table 5.1. The data shows that no naturally occurring NO_2^--N and NO_3^--N could be detected in any of the three test treatments on sampling days, indicating that either nitrification did not occur during the aerobic incubation or most of the ammonium ions formed were immobilised and/or volatilises as ammonia gas.

Table 5.1 Properties of various treatments before incubation (wet basis, n=4) (Number in brackets is the standard deviation).

Treatment	Moisture (%)	pH	NH_4^+-N (%)	NO_3^--N (%)	Dissolved organic carbon (%)	NO_2^--N (%)
Urine+dung	87.89 (0.013)	8.87	0.053 (0.0044)	nd	0.0746	nd
Urine+dung +soil	57.64 (0.024)	8.61	0.095 (0.0031)	nd	0.0417	nd
Urine+dung +sawdust	75.75 (0.012)	8.43	0.061 (0.0062)	nd	0.0415	nd

nd=not detected

Denitrification rate of without nitrate and carbon addition, or only with the addition of only glucose-C glucose

The emission of N_2O from urine+dung urine+dung+soil and urine+dung+sawdust treatments without substrate (nitrate and carbon) addition was carried out to determine the optimum time required to measure denitrification potential. The results showed that without the addition of substrate there was no significant emission of N_2O from the three treatments. The value was negligible with a maximum value of only 0.1μg N_2O-N/g tested material. Similarly, the maximum value of N_2O emission from the three treatments with glucose-C added was not more than 0.1 μg/N_2O-N g tested material. It indicated that glucose-C addition did not stimulate pre-existing microorganism denitrification activity. Soluble carbon was not a critical limitation factor affecting the denitrification activity of all treatments.

Accumulation of N_2O and denitrification rate with addition of NO_3^-

Fig. 5.1 illustrates that when NO_3^- was added to treatments under anaerobic condition, denitrifiers started to function to produce N_2O in all three treatments. The dilution factors resulting from addition of the sawdust and soil were taken into account to enable comparison between treatments. N_2O production in the presence of C_2H_2 from the three treatments occurred at a constant rate from 15 to 60 min monitoring period, so this period could be used to reflect original microorganisms activity. The switch-over from aerobic to anaerobic respiration by the facultative anaerobes appeared to be rapid and the microorganisms did not propagate during this incubation period. However, in all treatments, a dramatic increase in denitrification rate after 60 minutes of incubation especially in the case of urine+dung+sawdust treatment appeared. This implied that after 60 minutes the microorganisms in the treatments have begun to reproduce in the anaerobic condition. The activity after 60 minutes would not represent the activity of the pre-existing/original microorganisms in the treatments. The duration of the incubation used by Luo *et al.* (1996) in the short-term assay for soil treatment has ranged from one to five hours. Other workers obtained optimum incubation period ranging from one to eight hours (Limmer and Steele, 1982; Martin *et al.*, 1988) should be used.

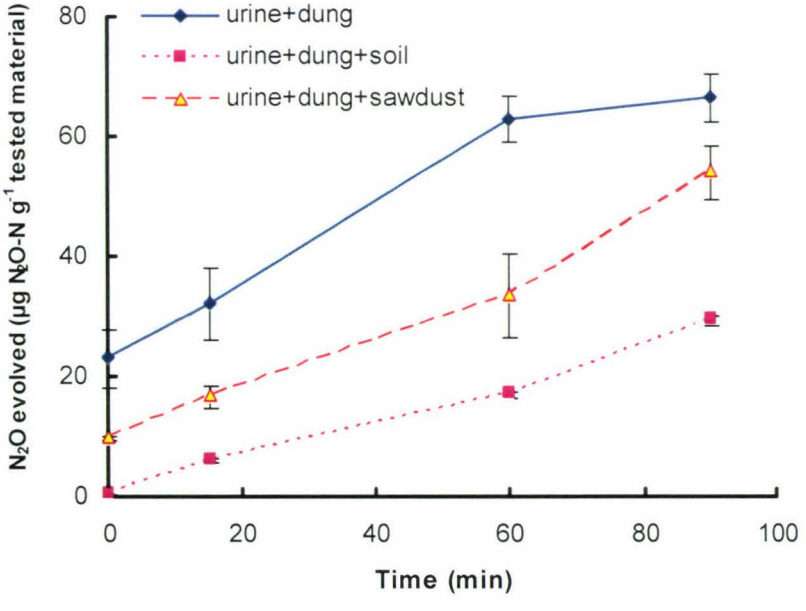

Fig. 5.1 N_2O evolved during anaerobic incubation when NO_3^- (50µg NO_3^- g^{-1} tested material) was added (bars represent standard deviation).

Denitrification rate with addition of NO_3^- and glucose-C

With the addition of NO_3^- and glucose-C, facultative anaerobes rapidly recovered and switched-over from aerobic to anaerobic respiration. From 15-60 minutes, all three treatments had a constant rate of N_2O production increase with the addition of NO_3^- and glucose-C, with maximum values are several times greater than those for the treatments with NO_3^- alone (Fig. 5.2).

After 60 minutes, the three treatments show a slight increase in denitrification rate. The activity then would not represent the activity of the original microorganisms in the test materials. The duration of the treatment with NO_3^- and soluble-C should be within 15 to 60 minutes, as this properly reflected the short-term denitrification potential of pre-existing microorganisms for three treatments.

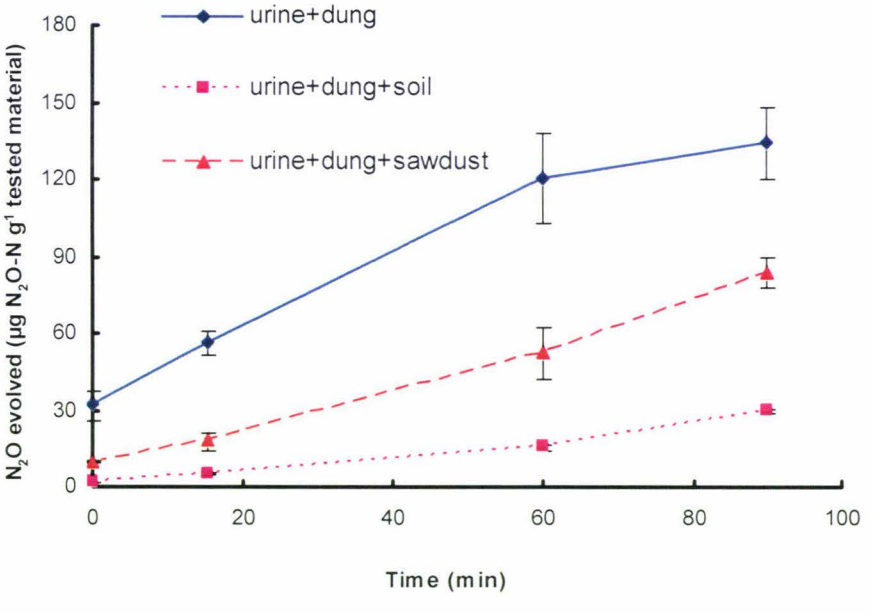

Fig. 5.2 N_2O evolved during anaerobic incubation when glucose and NO_3^- (300µg glucose-C and 50µg NO_3^- g^{-1} tested material) were added (bars represent standard Deviation).

In general, based on the above results, an incubation period between 15-60 minutes for all treatments should be used to measure the activity of pre-existing denitrifying organisms.

Denitrification potential among different treatments

Table 5.2 shows that the rate of N_2O emission from urine+dung increased from 43.81±4.93 (NO_3^- added) to 114.44±22.53 μg N_2O-N/g/hour with NO_3^-+glucose-C. The range varied from 39.56±7.18 to 68.75±14.69 μg N_2O-N/g/hour for urine+dung+sawdust treatment, and from 15.43±1.39 to 20.10±2.65 μg N_2O-N/g /hour for urine+dung+soil treatment. The denitrification rates from the three treatments with NO_3^- added were much higher than these with only glucose added. These results demonstrated that NO_3^- concentration was the main limiting factor affecting denitrifying bacteria activity. The rate of N_2O emission from addition of NO_3^- plus glucose was higher than that from addition of NO_3^- in the three treatments, suggesting although that soluble carbon was not a pivotal factor, the combination of glucose and NO_3^- would promote denitrification rates in all treatments.

Table 5.2 Denitrification rates among different treatments (μg N_2O-N g^{-1} tested material $hour^{-1}$).

Addition	Addition of NO_3^-	Addition of NO_3^- and glucose-C
Urine+dung	43.81±4.93	114.44±22.53
Urine+dung+soil	15.43±1.39	20.10±2.65
Urine+dung+sawdust	39.56±7.18	68.75±14.69
LSD (0.05)	7.978	24.76

5.3 Discussion

It can be concluded that NO_3^- is a crucial factor affecting the denitrification rate among the three treatments. Soluble-C is not a pivotal factor influencing denitrification rate. However, addition of NO_3^- and glucose-C could enhance the denitrification rate, as denitrifiers need to have energy from soluble-C to reduce NO_3^- to N_2O. Therefore, the denitrification rate from treatments with both NO_3^- and glucose-C added could represent the denitrification potential. Denitrification potential was in descending order: urine+dung> urine+dung+sawdust>urine+dung+soil. This may have been attributed to the high water content of urine+dung (87.89%) allowing diffusive movement of NO_3^- and soluble-carbon to the microsites where denitrification was occurring in the tested materials (Ruz-Jerez *et al.*, 1994; Luo *et al.*, 1999a). It could be assumed that little nitrous oxide was produced from excreta stored in the winter management systems as only a small amount of NO_3^- was detected during this period.

Chapter 6 Field experiment on the use of natural materials in stand-off pad for reducing nitrogen losses

Experimental studies are being carried out to develop effective stand-off pads constructed from natural materials so that cows can stand for extended periods of time when paddocks are not suitable for grazing (Fig. 6.1 and Fig. 6.2).

The success of stand-off pads depends on the effectiveness of pad materials in continuously retaining nutrients for a long period. The retention of nutrients means that after the stand-off period, pad materials could be then used as a natural source or be composted for gardening purposes. To be effective for this purpose pad materials should be chosen for maximum nutrient retention, to enhance their fertiliser value.

6.1 Objectives

The objective of this study was to identify naturally available materials of sufficient supply and with high adsorption potential that would be suitable for use as stand-off pad materials under field condition. In this chapter we present the results of a field-scale study carried out on a working dairy farm in which we compared two stand-off pads that differed only in their fill material, one being filled with pine bark and the other with sawdust (Fig. 6.3).

Fig. 6.1 Photo of a stand-off pad in Scott farm (R.D. Longhurst, AgResearch, Ruakura).

Fig. 6.2 Photo of a stand-off pad in South Island (R.D. Longhurst, AgResearch, Ruakura).

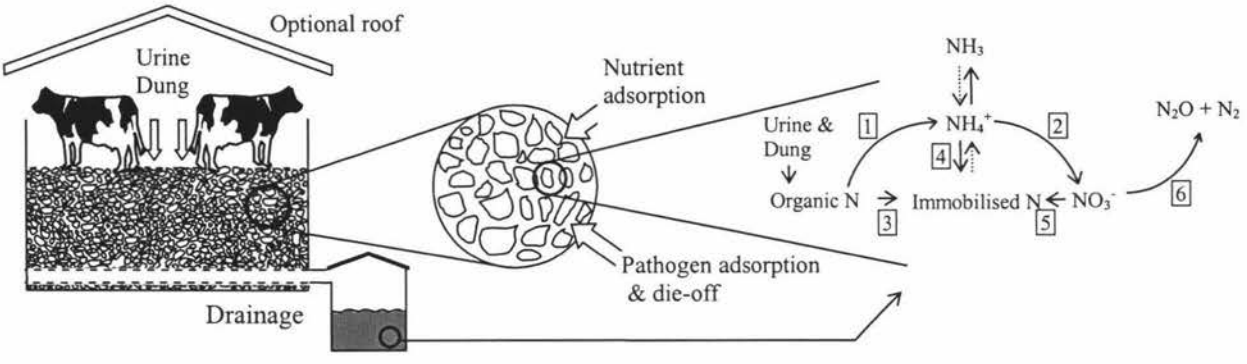

Fig. 6.3 Simplified diagram of a stand-off pad system.

6.2 Results and discussions

Field-scale study

The results from the field stand-off pad study showed that both bark and sawdust retained a considerable amount of N from cows' excreta (Table 6.1). During the 2005 winter season between late May and early August, we estimated that about 170 kg of excreta N was deposited by the cows on each stand-off pad containing either sawdust or bark. However, less than 5% (7.4-8.4 kg N) of the deposited excreta N was collected in the drainage from the pads. Gaseous N losses by denitrification in the sawdust pad were 20 kg N, accounting for about 12% of the deposited excreta N, while the losses in the bark pad were 7.1 kg N, accounting for about 4%. At nine months after use, the bark pad retained about 78% of deposited excreta N, while the sawdust pad retained about 51%. Mass balance calculation indicated that about 33% of the deposited excreta N were not accounted for in the sawdust pad, while about 14% were not accounted for in the bark pad. This N loss may have been due to ammonia gas losses or sampling and measurement errors. Other chemical analyses of materials also indicated that both sawdust and bark retained significant amounts of deposited excreta P, K and S (data not shown). Observation in April 2006 revealed that there was no indication of any breakdown of the sawdust and bark materials within the pads about eight months after commencing use of the pads. The results from the field study suggested that the C-rich materials (bark and sawdust) can be used as stand-off pad materials with effective retention of N and other nutrients.

Table 6.1 Nitrogen balance at nine months after 21 cows had been held on stand-off pads in the winter (31st May to early August 2005).

	Sawdust	Bark	Sawdust	Bark
	Amount (kg N)		Recovery (%)	
Deposited excreta N	170	170		
Drainage N	8.4	7.4	4.9	4.4
Denitrification N losses	20	7.1	12	4.2
Accumulative N in materials	86	133	51	78
Ammonia N losses and/or unaccounted for N[1]	56	23	33	14

[1] Unaccounted for N was due to errors of sample collection and analysis.

Table 6.2 Characteristics of materials in the field stand-off pad study.

	Sawdust	Pine bark
Total N (%)	0.023	0.103
Organic C (%)	23.0	36.0
C/N ratio	1000	350
pH	6.0	5.3
Water soluble C ($\mu g\ C\ g^{-1}$)	695	379

Discussion

The effectiveness of natural materials in retaining N can be attributed to enhanced microbial N immobilisation and/or direct absorption of ammonium ions (Bolan *et al.*, 2004a; Luo *et al.*, 2006). Bolan *et al* (2004c) have also demonstrated that treatment of farm effluent with pine bark achieves a considerable reduction in the N concentration, which they attributed to immobilization of N by the C- rich bark material (C:N ratio = 265:1). Crushed pine bark and sawdust both have large total surface areas and cations exchange capacities (CEC). Ammonium ions, mineralised from cows' excreta, can be adsorbed onto these surfaces, thereby decreasing the quantities of dissolved NH_4^+ ions and equilibrated NH_3 gas available for NH_3 volatilisation. Mass balance calculations indicated that lower NH_3 volatilisation losses could have occurred in the bark pad than in the sawdust pad (Table 6.1).

Denitrification also contributed to the loss of N in the field-scale stand-off pad (Table 6 1). Cow excreta and C-rich materials in the stand-off pads provided N and C for

denitrification. In addition, wetting and drying cycles in the stand-off pad materials would have favoured nitrification (to produce nitrate) and subsequent denitrification (to reduced nitrate to gaseous N). Sawdust contained more water soluble C than bark (Table 6.1), indicating that sawdust may have provided more readily available C as electron donor for denitrification bacteria than bark. Consequently, greater gaseous N losses through denitrification from the sawdust pad than the bark pad were found (Table 6.1). Bark and sawdust contain high levels of organic C (Tables 6.1 and 6.2) that would degrade slowly and could therefore be available to denitrifying bacteria for a longer period. Rates of denitrification N losses may also be increased over time due to decreased oxygen levels resulting from compaction of materials through animal treading on the pads. It is important to stress that since these bedding materials are rich in bioavailable carbon content, most of the nitrogen during the denitrification would have been lost as nitrogen gas (N_2).

6.3 Conclusions

A promising winter management practice that can reduce problems caused by cows grazing on wet soil is the use of stand-off pads constructed of natural materials. To be successful, natural materials must have the ability to retain the nutrients that are deposited in cow excreta. Field-scale stand-off pads were constructed of sawdust or bark and both retained large amounts of deposited excreta N. Drainage losses of N from the pads were minimal. More gaseous N losses through denitrification were found in the sawdust pad than those in the bark pad.

Chapter 7 Summary and conclusions

The overall objective of the study was to investigate the transformation and loss of nitrogen from excreta. The research focus was on the ability of natural materials with absorbent properties, such as sawdust and soil that could be used in winter management systems, as amendments or bedding materials to reduce nitrogen losses. Initially, a series of incubation studies were carried out under control conditions to quantify the transformation of nitrogen, to estimate the loss of nitrogen through ammonia emission and to measure denitrification potential from different treatments that represented various winter management systems. Excreta were used for these studies that constituted of a 1:1 mixture of fresh urine and dung. Following this, a field study was carried out to investigate nitrogen (N) retention and losses from cow excreta on stand-off pad material. This chapter summaries the results from these studies.

7.1 Nitrogen mineralisation and quantification of ammonia emission

Excreta-N was mineralised rapidly within three days that would cause ammonia loss. However excreta amended with sawdust and soil retained a large amount of ammonium ions by absorption and immobilisation. The ammonia emission rate from the excreta reached a peak value within three days under aerobic condition. This initial high rate of ammonia volatilisation may have been due to rapid hydrolysis of the urine component. When excreta was amended with sawdust or soil, the emission of ammonia was reduced. At the end of 78 days incubation, compared to the unamended excreta (urine:dung=1:1,w:w), the sawdust amendment (excreta: sawdust (1:2), w:v) and soil amendedment (excreta:soil (1:2), w:w) reduced ammonia emission by 19.5% and 32.9%, respectively. The results of the incubation study suggested winter management systems using amendments such as soil and sawdust would be effective for reducing ammonia emission.

In a separate experiment, urine was covered with a gas impermeable layer (i.e. vegetable oil) and this treatment was found to reduce ammonia emission by 67.5% compared with urine that had no oil covering.

7.2 Assessment of different ratios of soil or sawdust amendment on reducing ammonia losses

A second incubation study was carried out to investigate the effect of different ratios of manure to the two natural materials (soil, sawdust) in conserving nitrogen. Ammonia emission decreased with increasing addition of amendments. Although both materials were effective in reducing ammonia emission, the effect was more pronounced with the addition of these two amendments together. Excreta amended with soil and sawdust (1:1:1, w:w:v) reduced ammonia emission by 34%, followed by addition of soil (1:1, w:w) by 22%, sawdust and soil (2:2:1, w:v:w) by 17.1%, sawdust (1:1, w:v) by 12.7%, sawdust and soil (4:4:1, w:v:w) by 10.5%, soil (2:1, w:w) by 4% and soil (4:1, w:w) by 6%.

7.3 Assessment of denitrification potential among different treatments

Animal excreta and excreta with soil (1:1, w:w) and sawdust amendments (1:1, w:v) had a very low denitrification rate in the absence of glucose and NO_3^- addition. Results of study showed that NO_3^- concentration was the main limiting factor on demonstrating denitrification potential, although available soluble carbon had some influence on denitrification rates. Addition of glucose (300μg glucose/g excreta) and NO_3^- (50μg NO_3^--N/g excreta) significantly enhanced the denitrification rates with the values reaching 114.4μg, 68.75 and 20.14μg N_2O-N/g excreta/hour for excreta, excreta+sawdust and excreta+soil, respectively. Denitrification potential followed: excreta> excreta with sawdust> excreta with soil.

7.4 Stand-off pad study

At field-scale (on a dairy farm) the carbon-rich natural materials pine bark and sawdust were shown to retain nitrogen effectively when used as stand-off pad materials. Nine months after use, the bark had retained 78% of the deposited excreta nitrogen, while the sawdust pad had retained 51%. Gaseous nitrogen losses due to denitrification in both the sawdust and bark stand-off pads accounted for about 12% and 4% of the deposited excreta nitrogen, respectively.

7.5 Conclusion

Addition of either soil, sawdust, or a combination of both to animal excreta is effective in reducing nitrogen loss from animal excreta. The optimum ratio of excreta to natural materials was found to be 1:2. The reduction of ammonia emission in the stand-off pad was probably attributed to immobilisation of mineral nitrogen and adsorption of nitrogen compounds by the natural amendments (e.g. soil and bark). In addition, under optimum conditions in the absence of any limiting factors unamended excreta exhibited higher denitrification potential than amendment with soil or sawdust. From the results of incubation experiments carried out under laboratory conditions and a stand-off pad study, it can be concluded that reduction of gaseous emission (NH_3 and N_2O) could be achieved by using a stand-off pad or housing system (herd home) that incorporates the use of a carbon rich natural material or soil.

7.6 Future prospects

Since the loss of nitrogen from animal excreta under winter management systems is dominated by gaseous emissions of ammonia and nitrous oxide, regular monitoring of these gases should be undertaken. As these experiments were conducted mostly under controlled laboratory conditions, the effects of other factors, such temperature and wind velocity, needs to be verified in the field. Future research should focus on the technology of using natural materials to manage nitrogen dynamics at an operational scale.

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