CHAPTER 1 GENERAL INTRODUCTION

The world population in 2012 was about 7 billion and is projected to increase to 8.1 and 9.6 billion by 2025 and 2050, respectively (FAO, 2013; PRB, 2012). In south-east Asia, the population was 0.45 billion in 1990, increased to 0.6 billion in 2012 and is projected to be over 0.76 billion by 2050 (FAO, 2013; PRB, 2012). This rapid population growth has put enormous pressure on the agricultural sector to meet the growing demand for grains, meat and milk. The requirement for N has therefore substantially increased to meet this demand for greater production. However, the supply of N is unevenly distributed between the wealthy and poor countries, being more plentiful in developed countries and less available in developing countries (Perrings et al., 2014). The inequality of use of N for cropping and animal production has caused, and continues to cause contrasting environmental problems (Wang et al., 2011). For example, in intensive production systems in developed countries, crop and animal production is associated with N pollution whereas, in developing countries, such as in the tropics where production is by smallholder producers is based on a low-input system, N depletion from soils is occurring and the situation is not sustainable.

When N is overused, most of the surplus N is lost to the environment (Bouwman et al., 2011), and it is expected that this problem will increase in more developed countries as crop and livestock production increases up to 82% and 115%, respectively, over the next 40 years. However, it is also estimated that the recovery of N in crops will be improved across all farming systems over the next 40 years (35%) (Bouwman et al., 2011). Solutions to reduce the surplus of N would be alternative management strategies in livestock production systems, combining crops and livestock in intensive farming systems, better integration of animal manure in crop production and matching N and P supply to livestock requirements (Bouwman et al., 2011). Where N is under-supplied and N is being depleted from agricultural systems in developing countries, the maintenance and restoration of N in soil must also be taken into account (Perrings et al., 2014) and the priorities for N management could differ from more developed nations.

Improving diversified agricultural production, i.e. crop and livestock systems, requires knowledge of nutrient use efficiency. In general, most smallholders in tropical areas are located on infertile (mainly N and P nutrient deficiencies) and poorly structured soils, where owing to financial constraints, it is common for farmers to apply little or no inorganic fertiliser to their crops. Therefore, the challenge for smallholder farmers is to conserve N and increase its input into the farming system in order to increase productivity of animal and crop production. The traditional farming practice of applying livestock manures to fertilise crops and grasslands, i.e. the cycling of nutrients through the farming system, seems to be the

most sustainable way to improve or maintain soil fertility. However, when nutrients cycle through the farming system, i.e. crop and soil conservation, livestock and manure collection and storage, it is expected that the overall nutrient utilisation efficiency as a fraction of the nutrients lost within each sector is lowered, although the extent of nutrients loss largely depends on the nature of the farming system, i.e. beef-forage or beef-rice system (Tabata et al., 2009).

To quantify N cycling in mixed ecosystems, appropriate experimental procedures need to be established and this is a focus of investigations in this thesis. The work undertaken was part of a project entitled 'AH/2003/008 - *Improved feeding systems for more efficient beef cattle production in Cambodia* and ASEM/2010/049 - *Market-focused integrated crop and livestock enterprises for north-western Cambodia*, funded by ACIAR. One of the ACIAR's strategies is to focus on applied R&D that underpins agricultural diversification, particularly into soil, field crops besides rice and ruminant livestock (ACIAR, 2013).

Grain cropping (rice and maize) is the main activity in tropical areas such as Cambodia, resulting in an abundance of straw for feeding to ruminants (Chenost, 1995; Chenost and Kayouli, 1997). However, the digestible N from straw-based diet is not sufficient to support efficient production by livestock to produce meat or milk (Leng, 1990), and a high proportion of the feed N is excreted via faeces and urine (Barrow and Lambourne, 1962; Kebreab et al., 2009; Kebreab et al., 2002). It has been suggested that the nutrition of ruminants fed straw diets can be improved through the treatment of straw (Chenost, 1995; Devendra and Sevilla, 2002) or supplementation with available by-products, e.g. rice bran and palm kernel, produced on farm (Devendra and Sevilla, 2002). Forages from planted plots are also reported to be more successful and are more readily adopted by smallholder farmers (Devendra and Sevilla, 2002). However, the question remains: what are the most feasible, profitable and sustainable practices for resource-limited and financially challenged farmers to increase N conservation? In this thesis, it was hypothesised that improving the nutrient conservation and recycling efficiency would be the most feasible solution to reduce N losses from agro-ecosystems where the production is based on low nutrient inputs. The specific aim of the thesis was to investigate the effect of supplementing low-quality straw diets with green forages that are available on-farm or are by-products of nearby rice production, e.g. rice straw and rice bran.

This thesis is presented over eight chapters. Chapter 1 is this introduction. Chapter 2 is a review of literature that provides relevant background information concerning the N balance and N use efficiency in crop-livestock systems. These chapters are followed by five chapters describing experimental work (Chapters 3-7) addressing N utilisation in ruminant nutrition, crops and soil, and overall N use efficiencies. Finally, the implications of the results are discussed in Chapter 8.

THE RESEARCH OBJECTIVES

The objectives of this thesis are:

1. To investigate the effect of on-farm available supplements on the performance of ruminants offered low-quality straw-based diets.
2. To explore the effect of excreta nitrogen, from animals offered diets containing different supplements, on soil N mineralisation, plant N uptake and cycling efficiency, using isotopic and non-isotopic methods.
3. To investigate the effect of on-farm available supplements on the sustainability of crop-livestock production systems in ruminants based on low-quality straw-based diets.

CHAPTER 2 LITERATURE REVIEW

2.1. Introduction

Nitrogen (N) is a primary nutrient in all-living organisms. It is a necessary component of many biomolecules, including proteins, DNA, and chlorophyll (Bernhard, 2012). Nitrogen plays a major role in agricultural systems that produce food and supply protein for the human population (Follett and Hatfield, 2001). Increased use of N is directly in response to the increasing requirement for protein in the diets of the growing human population (Bouwman et al., 2011; Follett and Hatfield, 2001).

Crop and livestock production systems are the largest cause of human alteration of global N cycle (Bouwman et al., 2011). Global crop production is a key accelerator of N cycle, but livestock production drives global nutrient cycling (Bouwman et al., 2011). Crops and by-products are used for animal feeds and 30% of arable land is being used for producing animal feeds (Bouwman et al., 2011).

The increase in demand for food is having a significant impact on land use change and agricultural activities (Pezo et al., 2000), which in turn is having a negative impact on the environment in many parts of the world. This impact is putting serious pressure on the N cycle, especially in regions, such as Asia, that are experiencing high rates of population growth (Xiong et al., 2008; Zhu et al., 2005). Increasing food production in the Asian region also means there is an increasing production of crop residues and agro-industrial by-products and this could be used to sustain the increased animal numbers and intensify production (Blair and Kerridge, 1997). Some reviewed works show that the sustainable smallholder mixed crop-livestock farming systems could offer a key solution to tackle food crises, as well as address poverty and environmental issues in many parts of the world (Devendra and Thomas, 2002a; Devendra and Thomas, 2002b; Paris, 2002), especially by considering N as a part of sustainable development processes (Perrings et al., 2014).

Mixed crop-livestock systems refer to livestock production activity occurring in arable areas (Jahnke, 1982). Within this system, the major agricultural activity is directed towards crop production for food and cash generation with livestock playing an important supportive role. The majority of rural smallholder producers in developing countries rely on mixed crop-livestock systems for their food supplies (Altieri, 2008; Pimbert, 2008; World-Bank, 2007). In most smallholder systems, the major inputs of labour, capital and land are primarily aimed at crops. However, the role of livestock in the agricultural and rural economies is vital as they not only produce meat and milk, but they also provide key inputs to crop agriculture, i.e. animal power, manure fertilizer, capital accumulation and insurance (Udo et al., 2011). More importantly, livestock are important components of nutrient cycles, and especially the N

cycle, by returning nutrients to the soil via manure and urine. Therefore, N cycling in crop-livestock systems has biological, economic and environmental importance.

Nitrogen occurs in a variety of forms in the soil-plant-animal system *viz.* as gaseous (ammonia and nitrous oxides), cationic (NH_4-N) and anionic (NO_3-N) and mineral forms, and as urea, uric acid, amino acids, amino sugars, nucleic acids, chlorophyll and their derivatives in living organisms and in the soil (Beegle et al., 2008). Nitrogen is used by animals; they convert plant N to available compounds such as peptides and amino acids that are digested and absorbed by the animal and then excreted in various forms that can be re-captured immediately by plants, or mineralized more slowly in the soil. In so-called 'organic' agricultural production systems, the use of synthetic fertilizers is prohibited (Bruinsma, 2003). In these systems, organic fertilizers (e.g. animal manure and plant residues, especially N_2-fixing legumes) are the most important sources of N, along with soil OM.

The purpose of this review is to provide brief background information on gross N balance in agriculture. Nitrogen cycling efficiency in tropical mixed crop-animal, smallholder input and output systems will be reviewed. N use efficiency in the ruminant component of this eco-system and the recovery of N in excreta are considered along with factors affecting the conversion efficiency at each step. At the agronomic level, N conversion efficiency associated with manure storage and application to soil and efficiency of recovery by crops are reviewed. The overall N flow and its efficiency are shown by investigating the critical steps that need to be improved at the smallholder level in tropical areas.

2.2. Gross N balance: surplus or deficit

The global consumption of fertilizer N is about 84 Tg N annually (Smil, 1999). Chemical fertilizer supplies around 50% of the total N required for global food production (Mosier et al., 2004). The other annual inputs of N into crop production include biological N-fixation (25-41 Tg), recycling of N from crop residues (12-20 Tg), animal manures (12-22 Tg) and 21-27 Tg from other sources including atmospheric deposition and irrigation water (Smil, 1999). Of the 170 Tg N added as estimated above, half is converted into harvested crop (85 Tg), the rest is incorporated into soil organic matter or lost into the environment (Smil, 1999). The total N losses include: leaching, runoff and erosion (37 Tg); ammonia volatilization from soil and vegetation (21 Tg); denitrification as N_2 (14 Tg); nitrification/denitrification as N_2O and NO (8 Tg) (Smil, 1999). The surplus N (difference between input and output) is either lost to the environment or accumulated in the soil below the level of the plant roots. For some agricultural lands that are considered to be at, or near steady state for soil accumulation of N (i.e. United States and Western Europe), all inputs not removed by crops or animals are

likely to be lost to the environment. In the case of tropical lands, the surplus may contribute to N accumulation in soil (Tan et al., 2005).

The surplus of N increased from 18 to 36 Tg/year from 1900 to 1950 and to 138 Tg/year by 2000. This surplus is estimated to increase a further 23% by 2050 (Bouwman et al., 2011) and the most significant increase will occur in Africa (49%) and Latin America (75%). Much anthropogenic N is lost to air, water, and land causing a cascade of environmental and human health problems (Galloway et al. 2008).

2.3. N cycling efficiency

Nitrogen cycling can be quantified by measuring the fluxes of N between different nutrient pools in both crop and livestock component of systems (Oenema et al., 2003). To measure N use efficiency (NUE), four agronomic indices are commonly used in field studies, i.e. partial factor productivity (PFP_N), agronomic efficiency (AE_N), apparent recovery efficiency (RE_N) and physiologic efficiency (PE_N) (Krupnik et al., 2004). In this thesis, RE_N, which can be calculated using the difference method and ^{15}N isotope dilution method (Krupnik et al., 2004), is used, but other indices are also acknowledged for a completed N budget study for agriculture.

Factors affecting N use efficiency (NUE)

Cereals, such as rice, maize and wheat and other crops use, on average, 50% or less of applied N for producing above ground biomass (Krupnik et al., 2004). The other 50% is mostly lost to the environment. The N losses are an economic loss to farmers, especially for smallholder farmers in developing countries, where fertilizers represent a substantial part of the total production cost (Jayne et al., 2003). The NUE must be improved to produce enough foods to feed the growing population and avoid degradation of ecosystems caused by excess N (Tilman et al., 2001).

Fertilizer RE_N is controlled by three main factors, *viz.* crop N demand, N supply and N losses (Krupnik et al., 2004). Demand for N is related to total N uptake by the crop, which is strongly correlated with crop yield (Dobermann and Cassman, 2004). Factors affecting crop yield, and N demand, are light (energy), temperature, availability of water and other nutrients (P, K, Mg and S), pests, diseases and weeds.

Supply of N to the soil system can be achieved by the application of N fertilizer or from net mineralization of soil organic matter (SOM), crop residues or animal manure (Berry et al., 2002; Palm et al., 2001). The synchronization of N supply with crop demand for N is the main factor determining RE_N. Fertilizer N can be lost through denitrification, leaching, runoff, volatilization and soil erosion. By eliminating all the factors that are limiting for growth and

providing optimum delivery system, a maximum of RE_N up to 90 percent could be attained (assuming 10 percent of applied N remain in roots) (Balasubramanian et al., 2004). However, this theoretical maximum RE_N value can never be achieved because all the factors controlling N demand, N supply and loss cannot be optimized.

The protein in plants (approximately 5-45% of biomass) is converted to and deposited in animal protein with an efficiency that depends on animal species, balance of energy and nutrients in the animal's diet and animal management (Oenema and Tamminga, 2005). The other 55%-95% is excreted via urine and faeces and can potentially be used for plant production (Oenema and Tamminga, 2005). Three main contributors of animal manure N production are cattle (60%), sheep (12%) and pigs (6%) (Oenema and Tamminga, 2005). However, poultry and swine convert plant N more efficiently into animal protein than dairy cattle, which are more efficient than beef cattle and sheep (Oenema and Tamminga, 2005).

In general, the N output to input ratio (output:input) is relatively low. For milk protein production, output:input can vary from 13% for cows on poor-quality pasture to 31% for cows on improved-quality pasture (Nolan and Dobos, 2005). More efficient use of N in the ruminant diet can be achieved when N is provided to the rumen in appropriate forms and amounts so that the animal tissues are provided with amino acids (AA), especially all essential AA to meet requirements for tissue protein synthesis (Nolan and Dobos, 2005). Since improvement in efficiency is possible, increasing the efficiency of use of protein N by livestock, leading to lower N excretion, is an environmental priority (Castillo et al., 2001). The tissue requirements depend on the physiological state of the animal and the products being produced. Amino acid requirements rely on the genetic potential for protein deposition, intake of other substrates such as metabolizable energy (ME), mineral or vitamins (Nolan and Dobos, 2005).

2.4. Ruminant livestock nutrition and N use efficiency

In smallholder ruminant systems, low-quality feeds (crop residues and low-quality grasses) are important basal feeds (Blummel et al., 2009). Feed resources in mixed crop-livestock systems are classified into green feeds (cultivated fodder and grass), crop residues from cereals and legumes, and concentrates (grains, cakes and bran) (Blummel et al., 2009). Increasing production of crops such as rice, wheat and corn, has produced an under-utilized mass of straw and by-products which are not used efficiently for ruminant feeding because they have relatively low digestibility and low N, fermentable energy and mineral contents (Leng, 1990).

Most ruminant production systems in the tropics are faced with one or more seasons with low feed availability and quality; during these seasons animals depend almost totally on crop

residues and the production is absent or negative. During the cropping and harvesting season, more and better feeds are available but labour limitations and grazing land availability issues prevent animals from receiving adequate supplies of ME and nutrients (Owen et al., 2011; Tarawali et al., 2011). For economic and social reasons, the use of high quality feeds including concentrates and high quality forages into these systems is very low (Blummel et al., 2009).

2.4.1. Straw based-diet for ruminants

There are many types of crop residues and by-products that are potential ruminant feeds available to smallholder farmers. Most tropical countries grow rice (David, 1991), and therefore rice straw and rice bran are abundant.

Straw is deficient in the soluble N and minerals (P and trace minerals especially), which are needed to support an active and efficient rumen microbial ecosystem and therefore a high feed intake and digestibility (Perdok et al., 1988). It contains little or none of the nutrients which escape degradation in the rumen and it lacks nutrients that augment the products of fermentative digestion (Perdok et al., 1988). It is also low in lipid and, although it has a high content of potentially fermentable carbohydrate, it has a low actual digestibility due to the close association of the carbohydrates with lignin (Perdok et al., 1988).

Many strategies to improve the feeding value of low-quality feeds have been recommended. The application of these interventions should be considered in the farming system context (Devendra and Leng, 2011; Tarawali et al., 2011). The level of success of these interventions depends on their adoption by farmers, which are based on cost-effectiveness, safety and availability of labour, simplicity and observed results (Rangnekar, 2011). For example, treatment of rice straw using physical, chemical and biological methods could improve degradability and voluntary intake of rice straw, but the practical use of it is restricted in smallholder farms (Sarnklong et al., 2010).

Another recommendation to improve ruminant production in smallholder farming systems is forage technology. Since the 1980s, more legumes, grasses and shrubs have been incorporated into existing production systems in southeast Asia and may contribute to maintenance of soil productivity (CIAT, 1997). Remenyi & McWilliam (1986) predicted that the demand for forages in SE Asia would double by 2000 based on typical feed regime and projected trend in ruminant numbers. However, land and labour constraints were seen as a limitation to expansion of forage production (Remenyi and McWilliam, 1986). In mixed crop-livestock system, i.e. ruminants and pastures and forages crops, research on forage has focused on sustainable management of forage systems, the conservation of the genetic resources, the seasonality of forage production, and the management of grazing, relevant to the production system (Bolanos-Aguilar et al., 2010). There is evidence that intercropping of

perennial legume with grass, for example at 2 or 3 inter-row spacing increases yield and nutritive value of grass, hence improves productivity of ruminants consuming it (Alalade et al., 2013). The use of forages can be a tool to mitigate enteric methane emissions (Peters et al., 2013), hence improving the energy utilization in ruminants and reducing their effects on global warming.

2.4.2. Intake and digestion of N

Forage crude protein is divided into two fractions: fraction 1 is true protein (TP), which is water soluble and rapidly degraded in the rumen. Fraction 2 is mixed protein accounting for about 25% of plant true protein, which is slowly degraded (Pacheco and Waghorn, 2008). In the rumen, the crude protein is categorized into soluble (a fraction), insoluble (b fraction), undegradable (c fraction), rumen degradable protein (RDP), rumen undegradable protein (RUP), microbial crude protein (MCP) and metabolisable protein (MP) (Pacheco and Waghorn, 2008).

Dietary N can limit ruminant production if supply is insufficient relative to animal requirements; this is common for most feed regimes in southeast Asia. On the other hand, excess N intake relative to requirements in parts of the developed world can become a significant additional production cost (Pacheco and Waghorn, 2008). Dietary CP requirements (%CP in DM) are estimated to be 11% for maintenance, 14% for growing cattle and 18% for young or lactating cattle (McDonald et al., 2002; NRC, 2000).

Ingested feed proteins are modified by rumen microorganisms to supply AA to the intestines and tissues. Rumen microorganisms degrade nitrogenous material in feed (rumen degraded CP or RDP) and some protein escapes ruminal breakdown and flows into the abomasum and small intestines (undegraded dietary protein, UDP) (Figure 2.1). The UDP fraction is also termed ‘escape protein’, ‘bypass protein’, ‘protected protein’ and ‘rumen undegradable protein’. The rumen microbes assimilate RDP (as peptides, amino acids or ammonia) in order to synthesize proteins and other nitrogenous materials. Microbial CP (MCP) provides both essential and non-essential AA that is closely match the AA deposited in the animal tissues and additional AA are supplied by escape protein that passes to the small intestine (Nolan and Dobos, 2005).

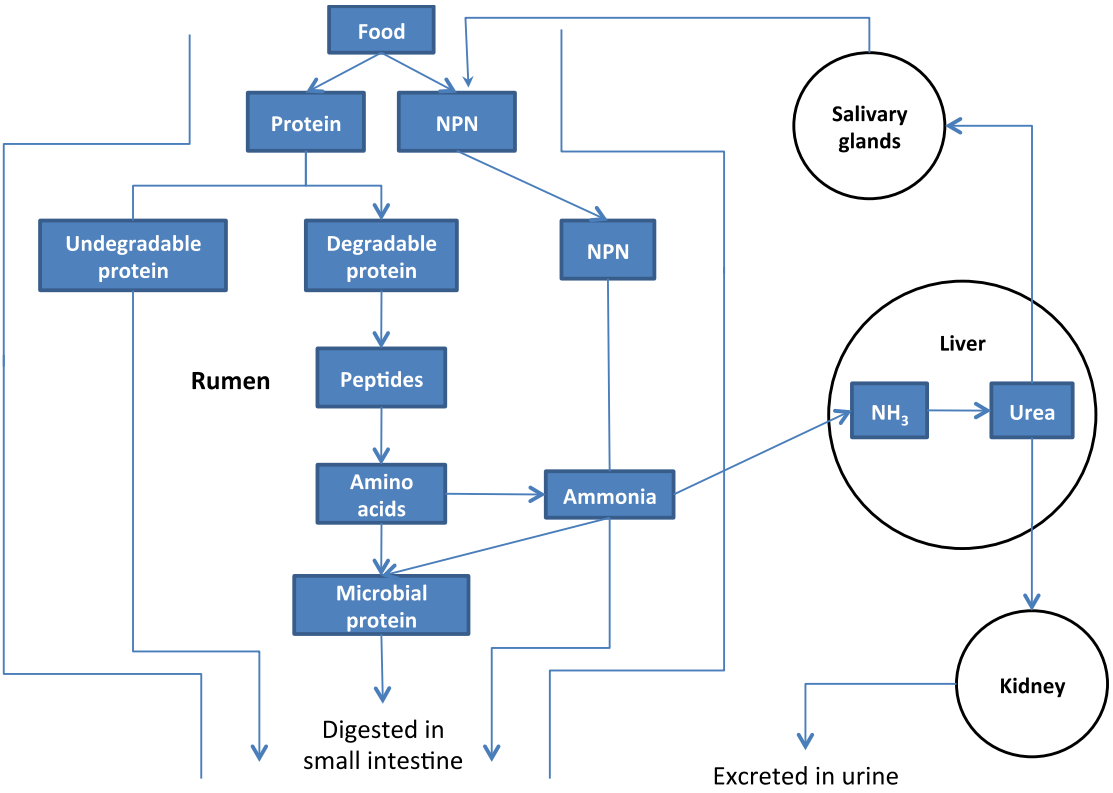

Figure 2. 1 Digestion and metabolism of nitrogenous compounds in the rumen

Rumen degradable protein (RDP)

The RDP is the proportion of a feedstuff that is degraded in the rumen to peptides, amino acids and ammonia by the action of microbial and plant proteolytic enzymes (Pacheco and Waghorn, 2008). If ruminants are fed on high-protein diets, RDN supply can exceed the requirements for microbial protein synthesis, especially if fermentable energy or nutrients are limiting for microbial synthesis. In this case, excess N in the form of ammonia may be absorbed across the rumen wall into the bloodstream and converted to urea in the liver (McDonald et al., 2011). The urea formed in this way may be excreted in urine or recycled to the digestive tract where it is hydrolysed back to ammonia (Cocimano and Leng 1965).

Rumen ammonia-N

Bacteria and fungi assimilate ammonia in form of unionized (NH_3). Fungi may also use the ionic form (NH_4^+) (Hackette et al., 1970). Rumen ammonia concentrations below 50 mg/L are may limit the rate of microbial growth (Satter and Slyter, 1974). In ruminants on low protein diets, the recycling of urea helps to maintain higher rumen ammonia concentrations and this reduces their dietary protein requirements for maintenance.

Microbial crude protein (MCP) synthesis

The synthesis of MCP in the rumen needs energy-rich substrates, protein compounds (NH_3, AA or peptides) and other essential nutrients including sulfur and trace minerals, fatty acids and certain growth factors (Nolan and Dobos, 2005). The estimation of MCP flow to the intestines can be determined in animals fitted with abomasal or duodenal cannulas or it can be predicted in non-fistulated animals from the rate of excretion of purine derivatives in the urine as described in Chen et al. (1990). Prediction equations for different species and types of ruminants have been reported (Chen and Ørskov, 2004). Microbial growth efficiency can be expressed as the yield of cell OM per unit of feed OM truly fermented (OMTDR) in the rumen, or alternatively as microbial DM yield per unit (OMADR) or as MCP/MJ ME. Previous studies show that increased MCP synthesis could improve N utilisation efficiency provided that the amount of energy in the rumen (mainly water soluble carbohydrate) is sufficient to support microbial growth and hence intake (Calsamiglia et al., 2010).

Escape or by-pass protein

Protein-rich materials that are highly resistant to ruminal fermentation can contribute significantly to the total AA flow from the rumen to the small intestine. Agro-industrial by-products are often sources of escape/by-pass protein (and digestible energy) in tropical countries. Feeding these protein sources to ruminants provides essential AAs that complement the amino acid supply from MCP. Animals most likely to benefit from supplements rich in escape protein are those that are growing rapidly or are pregnant or lactating. Previous results have shown that supplementary by-pass protein increase the growth rate and milk yield (Clark, 1975; Leng, 1991), while conserving dietary N and reducing excretion of urea and N wastage.

Energy × N on N utilization

The protein:energy ratio in the nutrients absorbed from the gut determines intake and digestion in ruminants (Egan, 1977). Carbohydrates and proteins from ingested foods are modified by microorganisms such as anaerobic bacteria, protozoa and fungi, in the rumen. Microorganisms ferment polysaccharides, sugars and proteins in order to maintain energy (as ATP or transmembrane potentials) and to generate intermediates for synthesis of cell constituents such as polysaccharides, lipids, proteins and nucleic acids. End-products of the fermentation process are short-chain fatty acids (volatile fatty acid) and rumen ammonia. The microbial cells are either recycled to be re-used in the rumen or are absorbed and metabolized by the animal at the tissue level.

Efficient N utilisation in ruminants depends on a complex interaction between energy and various nutrients in the gut and in the tissues. Adequate and synchronous energy rich

substrates need to be supplied to enable N to be efficiently used by microbes (Nolan and Dobos, 2005).

If N and other nutrients are non-limiting, microbial growth is usually directly dependent on digestible energy intake (Russell, 2002). By contrast, the diets lacking N (peptides and AA) with an excess of rapidly fermentable carbohydrate, the additional energy expenditure serves to prevent microbes from 'eating themselves to death', but massively reduce microbial growth (Russell, 2002). An excess of degradable N in the diet along with energy-rich substrate also cause inefficient assimilation of N by rumen microbes (Nolan and Dobos, 2005). Under grazing conditions in the humid tropics, the supplement of medium feed (2.26 MJ ME/day and 31.6 g CP/day) did not improve the performance (daily weight gain) of post-weaning ewe compared to supplement of low feed (i.e. 0.75 MJ ME/day and 10.5 g CP/day) (Klausner et al., 1985).

N utilization and CH_4 emissions

Carbohydrate degradation is directly related to CH_4 production but the interactions in carbohydrate digestion involve source (structural *vs* non-structural), rumen pH, dietary protein composition and N availability in the rumen, and extent of ruminal escape and site of carbohydrate digestion. In the case of feeding practices in the tropics, the dietary fibre/concentrate ratio is relatively high, hence the methane production will be increased. Tropical forages contain low fiber digestion in the rumen. This is the main factor contributing CH_4 production. Dietary strategies to reduce N excretion in faeces and urine might have implication on enteric methane (Dijkstra et al., 2011). Methane production declines when starch or digestible nutrients escaping rumen fermentation process, but it is increased when the level of fibre increases. The mitigation options to reduce urinary N excretion, that involve increasing fibre level, might augment methane emission level (Dijkstra et al., 2011).

2.4.3. Nitrogen absorption and excretion

N transactions and absorption in ruminants

The major pools of N in the gut of a ruminant are likely to be involved in ruminal digestion, rumen microbial growth, intestinal absorption and faecal excretion. Crude protein (CP) from digested feed is either degraded in the rumen (becoming RDP, which includes NH_3) or leaves the rumen undegraded (as 'bypass protein'). Some RDP is directly absorbed as NH_3 from the rumen, the rate of absorption depends mainly on the NH_3 concentration in rumen digesta (Satter and Slyter, 1974). CP also passes distally from the rumen in the form of microorganisms that have incorporated RDP (and small amounts of recycled endogenous

urea and non-urea-N) during their growth in the rumen (Leng and Nolan, 1984). A small fraction of the microbial CP (15%) is present as purines (Leng and Nolan, 1984).

After leaving the rumen, microbial CP is extensively degraded in the small intestine and about 80% of the microbial CP is absorbed, mainly as peptides, amino acids and purines. Undigested microbial CP passes through the intestines and, after a finite delay, reaches the rectum and is excreted.

Absorbed NH_3 passes via portal blood to the liver and is quantitatively converted to urea. Peptides and amino acids absorbed from the small intestine are available for tissue protein synthesis. This includes enzyme proteins that have been secreted into the gut. The amounts incorporated into tissues will depend on the availability of energy sources to support tissue growth, and the amino acids not retained in tissues will be largely deaminated, with the resulting NH_2 groups being returned to the liver for urea synthesis. Protein-bound amino acids in tissues are turned over the time and may be re-used for protein synthesis or further degraded and the NH_2 groups incorporated into urea. Urea circulating in the bloodstream is excreted in urine or is recycled to the rumen and to distal parts of the digestive tract. Absorbed purines are derived predominantly from rumen microbes and are almost completely excreted as purine derivatives in urine. Purines in feed are almost completely degraded in the rumen and are therefore not available for intestinal absorption. The transactions are summarized in Figure 2.2.

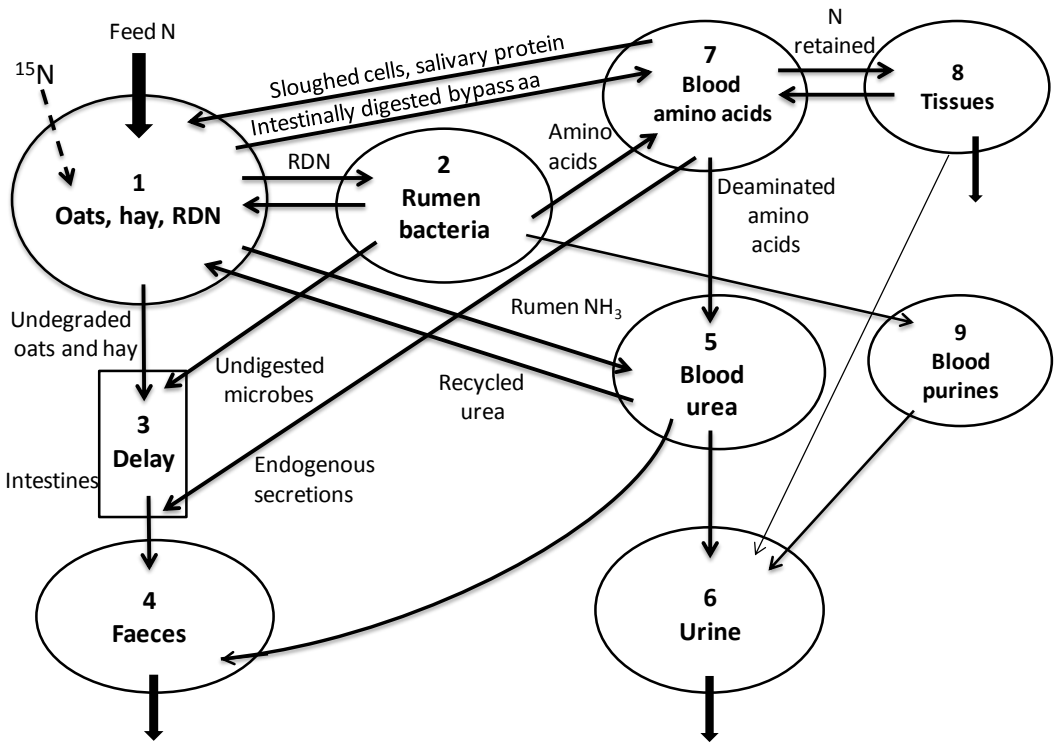

Figure 2. 2 A diagram showing N inputs and outputs and major N transactions in sheep ingested ^{15}N-labeled oats (routes identified in text).

N excretion in urine and faeces

As discussed above, N excretion in faeces and urine varies in response to feed quality and intake. Total N content of manure varies from 0.5 to 4% (Beegle et al., 2008). Many published reviews aimed at identifying the factors affecting N utilization and excretion in farm animals and ways of prediction by using meta-analysis method. Decandia et al. (2011) used data from 49 papers with 154 observations to find the effect of nutritional and animal factors affecting N excretion in sheep and goats. They found that urinary N excretion was greater than faecal N excretion in each physiological stage (growing *vs.* mature and dry *vs.* lactating stage) both in sheep and goats. Total N excretion, urinary N excretion was positively correlated with dietary CP (Decandia et al., 2011).

Lignin is the most important fibre component limiting degradation and feed nutrient availability for ruminant ingesting straw based diet in the tropics because it protects cell wall structural polysaccharides from enzymatic attack. Almost all lignin-N is recovered in the faeces (Somda et al., 1995). In this regard, lignin in feed reduces feed utilization at the ruminant level, but increases feed recovered in faeces. Nitrogen attached to lignin is not absorbed for body storage, hence it is excreted as faecal N. The faecal lignin-N breaks down slowly when applied to soil.

N use efficiency in ruminants and recovery in excreta

Nitrogen balance method has been used widely to quantify N utilisation efficiency in ruminants. In general, livestock in tropical smallholder systems retain less than 20% of ingested N and the remaining 80% is excreted (Reynolds and de Leeuw, 1995). Ruminant types, basal diets, supplements and chemical treatments of straw based-diets are factors affecting N retained in the animal body and N recovered in urine and faeces. At the manure collection stage, Spanghero and Kowalski (1997) argued that underestimation of faecal N and urinary N and unaccounted dermal losses are possible sources of error of using N balance method.

The N retained in ruminants taken from selected studies could range from -107 to 49% (average 8%) depending on %N digestibility of the ingested diet (Table 2.1). For poor quality diets (<30% of N apparently digested), the amount of N retained in animal was negative due to use of body N reserves, resulting in weight loss. When poor quality non-legume or cereal straws were fed, animals excreted more N in faeces than in urine. The improvement of N retained in animal is achieved through supplementing straw-based diets with leguminous forages, protein meal or starch (Table 2.1). These supplements, in most cases, decrease N excretion in faeces but maintain or even increase N excretion in urine.

However, animals fed on legume-based diets may retain N or use the body reserves of N depending on the digestibility of the basal diet itself. Most animals on these diets excrete more N in urine than in faeces (Table 2.1).

Table 2. 1 Dietary effect on DM and N digestibility (%DMD, %N digest.), %N retained in ruminant (%Nret), % recovery of N in excreta (%RNE), partitioning in urine (%RNU) and faeces (%RNF) in selected studies

References	Location	Animal Species	Diets	n	%DMD	%N digest.	%Nret	%RNE	%RNU	%RNF
Moran et al. (1983)	Indonesia	Cattle (Ongole)	Rice straw	5	38	24	-10	110	34	76
			Alkali-treated rice straw (1:1 NaOH 4%:RS)	5	47	20	-44	144	65	80
			70% rice straw + 30% Leucaena	5	42	47	22	78	24	54
			70% Alkali-treated rice straw + 30% Leucaena	5	48	46	23	77	23	54
			Elephant grass	5	53	64	19	81	45	36
		Swamp buffaloes	Rice straw	5	37	21	-22	122	42	79
			Alkali-treated rice straw (1:1 NaOH 4%:RS	5	50	25	-25	125	50	75
			70% rice straw + 30% Leucaena	5	39	43	9	91	34	57
			70% Alkali-treated rice straw + 30% Leucaena	5	51	50	28	72	23	50
			Elephant grass	5	53	62	16	84	45	38
Ramírez-Rivera et al. (2010)	Mexico	Sheep (Pelibuey)	65% Taiwan grass hay + 20% concentrate	4	48	48	10	90	39	51
			65% Taiwan grass hay + 20% *T. diversifolia* hay	4	53	53	23	77	30	47
			50% Taiwan grass hay + 35% *T. diversifolia* hay	4	52	53	18	81	34	48
			35% Taiwan grass hay + 50% *T. diversifolia* hay	4	52	53	16	85	38	47
Umunna et al. (1995)		Sheep (Ethiopian Menz)	Oats hay	4	56	30	2	96	27	69
			Oats hay + 250 g lablab	5	58	54	32	60	15	46
			Oats hay + 250 g wheat middlings	5	61	52	25	71	23	48
			Oats hay + 250 g sebasnia	5	57	54	13	65	20	46
			Oats hay + 250 g tagasaste	5	62	60	30	84	44	40
			Oats straw + lablab	5	53	49	32	96	44	52
Souza et al. (2010)	Brazil	Cattle (Holsein x Zebu)	Signal grass hay	4	32	17	-100	200	117	83
			Signal grass hay + N compounds 10%	4	43	20	-107	207	126	81
			Signal grass hay + corn starch 20%	4	44	80	3	97	77	20
			Signal grass hay + N compounds 10% + 20% cornstarch	4	48	75	15	85	60	25
Wickersham et al.	USA	Cattle	Tall grass prairie hay	4			7	93	43	50

(2009)		(Angus x Hereford)	Tall grass prairie hay + 62 mg N/kg BW/d casein	4			23	77	33	44
			Tall grass prairie hay + 124 mg N/kg BW/d casein	3			31	70	30	40
			Tall grass prairie hay + 186 mg N/kg BW/d casein	4			33	67	31	36
McMeniman et al. (1988)	Australia	Sheep	Mungbean straw	3	64	34	13	87	53	34
			Cowpea straw	3	64	53	14	86	33	53
			Peanut straw	3	65	50	14	86	36	50
			Pigeonpea straw	3	54	23	8	91	68	23
			Lucern straw	3	64	25	12	88	63	25
Khan et al. (2013)	Pakistan	Sheep (Ramghani)	70:0 Wheat straw & Peanut hay	4	43	40	29	71	31	40
			46:24 Wheat straw & Peanut hay	4	51	50	33	67	27	40
			24:46 Wheat straw & Peanut hay	4	62	65	35	65	19	46
			0:70 Wheat straw & Peanut hay	4	74	76	37	68	19	43
Getachew et al. (1994)	Ethiopia	Sheep (Ethiopian highland)	Maize stover + 472 g *D. intortum*	8	53	45	28	72	18	54
			Maize stover + 472 g *M. axillare*	8	52	47	11	89	36	53
			Maize stover + 472 g *S. guianensis*	8	59	48	21	170	73	97
			Maize stover + 472 g *D. intortum* + 60 g conttonseed cake	8	54	53	22	78	31	46
			Maize stover + 472 g *M. axillare* + 60g cotton seed cake	8	56	58	21	79	37	43
			Maize stover + 472 g *S. guianensis* + 60g cotton seed cake	8	54	57	21	80	36	44
			Maize stover + 100 g cottonseed cake (control)	8	57	66	18	82	48	34
Foster et al. (2009)	Florida, USA	Sheep (Dorper x Katadhin)	Bahiagrass	6	59	47	23	78	24	55
			Bahiagrass + soybean meal	6	60	57	35	64	22	42
			Bahiagrass + Annual peanut	6	64	62	40	61	24	37
			Bahiagrass + Perennial peanut	6	68	67	49	51	17	34
			Bahiagrass + Cowpea	6	59	54	36	64	19	45
			Bahiagrass + Pigeonpea	6	56	56	35	66	22	44
			Bahiagrass + Soybean	6	61	58	33	67	26	41
Mupangwa et al. (2000)	Zimbabwe	Sheep (Dorper)	Cassia	4	55	19	-96	196	177	19
			Lablab	4	64	29	-69	169	140	29
			Siratro	4	58	19	-35	135	116	19
			Stylo	4	58	15	-2	101	86	15

2.4.4. Ruminant excreta collection and handling by smallholder farmers

Partitioning of excreta between stall and pasture

In systems where cattle are kept in pens most of the N recovered in excreta may be collected and used. The faeces can be stored for a period of time or be composted before being applying to the field. In some developing countries, where electricity is not accessible in rural areas, faeces can be placed in a bio-digester to produce biogas for cooking and lighting (NBP, 2010).

In systems where cattle are grazing, excreted N falls onto the soil but the loss of N may also be substantial. In general, faecal output is proportional to feed intake but is dependent on both production system (e.g. grazing *vs* intensive), which determines the level of intake, and on diet quality (i.e. apparent digestibility of diet).

Betteridge et al. (1986b) found that urination of ruminants during the night was less frequent, but with higher volume and N concentration than during the day so N output in urine was similar during day and night.

Factors affecting the efficiency of manure N collection

In tethered animals, losses of N before collection may be significant depending on the manure management system and the design of the stall. Urinary N is very susceptible to losses especially under humid tropical conditions (Dubeux et al., 2007). N loss during the excretion time and storage can be minimised through roofing and concrete flooring (Rufino et al., 2007). The volatilization of ammonia can be minimized using straw to absorb ammonia from freshly excreted faeces and urine (Jeppsson, 1999), but the amount of reduction depends on bedding type (Misselbrook and Powell, 2005). However, the use of straw for bedding to capture urine in smallholder system could be limited by availability of straw and labour, despite the abundance of straws produced in fields.

Bio-digester technology, which has become popular among crop-livestock smallholder farmers in developing countries (i.e. National Bio-digester Program in Cambodia), may be an effective way of avoiding the loss of N during the collection and handling step. The suggested design of bio-digesters facilitates the collection of manure from stalls and transfer of manure directly to the anaerobic underground storage that could minimise N loss. The installation cost of this technology is expensive but subsidies from governmental or private organizations are available in some developing countries (NBP, 2010).

2.5. Agronomic value of manure and N use efficiency

The inefficiency of agronomic N application is mainly due to N losses which are the direct and indirect result of microbiological processes in the soil. Losses can be high in tropical agro-systems because microbiological processes are accelerated under high temperature and humid conditions (Wetselaar and Ganry, 1982). In addition, denitrification is enhanced under anaerobic conditions that exist under wet soil conditions associated with flooded rice fields, one of the most important agro-systems in the tropics (Wetselaar and Ganry, 1982).
The efficiency of conversion of N from animal manure into plant protein following its application to soil ranges from 0 to 60% and the global mean is 15% (Oenema and Tamminga, 2005). The other 40%-100% is lost to the environment via ammonia volatilization, denitrification, leaching and run-off in pasture or during storage (Oenema and Tamminga, 2005). It is estimated that only 40%-50% of N excreted from animals globally is collected in barns, stables and paddocks and only half of the 40% to 50% (20%-25%) is recycled to crop lands (Oenema and Tamminga, 2005).

2.5.1. Manure storage by smallholder farmers

Nitrogen and C losses during manure collection and storage depend on manure management (Burton and Turner, 2003). N losses vary widely from less than 10% to 90% (Eghball et al., 1997). Manure N losses are lower for manure with high C/N ratio and when the manure storage systems are more compact or more anaerobic (Eghball et al., 1997; Thomsen, 2000). The majority of N lost from manure storages is via ammonia volatilization, rather than through leaching of mineral N (Eghball et al., 1997). Losses could be reduced by covering manure or slurries or using additives to solid manure composting (Tran et al., 2011). CO_2, CH_4 and NO_2, are also gases lost during composting. A study on composting of feedlot manure, which consist of high GHGs concentration, has shown that production and emission rate of gases was increased when the compost was turned to allow aeration (Hao et al., 2001).
According to review by Rufino et al. (2006) losses of N after composting could range from 13-70% in African smallholder crop-livestock production. N losses at this stage could be reduced by adding straw to manure to increase C pool, promoting N immobilisation.

2.5.2. Manure N transformation and losses when applied to soil

Improved management of plant materials for animal feed and soil amendments is necessary in regions where inorganic fertilizers are largely unavailable and expensive, and farmers rely on organic matter recycling to sustain cropland productivity. Feeding crop residues to animals could enhance nutrient cycling compared to applying crop materials directly to soil because manure-bound N and P mineralize faster than plant-bound N and P (Somda et al.,

1995). Organic compounds present in organic fertilizers (manure and green forage) and in native soil OM have to be mineralized by microorganisms before becoming available for plants and this availability delayed by immobilization processes (Mary et al., 1996).

Mineralization/immobilization of N from organic materials

N mineralization is the production of inorganic N (NH_4^+ and NO_3^-) from organic N. *N immobilization* is the incorporation of inorganic N into organic forms (Jansson and Persson, 1982). *Net N mineralization* is the sum of these two opposing processes and may result in higher concentrations of NH_4^+ and NO_3^- during the course of soil incubation. In cases where the soil inorganic N concentrations decrease during soil incubation, the term *net immobilization*, or sometimes negative net N mineralization, is used.

When N dynamics in soil are traced with ^{15}N, *gross N mineralization* or *gross N immobilization* are terms used to describe production of NH_4^+ or consumption of NH_4^+. The NO_3^- pool is not considered to be part of the N mineralization and N immobilization processes in this manner (Myrold and Bottomley, 2008).

Microorganisms prefer NH_4^+ as main source of inorganic N, but also take up NO_3^- and amino acids to satisfy their requirement of N. Organic matter mineralization and immobilization processes are also based on the energy (C) in the organic bonds required by heterotrophic microorganisms (Beegle et al., 2008).

Factor affecting mineralization of organic materials

Estimation of N mineralization from organic materials in soil is difficult because manure N occurs in both organic and inorganic forms and is strongly dependent on environmental factors that influence microbial activity, including aeration, moisture content, temperature, the chemistry of soil and composition of the manure (Beegle et al., 2008). Researchers have tried to identify and understand the main factors affecting the rates of N mineralization of plant materials (Palm and Sanchez, 1991) and animal manure. Manure C/N ratio is the key characteristic that determines the net mineralization or net immobilization of manure when applied to soil (Fog, 1988). A C/N ratio of less than 25:1 could result in net mineralization of organic N to mineral N (Hodge et al., 2000). The C/N ratio in manure ranges from 13:1 to 25:1, C/N ratio may be higher than this range when ruminants consume low-quality diets.

The lignin in OM is resistant to microbial degradation (Van Soest, 1994) and slows N mineralization. Tropical grasses or legumes contain high lignin and lignin/N ratios (Palm and Sanchez, 1991; Thomas and Asakawa, 1993). Soluble polyphenols which bind to proteins also reduces N mineralization (Constantinides and Fownes, 1994; Palm and Sanchez, 1991). However, other materials such as urea, simple peptides, amino acids, protein supplements,

ruminal bacteria and colonic cells in manure are readily available for mineralization (Van Kessel et al., 2000).

Soil texture also affects N mineralization, being higher in coarser-textured soils (Sørensen et al., 1994a). Soil pH and the proximity of soil moisture content to field capacity also affect mineralization of manure N (Cassman and Munns, 1980; Myers et al., 1982). The influence of texture on soil moisture and aeration which directly affect microbial activity in the soil is likely to be the reason for variations in N mineralization of manure (Beegle et al., 2008).

Soil temperature is also factor affecting mineralization of organic N (Cassman and Munns, 1980). The optimal temperature for microbial activity is 30-40 ^{o}C and temperature below 5 ^{o}C stops mineralization of organic N (Brady and Weil, 1996). In tropical warm climates, the mineralization of manure applied to field is relatively fast, within a year, except during times of drought due to the interaction between soil moisture and temperature (Agehara and Warncke, 2005).

In the short term, the rate of mineralization relative to the N requirements of crops is important. The synchrony of N release from OM and N requirement of crops is difficult to achieve. The kinetics of the mineralization-immobilization process were described by different models. The most common one is the first-order reaction with mineralization decreasing with time for short-term net mineralization of organic N (Cabrera et al., 2005). A two-pool model, first-order kinetic model with a fast pool and a slow pool, has been suggested as a better description of the mineralization of manure organic N (Gil et al., 2011). A three-phase model is also suggested for this process (Gutser et al., 2005). The mineralization of organic N is slow and may continue for many years, so the residual effect in the years following application of manure to fields must be taken into account.

Volatilization of ammonia and gaseous nitrogen

Ruminant production emits N as ammonia (NH_3), nitrous oxide (N_2O) and di-nitrogen (N_2) to air and excrete nitrate (NO_3^-) to groundwater pathways. Urinary N components are decomposed by microorganisms and enzymatic action in soil and water and transformed to NH_4^+ and thereafter to NO_3^- and ultimately in N_2 accompanied with the release of N_2O.

The majority of N loss is from urinary and faecal N excretion and the amount of N excretion is directly affected by variations in dietary N intake (Dijkstra et al., 2013). The N in urine is present in the form of urea (50-90%) and remaining nitrogenous compounds are purine derivatives (PD, i.e. hypoxanthine, xanthine, uric acid and allantoin), hippuric acid, creatine and creatinine (Dijkstra et al., 2013). Major dietary strategies to mitigate N_2O emission from cattle include reducing dietary N supply or increasing energy supply to the microorganisms and to the host animals in order to decrease urinary N excretion, in particularly that of urea-

N. Non-legume straw-based diets are low in N content and low digestibility, but high in energy content which promotes more N excretion in faeces than in urine (Table 2.1).
The mitigation of N_2O can also be done by increasing dietary mineral content through adding salt to the diet (i.e. Na or K) to increase urine volume, hence the concentration of N is lower (Dijkstra et al., 2013). Cattle consuming high minerals in diet drink more water than cattle consuming low minerals and the mineral load that needs to be excreted determines the volume of urine.

Nitrification and leaching of urinary and faecal N

Leaching of NO_3-N from soils where manure has been applied can lead to ground water NO_3-N contamination, especially in areas where there is intensive animal production (Stout et al., 1997). The leaching losses from manure could be as high as from fertilizers in this system (Bergström and Kirchmann, 1999; Randall et al., 2000). Bergström and Kirchmann (1999) added that the leaching loss can be greater from manured than fertilized areas in the long term, due to continued mineralization of organic N from manure and subsequent nitrification and leaching.
The type of manure i.e. composted or non-composted, dry farmyard manure or slurry manure also affects NO_3-N leaching due to the different amount of NH_4-N content of manure and the extent of immobilization of the N in stable organic forms. Soil properties are also important to determine the level of NO_3-N leaching losses; for example, soil with greater infiltration will lead to higher leaching of available NO_3-N. Well-drained soils are more conducive to mineralization and nitrification, which subsequently would increase pool of NO_3-N for leaching (personal communication – Associate Professor Chris Guppy).
Management is the key role to reduce NO_3-N leaching. For example, timing of manure application to avoid high levels of NO_3-N in the soil when high precipitation is expected can reduce leaching. Tillage can also reduce NO_3-N leaching due to the fact that no-tillage may increase water filtration that will increase NO_3-N leaching (Hansen and Djurhuus, 1997; Stenberg et al., 1999). Cover crops can also reduce leaching of NO_3-N by taking manure N from continuous mineralization and nitrification during the time that crops are not being grown (Baggs et al., 2000; Chambers et al., 2000).
A major concern of NO_3-N leaching is when the agro-system is involving with animals, for example, in intensive grazing systems, due to the high rate of urea N in urine. In smallholder systems, where dietary N fed to animals remains low, leaching of NO_3-N from urine was not as high as in intensive system. However, NO_3-N leaching from long-term application of faeces is more concerning.

Denitrification of urinary and faecal N

When soil amended with manure, dinitrification is a potential loss pathway for NO_3-N from soil (Paul et al., 1993). Loss of N through denitrification represent a large loss of available N for crops. NO_2 is a potent greenhouse gas that is reported to cause global warming and ozone depletion. Then level of loss of manure N through denitrification can be small or large. Denitrifying bacteria utilize NO_3 as an electron acceptor under anaerobic conditions and then reduce NO_3^- to N_2 and N_2O (Beegle et al., 2008). As the bacteria use NO_3 as an energy source, so it is likely that the level of NO_3 determine the level of denitrification loss. Manure application can result in a more sustained supply of NO_3-N that can result in a longer periods of denitrication following manure application compared to fertilizers.

Runoff of manure N

Manure N is susceptible to losses via surface runoff following the application of animal manure to arable land (Sitrensen and Jensen, 2013). The amount of manure N lost in run off is highly variable and affected by many factors (Beegle et al., 2008). The two most important factors are the timing of the manure application and the degree to which the manure is incorporated. Rainfall events after manure application determine the amount of nutrient runoff losses (Sitrensen and Jensen, 2013). The runoff losses of cattle slurry or farmyard manure is mainly in form of solid, while runoff losses of inorganic fertilizers is in form of NO_3^- (Smith et al., 2001). Higher losses of NO_3^- occurred when nitrification of manure N occurred before a runoff event (Edwards and Daniel, 1992). Surface application of manure usually results in greater N loss by runoff than incorporated manure (Eghball and Gilley, 1999), because surface manure is directly in contact with runoff water.
The reduction of manure rate per spreading event or incorporating the manure can reduce the risk of runoff losses of manure N (Eghball and Gilley, 1999). Slurry can be separated into fractions with low solids content that can promote rapid infiltration in soil and incorporation of solid fractions in soil in order to reduce runoff losses (Eghball and Gilley, 1999).

2.5.3. Methods of quantifying N availability of manure and N use efficiency by crop

N availability of manure

Manure available N originates from the urea or inorganic N present in the manure and the organic N that is mineralized in sufficient time to be utilized by the crop, minus the N that is lost following the application (Klausner et al., 1985). The amount of manure N available to crops depends on the forms, amounts and locations where manure is applied. Hence, only the potential N utilized by a crop is considered available N. In general, the inorganic forms of manure N (NO_3-N and NH_4-N) are considered as available N to the crop (Fouda, 2011).

Measuring N use efficiency by crop

There are three main methods for measuring the efficiency of fertilizer use, i.e. the classical or conventional method, difference method and isotopic method. *The classical or conventional method* based on yield measures the biological response of increasing fertilizer rates on crop yield (IAEA, 2001). Difference and isotopic method are methods based on nutrient uptake.

Difference method measures the nutrient uptake difference by the crop with and without (control treatment) fertilizer application. In this indirect method, it is assumed that the nutrient uptake of the control treatment measures the amount of nutrient available from the soil, whereas that of the fertilized treatments, the amount of nutrient available is from soil and fertilizer. Furthermore, the method assumes that all nutrient transformation of both soils, such as N mineralization, immobilization and other process, is the same. Recovery data estimated using this method are best referred to as "apparent utilization". Isotopic methods commonly provide more accurate manure N recovery values.

Isotopic method is the only direct method of measuring nutrient uptake from applied fertilizer. Extensive research work has been conducted using N-fertilizers labelled with stable isotope ^{15}N and P-fertilizers labelled with the radioactive isotopes ^{32}P and ^{33}P. This method assumes that the behavior of isotope and the carrier is identical to unlabelled nutrients in the soil-plant system. It is argued though that the labelled fertilizers could lose their identity in the soil, i.e. becoming one pool of nutrients, after they incorporate into organic matter, soil solution, ion exchange processes etc. (IAEA, 2001).

2.5.4. Efficiency of manure N uptake by plants

Nitrogen utilization by crops has to be improved to minimize losses to the environment (Bosshard et al., 2009; Nannen et al., 2011). Indicators for assessing the N status of crops or soil provide valuable information (Herrmann and Taube, 2005; Samborski et al., 2009; Schröder et al., 2000) and accurate fertilizer N recovery in crops is important. Smallholder crop-livestock mixed farming systems rely on recycling organically bound nutrients to maintain soil productivity. In crop-livestock mixed systems the passage of plant biomass through ruminant livestock plays a major role in nutrient cycles.

N use efficiency (NUE) in tropical farming system is generally poor and varies widely depending on the method of measurement of the efficiency (difference method or isotope dilution), treatments of manure (faeces, urine, slurries), mode of application (surfaced or incorporated), experimental condition (glasshouse or field) and species of tested crops. Nitrogen use efficiency in crops across different studies is difficult to compare due to the different experimental materials used by researchers, hence some selected studies are

presented in this review (Table 2.2). However, only within individual studies is it possible to test the hypothesis such as the effect of sources of diet fed to ruminant to produce faeces and urine applied to soil or methods of manure treatment before applying to soil in order to maximize the NUE.

Nitrogen use efficiency of faeces is negative when measured with the difference method, due to the effect of immobilisation-mineralisation processes, for example Jost et al. (2013) measured NUE of faeces of dairy heifers fed different quality diets in Italian ryegrass, negative NUE was found, even though better quality ruminant diets improved the NUE compared to poorer quality diet.

Using ^{15}N dilution method gives positive NUE. Sorensen and Jensen (1998) has shown that treatment of manure to slurry could increase the NUE of faeces, which was lower than 15% in barley and grain, the NUE of urine was very variable, ranging from 30-50%, which was almost comparable to fertilizers (Table 2.2). Effect of crop species on NUE of faeces and urine was also shown in this study, being higher in grain crop (barley) than in non-grain crop (ryegrass). The NUE is higher in coarser soil compared to finer soil, i.e. the NUE of manure in sandy soil was higher than sandy loam.

Thomsen et al. (1997) reported the difference of NUE of animal slurry labelled with ^{15}N-labeled urine, faeces or fertilizer in spring barley (12-49%) only during the first year. Then the NUE of all treatments was less than 4% and not different during the second year, showing the effect of year of application of slurry on NUE. Another study by Catchpoole and Blair (1990a) reported the effect of legume species fed to ruminant (Gliricidia or Leucaena), mode of application (surfaced or incorporated), materials applied (leaf, faeces or urine) on NUE in grass. The interactions of the three factors were obvious in this study.

Table 2. 2 Effect of different amendments (faeces, urine, slurry or fertilizers) on nitrogen uptake efficiency (NUE) in glasshouse and field conditions in selected studies

Reference	Soil type	Treatments	n	Method used	Amount N added	Growing duration (d)	Crop type	NUE (%)
(Jost et al., 2013)		Faeces from Heifer [1] (incorporated)	6 pots	Difference	130-252 mg /pot (2.4 kg soil)	62	Italian ryegrass	-4.5
		Faeces from low-yielding dairy cows[2] (incorporated)	6 pots	Difference	130-252 mg /pot (2.4 kg soil)	62	Italian ryegrass	-3.1
		Faeces from high-yielding dairy cows[3] (incorporated)	6 pots	Difference	130-252 mg /pot (2.4 kg soil)	62	Italian ryegrass	-2.1
		Control	6 pots	Difference	0	62	Italian ryegrass	0
(Sørensen and Jensen, 1998)	Sandy loam	$(NH4)_2SO_4$, incorporated (labeled NH_4-N)	4 plots	^{15}N dilution	120 kg N/ha	90	Barley + Grain	48.6
		Slurry incorporated (labeled faeces)	4 plots	^{15}N dilution	120 kg $(NH_4)_2SO_4$-N + 118 kg faecal N/ha	90	Barley + Grain	6.5
		Slurry injected (labeled faeces)	4 plots	^{15}N dilution	120 kg $(NH_4)_2SO_4$-N -N + 118 kg faecal N/ha	90	Barley + Grain	13.4
		Slurry surface (labeled faeces)	4 plots	^{15}N dilution	120 kg $(NH_4)_2SO_4$-N -N + 118 kg faecal N/ha	90	Barley + Grain	9.7
		Slurry surface (labeled NH_4-N)	4 plots	^{15}N dilution	120 kg $(NH_4)_2SO_4$-N -N + 118 kg faecal N/ha	90	Barley + Grain	44.8
		$(NH_4)_2SO_4$, surface (labeled NH_4-N)	4 plots	^{15}N dilution	120 kg N/ha	90	Barley + Grain	52.9
	Sandy	$(NH_4)_2SO_4$, incorporated (labeled NH4-N)	4 plots	^{15}N dilution	120 kg N/ha	90	Barley + Grain	51.2
		Slurry incorporated (labeled faeces)	4 plots	^{15}N dilution	120 kg $(NH_4)_2SO_4$-N + 118 kg faecal N/ha	90	Barley + Grain	12.6
		Slurry injected (labeled faeces)	4 plots	^{15}N dilution	120 kg $(NH_4)_2SO_4$-N + 118 kg faecal N/ha	90	Barley + Grain	16.2
		Slurry surface (labeled faeces)	4 plots	^{15}N dilution	120 kg $(NH_4)_2SO_4$-N + 118 kg faecal N/ha	90	Barley + Grain	10.3
		Slurry surface (labeled NH_4-N)	4 plots	^{15}N dilution	120 kg $(NH_4)_2SO_4$-N + 118 kg faecal N/ha	90	Barley + Grain	44.1
		$(NH_4)_2SO_4$, surface (labeled NH_4-N)	4 plots	^{15}N dilution	120 kg $(NH_4)_2SO_4$-N + 118 kg faecal N/ha	90	Barley + Grain	53.8
	Sandy loam	$(NH_4)_2SO_4$, incorporated (labeled NH_4-N)	4 plots	^{15}N dilution	120 kg N/ha	90	Ryegrass	4.5
		Slurry incorporated (labeled faeces)	4 plots	^{15}N dilution	120 kg N/ha	90	Ryegrass	1.4
		Slurry injected (labeled faeces)	4 plots	^{15}N dilution	120 kg $(NH_4)_2SO_4$-N + 118 kg faecal N/ha	90	Ryegrass	1.7

		Slurry surface (labeled faeces)	4 plots	^{15}N dilution	120 kg $(NH_4)_2SO_4$-N + 118 kg faecal N/ha	90	Ryegrass	2.7
		Slurry surface (labeled NH_4-N)	4 plots	^{15}N dilution	120 kg $(NH_4)_2SO_4$-N + 118 kg faecal N/ha	90	Ryegrass	5.7
		$(NH_4)_2SO_4$, surface (labeled NH_4-N)	4 plots	^{15}N dilution	120 kg N/ha	90	Ryegrass	6
	Sandy	$((NH_4)_2SO_4$, incorporated (labeled NH_4-N)	4 plots	^{15}N dilution	120 kg N/ha	90	Ryegrass	4.4
		Slurry incorporated (labeled faeces)	4 plots	^{15}N dilution	120 kg $(NH_4)_2SO_4$-N + 118 kg faecal N/ha	90	Ryegrass	4.4
		Slurry injected (labeled faeces)	4 plots	^{15}N dilution	120 kg $(NH_4)_2SO_4$-N + 118 kg faecal N/ha	90	Ryegrass	4.1
		Slurry surface (labeled faeces)	4 plots	^{15}N dilution	120 kg $(NH_4)_2SO_4$-N + 118 kg faecal N/ha	90	Ryegrass	6
		Slurry surface (labeled NH_4-N)	4 plots	^{15}N dilution	120 kg $(NH_4)_2SO_4$-N + 118 kg faecal N/ha	90	Ryegrass	4.6
		$(NH_4)_2SO_4$, surface (labeled NH_4-N)	4 plots	^{15}N dilution	120 kg N/ha	90	Ryegrass	5.9
(Thomsen et al., 1997)	Coarse sand	Animal slurry (labeled faeces) Year 1	2 lysimeters	^{15}N dilution	12 g N/m^2	120	Spring barley	12
		Animal slurry (labeled urine) Year 1	2 lysimeters	^{15}N dilution	12 g N/m^2	120	Spring barley	32
		$^{15}NH_4{}^{15}NO_3$ Year 1	2 lysimeters	^{15}N dilution	6 g N/m^2	120	Spring barley	36
	Sandy loam	Animal slurry (labeled faeces) Year 1	2 lysimeters	^{15}N dilution	12 g N/m^2	120	Spring barley	14
		Animal slurry (labeled urine) Year 1	2 lysimeters	^{15}N dilution	12 g N/m^2	120	Spring barley	36
		$^{15}NH_4{}^{15}NO_3$ Year 1	2 lysimeters	^{15}N dilution	6 g N/m^2	120	Spring barley	49
	Coarse sand	Animal slurry (labeled faeces) Year 2	2 lysimeters	^{15}N dilution	12 g N/m^2	120	Spring barley	4
		Animal slurry (labeled urine) Year 2	2 lysimeters	^{15}N dilution	12 g N/m^2	120	Spring barley	3
		$^{15}NH_4{}^{15}NO_3$ Year 2	2 lysimeters	^{15}N dilution	6 g N/m^2	120	Spring barley	3
	Sandy loam	Animal slurry (labeled faeces) Year 2	2 lysimeters	^{15}N dilution	12 g N/m^2	120	Spring barley	3
		Animal slurry (labeled urine) Year 2	2 lysimeters	^{15}N dilution	12 g N/m^2	120	Spring barley	3
		$^{15}NH_4{}^{15}NO_3$ Year 2	2 lysimeters	^{15}N dilution	6 g N/m^2	120	Spring barley	3
(Catchpoole and Blair, 1990a)	Typic Ustifluvent	Urine derived from Gliricidia - Surface	4 pots	^{15}N dilution	775 mg N/pot (2.5 kg soil)	70	Panicum max. cv. Riversdale	38
		Faeces derived from Gliricidia - Surface	4 pots	^{15}N dilution	775 mg N/pot (2.5 kg soil)	70	Panicum max. cv. Riversdale	8
		Gliricidia leaf - Surface	4 pots	^{15}N dilution	775 mg N/pot (2.5 kg	70	Panicum max. cv.	36

			soil)		Riversdale	
Urine derived from Leucaena - Surface	4 pots	^{15}N dilution	775 mg N/pot (2.5 kg soil)	70	Panicum max. cv. Riversdale	37
Faeces derived from Leucaena - Surface	4 pots	^{15}N dilution	775 mg N/pot (2.5 kg soil)	70	Panicum max. cv. Riversdale	11
Leucaena leaf - Surface	4 pots	^{15}N dilution	775 mg N/pot (2.5 kg soil)	70	Panicum max. cv. Riversdale	22.5
Urine derived from Gliridia - Incorporated	4 pots	^{15}N dilution	775 mg N/pot (2.5 kg soil)	70	Panicum max. cv. Riversdale	55
Faeces derived from Gliricidia - Incorporated	4 pots	^{15}N dilution	775 mg N/pot (2.5 kg soil)	70	Panicum max. cv. Riversdale	14
Gliricidia leaf - Incorporated	4 pots	^{15}N dilution	775mg N/pot (2.5 kg soil)	70	Panicum max. cv. Riversdale	45
Urine derived from Leucaena - Incorporated	4 pots	^{15}N dilution	775mg N/pot (2.5 kg soil)	70	Panicum max. cv. Riversdale	94
Faeces derived from Leucaena - Incorporated	4 pots	^{15}N dilution	775mg N/pot (2.5 kg soil)	70	Panicum max. cv. Riversdale	20
Leucaena leaf - Incorporated	4 pots	^{15}N dilution	775mg N/pot (2.5 kg soil)	70	Panicum max. cv. Riversdale	22

[1] Mixed grass and straw silage, [2]Mixed grass and straw silage + 5 kg concentrate/day, [3]Mixed grass and straw silage + 9 kg concentrate

2.6. Overall efficiency and critical steps in the flow of N in smallholder systems

In smallholder farming systems, where resource utilization is poor, successful and sustainable agricultural production is based on the efficiency of conservation and recycling. Each transfer of nutrients across farming system promotes losses depending on type of farming, management practices and site conditions. Below the comparison of N cycling efficiency investigated by previous research and the critical step of N flow are given.

2.6.1. Overall efficiency of N

Some researchers calculated the partial N cycling efficiency using meta-analyses methods. A few conducted a series of experiments using apparent recovery efficiency of each production level to calculate the overall efficiency.

The partial N cycling efficiency, calculated as the ratio of nutrient output to input, was variable for each step of farming system, i.e. livestock (46-121%), manure handling (6-99%), manure storage (30-78%), soil and crop conversion (3-76%) in an African farming system (Rufino et al., 2006). The overall efficiency of N was from 1% to 44% when combination of livestock, manure handling and crop response was calculated. The highest N efficiency, i.e. 44%, is unlikely to occur simply because the efficiency at each level was overestimated (i.e. nitrogen cycling efficiency at livestock level was 121% recovery in excreta). Other reviews show that legume crop residues and green manures directly incorporated in soil in tropics might have better N cycling efficiency, i.e. 20-40% (Catchpoole and Blair, 1990a), 6-28% for first year and 2-15% for second year (Giller and Cadisch, 1995).

Using difference method, the recovery of N in crop from faeces derived from different plant materials fed to sheep was negative (Abbeddou et al., 2013). At the composting level, the recovery efficiency was between 20-30% and the efficiency of N of composted manure was less than 5% (Abbeddou et al., 2013).

2.6.2. Critical steps of N flow

After reviewing the literature, it seems that direct application of plant materials to soil provides more efficient cycling of N than materials fed to livestock. This is because more losses occur when plant materials are passing through animal digestion. However, livestock provides more value added products to farmers (milk and meat). Manures can at least maintain, or even increase soil organic C pool. The traditional methods used to estimate the recovery efficiency at animal level normally generate controversial results. Using an isotope dilution method provides a more accurate estimate of N cycling through different production levels.

Losses of N and C occur during rumen digestion and also when N and C are excreted in faeces and urine. In smallholder crop-livestock systems, the process of manure collection, handling and storage can be technically challenging. For instance, the good-quality feeds are fed to cattle during night time, and cattle are brought to grazing pasture outside the farm during the day, which means that a substantial amount of N and C are transported outside the farm.

It may be possible to improve the N use efficiency at each step of production in mixed crop-livestock ecosystem. For example, changing the animal feeding regime by providing more N to the diet to achieve better animal production as well as increased manure N. Feeding only the plant materials to animal might slightly affect the recovery efficiency of N at composting or crop level. Keeping cattle in pen and providing good feeds may initially increase cattle production and then allow increased collection of excreta in stall. Anaerobic storage might be a great opportunity to improve N cycling efficiency while providing extra energy to the households of smallholder.

Making more efficient use of animal manures depends critically on appropriate manure handling and storage, and on synchrony of mineralisation with crop uptake (Rufino et al., 2006). Measures to improve manure handling and storage are generally easier to implement than measures to improve crop recovery of N, and should receive much greater attention if overall system nitrogen cycling efficiency is to be improved.

2.7. Identifying the research gap

Within the crop-livestock systems, the balance of N is maintained either through the cycling and regeneration of internal resources, or through external supply of inorganic fertilizer. The N cycling practices used in mixed crop-ruminant-soil ecosystems are shown in Figure 2.3. The understanding of the effect of animal nutrition on the N flow in the whole system is a complex process. Smallholder farmers use a wide range of feed qualities, which differ in N retained in animal and the amount of N recovered in excreta and N use in subsequent soil and plants. As observed in Table 2.1, diets contain low N and low %DMD, such as straws and low-quality roughage, resulted in negative N retention in animal body, but high N recovered in faeces and urine. With low N diet, the N retained in animal body could be improved a little bit when %DMD is higher (60-70%), even though the N excreted in excreta is still substantial (higher N excreted in urine than in faces). Similarly, animals consuming diets containing high N but low %DMD, i.e. straw diets supplemented with N and starch compounds or legumes, retained low N in the body still and excreted high N in urine rather than in faeces. Diets contenting high N and high %DMD promote high N retained in animal body, and lower N recovered in excreta. The proportion of N excreted in faeces and urine is

equal. These arguments are not warranted due to complex nature of feed ingredient, which interact with %DMD and intake.

From Table 2.2 there is not much focus on the effect of different qualities of diets of ruminant on N use efficiency in subsequent crops. For most of the case the excreta is treated before applying to soil and crops. In view of great uncertainty attached to the different stages of N flow through ruminant-soil-crop ecosystems of smallholder farmers, this thesis aimed to quantify the N use efficiency starting from forage to ruminant then back to forage crop by a reliable method, such as tracer technique, is warranted.

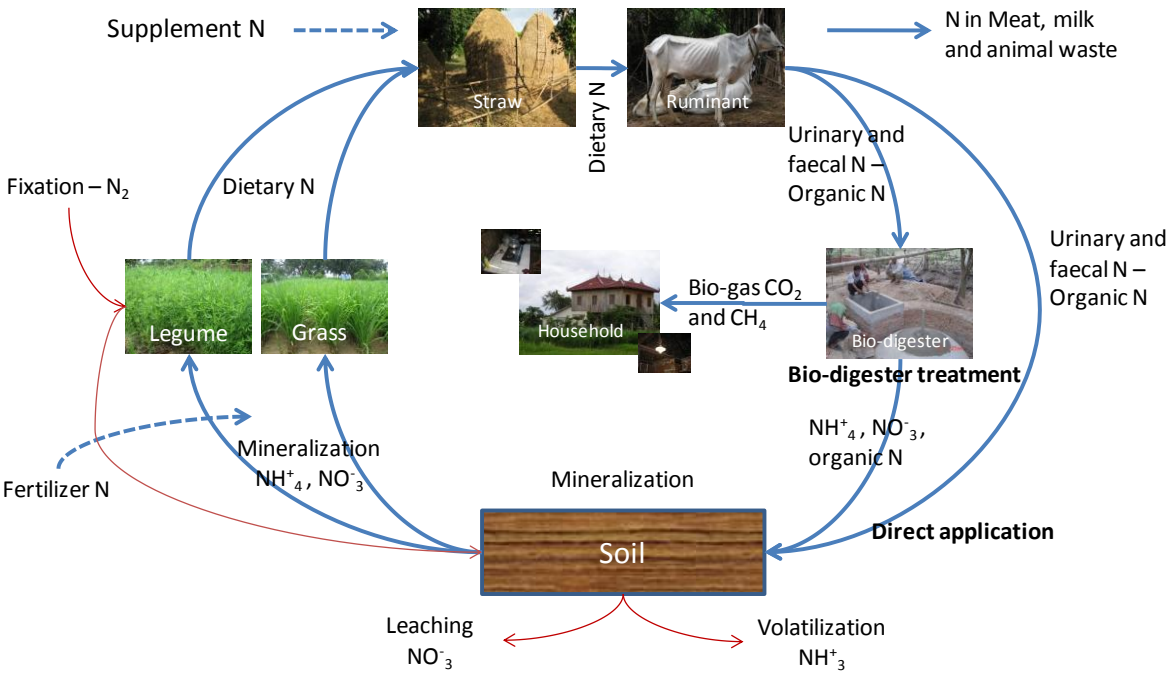

Figure 2. 3 Flow diagram of N cycle in smallholder soil-plant-ruminant system and bio-digester

CHAPTER 3 USE OF FORAGE OATS TO IMPROVE INTAKE, NITROGEN METABOLISM IN SHEEP AND ^{15}N RECOVERY FROM ^{15}N-LABELLED OATS IN EXCRETA FOR NUTRIENT CYCLING

3.1. Introduction

In most tropical regions, ruminant livestock are raised on low-quality diets, such as rice straw, that result in low animal production and low levels of N available in manure for crop production. In some areas, ruminants fed on straw are often supplemented with forages to improve animal production (Pezo et al., 2000; Stür et al., 2002). However, in most cases, the supplemented forages are mature at harvest and although the DM biomass yield is high, the mature material contains relatively low levels of the digestible nutrients needed for animal production, especially nitrogen (N). The N is often bound to other components and hence the feed has lower N digestibility (Enoh et al., 2005).

Utilisation of straw and forages are affected by their chemical and physical characteristics as these factors determine the proportion of feed fermented by microbes or the amount of feed escaping the fermentation process (Leng, 1990). Anti-nutritional substances, including lignin, tannin, saponin, polyphenols and silica, present in the straw and forages can bind with N and reduce the digestion of N by microorganisms (Bae et al., 1997; Barry and McNabb, 1999; Buranov and Mazza, 2008; Cheeke, 1996; McSweeney et al., 2001; Min et al., 2003; Reed, 1995; Van Soest, 2006; Wina et al., 2005). Consequently, the N bound with these substances is not utilised by the animal and is excreted in faeces (Powell et al., 2004). When farmers use this type of faeces as a main source of organic manure for croplands, the anti-nutritional substances also slow the process of N mineralisation in soil and thus N uptake by crops (Hättenschwiler and Vitousek, 2000; Sørensen et al., 2003).

In mixed crop-livestock systems, recycling of nutrients is key to achieving sustainability because the external input of nutrients (fertilisers and concentrate feeds) is normally low or unavailable (Pfeffer and Hristov, 2005). Compounding this situation is the removal of nutrients from cropland when farmers cut-and-carry forages or straw to feed their livestock. Therefore, the manure excreted from the ruminants must be collected and carried back to the soil to sustain the cycle, especially as the harvest of nutrients in crops often exceeds nutrient inputs, giving rise to soil nutrient depletion (Powell et al., 2004a). In general, farmers give little regard to the value of manure as a fertiliser the release of N is slow and does not synchronise with crop requirements and therefore crop yield response is poor. Furthermore, in smallholder systems, most farmers have insufficient livestock and therefore an inadequate manure supply to sustain the cycle of their food production. Nevertheless, recycling available

nutrients within a farm is a key component to achieving sustainable and profitable food production.

To investigate N recycling in farming systems, feeds with ^{15}N have been used to trace patterns of the rate of ^{15}N excretion in urine and faeces and subsequent use of these excretion products in soil and plants (Powell and Wu, 1999; Powell et al., 2004). The labelling of excreta with ^{15}N consists of two methods: 1) feeding animals herbage that has been fertilised with ^{15}N enriched fertiliser and 2) feeding animals ^{15}N-enriched urea (Powell et al., 2004). These two methods have both advantages and disadvantages. For example, feeding ^{15}N enriched forage results in increased ^{15}N enrichment in urine, endogenous N and undigested feed N, whereas feeding ^{15}N-enriched urea via rumen fistula labels the urine and endogenous N, but not the undigested feed. This study will therefore use ^{15}N enriched forage as the tracer carrier.

Faecal N originates from indigestible feed N and digested endogenous N consists of microbial debris from the rumen and large intestine and materials such as sloughed epithelial cells, mucus and bile pigments (Freer et al., 2007; Mason, 1969; NRC, 1991). To further explore the complexities of N cycling in ruminants and associated N pools, isotopic methods can by utilised. When ^{15}N-labelled feed is offered to ruminants, ^{15}N in faeces is at first diluted with unlabelled feed N remaining in the digestive tract (from the period before feeding ^{15}N) and by endogenous N secreted into the digestive tract. During the days following, the ^{15}N in faeces is further diluted by partly labelled endogenous N.

These N pools in ruminant faeces can be divided into two: Pool 1 consists of undigested feed N and undigested enriched microbial N from the rumen. This pool slowly decomposes in soil because the N component has already passed through the digestive tract and been exposed to microbes, acids and digestive enzymes without being assimilated. Pool 2 consists of N compounds in living microbes, partly-decomposed microbial tissues, digestive secretions and dead cells sloughed off the wall of the digestive tract, and is more easily decomposed in soil than Pool 1. This N in Pool 2 can also be diluted by endogenous N from the animal and therefore the labelled fraction of N is always less than the feed N (Nolan, 1975).

The variation in the proportion of different forms of N in faeces is complex, as is the release of N from feaces to the soil solution and to plants, which can be highly variable. Generally though, short term measurements often show limited effects of the N forms present in faeces on crop N nutrition (Bosshard et al., 2009; Langmeier et al., 2002).

The amount of enrichment and recovery of ^{15}N in urine and faeces derived from herbage is dependent on the type of herbage, labelling procedure and type of animals, i.e. enriched forage method or enriched urea method, single dose or multiple dose injections of enriched urea, empty rumen or not, shorter or longer feeding periods of enriched forage. To study nutrient cycling using ^{15}N-labelling, uniform labelling of the manure materials is required

(Powell and Wu, 1999; Sørensen and Jensen, 1998). Urine is generally uniformly labelling with ^{15}N (Sørensen and Jensen, 1996), however, it is difficult to achieve such homogenous labelling of faeces, even when ruminant animals are fed enriched feed for up to 9 days (Bosshard, 2007). Bosshard (2007) suggested that in their case this was due to heterogenous labelling of feeds and inhomogeneity of ^{15}N-labelling of sheep caused by the metabolic process of the animal. However, as long as the ^{15}N-abundance of total faecal N was within the range of mineral faecal N, N cycling could be still be accurately performed (Bosshard, 2007). For this reason, and the availability of cannulated sheep and appropriate facilities at UNE, and the cost effectiveness of performing this experiment with sheep rather than cattle, sheep were chosen as the experimental ruminant this study.

The introduction of forages to animals fed a low quality fibrous diet has been shown to improve animal production (Blair et al., 1986; Shelton and Stur, 1991). However, the potential impact on the environment and therefore capacity to subsequently grow food (animals and plant) deserves further investigation. The available literature above demonstrates the importance of manure management for N cycling in a ruminant-plant system. However, investigations that are relevant to low-input agriculture, i.e. quantifying the amount of forage N recovery after ruminant digestion of the base straw diet supplemented with different amounts of mature green forage, are scarce. In this experiment, we aim to quantify 1) forage oats utilisation by sheep fed a basal diet of low-quality grass hay supplemented with different levels of forage oats, and 2) the recovery of N from ^{15}N-labelled forage oats in faeces and urine. It is hypothesised that nutrient intake and digestibility, rumen ammonia-N, microbial protein production and efficiency, rumen fermentation and N retained in sheep would be improved with higher ratios of oats in hay compared to the basal grass hay diet. By using ^{15}N-labelled forage oats, the ^{15}N from plant material digested and then excreted in faeces and urine can be traced, and the labelled excreta can then be used for subsequent soil-plant cycling studies.

3.2. Materials and Methods

The following procedures were reviewed and approved by the University of New England (UNE) Animal Ethics Committee (AEC10/108).

Establishment of unlabelled and ^{15}N-labelled oats

Forage oats (*Avena sativa L.*) were regrown from a previous planting, on a black, medium clay soil (330 m^2) at 'Laureldale' Farm, UNE, in October 2010 with forage in a sub-plot (10 m^2) (within the main 330 m^2 plot), enriched with ^{15}N fertiliser ($^{15}NH_4{}^{15}NO_3$, 99.23 atom% ^{15}N). The oat plot was receiving rain for three months during the regrowth period. To supply

adequate N, P and K to the plants, the whole growing area was fertilised once with KH_2PO_4 in the first week at a rate 50 kg ha^{-1} and once per week for three weeks with urea equivalent to 30 kg ha^{-1}. To produce the ^{15}N-labelled oats feeds in the 10-m^2 sub-plot, 23 g of $^{15}NH_4{}^{15}NO_3$ was mixed with 51 g of urea-N in water and applied to this section using a watering can. Both ^{15}N-labelled and unlabelled oat plants were harvested after the 3 weeks of fertiliser application then air-dried in a glasshouse for seven days. The dried plants were chopped to 2-cm in length and stored in chaff bags for feeding to sheep during the experimental period.

At the time of harvest, subsamples of ^{15}N-labelled oaten hay, and samples of the roots and soil where the oat forage was grown were collected for analysis of atom % ^{15}N. The samples of labelled oat plants were divided into three parts, i.e. shoot bottom (0-25 cm above ground), middle (25-50 cm) and top (50-75 cm), so that ^{15}N concentration in the plant components could be determined and even-ness of labelling quantified. The analysed DM, N content and atom% ^{15}N enrichment in soil and different plant parts are given in Table 3.1.

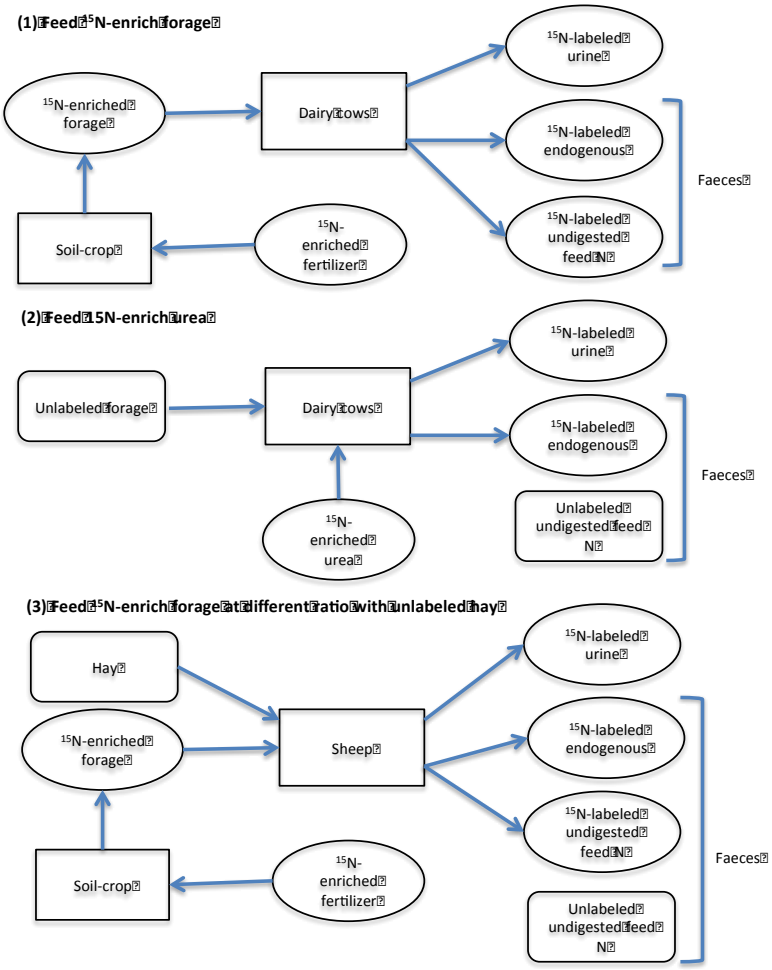

Figure 3. 1 Differential ^{15}N labelling of urine N and faecal N components by feeding sheep ^{15}N-enriched forage or ^{15}N-enriched urea method (Powell et al., 2004). (1) and (2) are the labelling procedures employed by the Powell's team that involved multiple days of feeding dairy cows 15N-enriched forage (1) or injecting ^{15}N-enriched urea (2). (3) is the labelling procedure following the forage method, which involves using unlabelled feed (hay) to further dilute the isotope ^{15}N in faeces.

Table 3. 1 Dry matter (DM), N content (N) and atom% ^{15}N enrichment in soil and various parts of oat (*Avena sativa*) plants supplied with $^{15}NH_4{}^{15}NO_3$ at weekly intervals over a 3-week period

Parameters	**DM (g/kg)**	**N (g/kg DM)**	***Atom % ^{15}N enrichment***
Soil from enriched plot	779	2.1	0.0959
Oats – root[1]	-	7.5	2.3043
Cut oats - lower (0-25cm)	253	4.9	3.4161
Cut oats - middle (25-50cm)	228	9.2	3.8821
Cut oats - top (50-75cm)	310	15.9	4.1277
Oats - whole cut plant	261	8.5	3.9790

[1] The DM root was not determined as the roots were washed with water to clear the attached soil

Animals, diets and ^{15}N-labelled oats feeding

Eight rumen-cannulated crossbred (Merino × Dorset) sheep, 3-4 years of age and 47 ± 3.8 kg (mean ± SD) liveweight at the start of experiment were used. The experiment was conducted in November 2010 in the Animal House Complex at UNE. Animals were randomly allocated to two dietary treatments with four replicates per treatment, consisting of either a low level of oats inclusion to hay (25%oats-75%hay) or a high level of oats to hay (75%oats-25%), mixed together on a DM basis.

The animals were adapted to the dietary treatments during 10 days (Days 1-10) in pens grouped by treatment, after which they were maintained individually for a total of 15 days in metabolism crates (Days 11-25) with individual single feeding bins and water facility. The amount of feed offered to sheep was based on the live weight (LW) of the animals (3% LW per day) and fresh water was always available. The daily DM and N amount in the two dietary treatments are listed in Table 3.2. The animals were weighed every week using electric scale so that the amount of feed provided to animals could be adjusted accordingly.

The 15-day period in crates consisted of five days of adaptation to the treatment diets and metabolism crates (Days 11-15); six days for feed intake measurement and urine, faeces and rumen fluid collection (Days 16-21), and four days for ^{15}N-labelled urine and faeces collection (Days 22-25). The unlabelled feeds were provided *ad libitum* twice per day at 10:00 h and 16:00 h. On Day 22, ^{15}N-labelled oats were substituted into feeds and sheep were fed a single meal (669 g DM) at 10:00 h to produce ^{15}N-labelled faeces, as per the forage method (Figure 3.1). The meal consisted of 63.2 and 183.3 mg ^{15}N and an average total N (unlabelled plus labelled feed) of 32.7 and 35 g N per animal for the 25%Oats:75%Hay and 75%Oats:25%Hay diets respectively (Table 3.2). All the sheep had finished the meal by 13:00 h after which time the unlabelled diets were resumed.

Table 3. 2 Composition of two treatment diets offered *ad libitum* to sheep during the whole experimental period, and the amount of N and ^{15}N fed to sheep as a single meal on Day 22 in order to measure the ^{15}N recovery in faeces and urine.

Treatments	25%Oats:75%Hay	75%Oats:25%Hay
Experimental diet		
Green oat (g DM/d)	310	973
Low-quality hay (g DM/d)	932	311
Total (g DM/d)	1242	1283
^{15}N-Labelled diet allowance		
Total (g DM/d)	669	669
N (g/d)	32.7	35
^{15}N (mg/d)	63.2	183.3

Both treatment diets were iso-nitrogenous (Table 3.3). The fibre components (NDF, ADF and ADL) of both diets were relatively high compared to the diet of sheep in an Australian grazing context. As would be reasonably expected, the 75% oats inclusion-diet had higher DMD, DOMD and ME concentrations and lower NDF, ADF and ADL concentrations.

Table 3. 3 The nutrient composition of hays and oats (*Avena Sativa L.*) offered to sheep.

DM, dry matter; OM, organic matter; N, nitrogen; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; DMD, digestibility of DM; DOMD, dry organic matter digestibility

Parameters	Chemical analysis		Calculated values	
	Hays	Oats	25%Oats:75 %Hay	75%Oats:25%Hay
DM (g/kg)	891.5	850.0	881.1	860.4
OM (g/kg DM)	918.7	929.5	924.7	933.6
Ash (g/kgDM)	81.3	70.5	75.3	66.4
N (g/kg DM)	8.0	8.4	8.3	8.4
CP (g/kg DM)	50.0	50.2	51.6	52.2
NDF (g/kg DM)	620.0	600.0	615.0	605.0
ADF (g/kg DM)	360.0	340.0	355.0	345.0
ADL (g/kg DM)	83.0	68.0	79.3	71.8
DMD (g/kg DM)	540.0	600.0	555.0	585.0
DOMD (g/kg DM)	520.0	570.0	532.5	557.5
ME (MJ/kg DM)	7.6	8.6	7.9	8.4

Sampling procedures and measurements

Feed intake was recorded daily and subsamples of feeds offered and refusals were also collected in the morning before feeding. The feed refusals from each sheep were carefully collected, weighed and mixed. Sub-samples of feed offered and refused were sealed in

plastic sample bags then stored at 4ºC, to be later dried, bulked (samples from the same day) and ground in a 1-mm hammer mill. Samples were then stored in air-tight sample containers at 4 ºC, pending analysis for DM, OM, N, NDF, ADF, ADL, ME, DMD, DOMD, ash and mineral contents. Rumen fluid samples were also collected every morning at 10:00 h during this 6-day period and then at 0, 4, 8, 16, 24, 32, 48, 56, 72 and 96 h after the single meal of ^{15}N-labelled oats was offered. The rumen fluid samples were analysed for pH immediately after collection and for rumen ammonia-N and VFA concentration.

In addition, urine and faeces were collected daily from all animals during Days 16-21 to give separate total volumes The allantoin concentrations in urine were determined using the method of Chen and Gomez (1995) and this urine derivative was further used to estimate purine absorption in animal and microbial protein production (Chen and Gomes, 1995). Subsamples of the faeces were analysed for DM, OM and N content.

During the ^{15}N-labelling period for excreta (Days 22-25), ^{15}N-labelled urine and faeces were collected at 0, 4, 8, 16, 24, 32, 48, 56, 72 and 96h after sheep ingested the single meal of labelled feed. At each four hour interval, fresh faeces contained in collection bags (20×50 cm) were removed (and new bags inserted), weighed and sub-samples (from the top of bags) collected and stored at -20 ^{o}C for later chemical analysis. Urine was collected at each interval in bottles and sub-samples taken for immediate acidification using 18M H_2SO_4 then stored at -20 ^{o}C pending analysis. The remaining urine was not acidified but embedded in containers of ice to reduce volatilisation. The fresh unacidified urine, along with the bags of faeces, were stored at -20 ^{o}C pending their use in a subsequent study of nutrient cycling using maize grown in pots in a glasshouse.

Chemical analysis

Sub-samples of feed offered, feed refused and faeces were dried to a constant weight at 60^{o}C in a fan-forced drying oven for 48 h to determine DM content, and then combusted in a muffle furnace at 550^{o}C for 4.5 h to determine OM and ash content. Total N content in feed, faeces and urine samples was measured following the Dumas method using a LECO analyser (TruSpec Carbon and Nitrogen Analyser by LECO Corporation, 3000 Lakeview Avenue St. Joseph, Michigan 49085) (Etheridge et al., 1998). Total CP content in samples was calculated by multiplying N by 6.25.

Neutral detergent fibre,, ADF and lignin content (ADL) in feed samples were determined using near infrared (NIR) spectroscopy following CSL Method LMOP 2-1129 (service provided by Wagga Wagga Agricultural Institute). Crude fat was quantified by extraction using petroleum following CSL Method LMOP 2-1122. Metabolisable energy was calculated according to AFIA Method 2.2R based on DOMD (AFIA, 2011). Volatile fatty acid

concentration in the rumen fluid was determined by mixing 0.45 mL of rumen fluid supernatant with 0.9 mL of internal isocaproic standard before reading on a Varian CP-3800 Micro Gas Chromatograph [following the AOAC Official method 971.11 (AOAC, 1990). Centrifuged rumen fluid was diluted 1:5 with distilled H_2O (using an automated procedure) and placed in a SKALAR continuous flow analyser to determine the concentration of ammonia-N. This procedure is based on a modified Berthelot reaction using salicylate reaction, with colour measured at 660 nm. To determine mineral concentrations in feeds, 200 ug of ground feed was oxidised in a modified sealed chamber digester (SCD) using perchloric acid and hydrogen peroxide (Anderson and Henderson, 1986) before analysis using Inductively Coupled Plasma Optical Emission Spectroscopy (Vista Radial MPX, Varian, USA).

The pH of rumen fluid and urine was measured using pH meter (ECpH6, Eutech Instruments Pty 40 Ltd, Singapore) calibrated with pH 4.01 and pH 7.00 buffer solutions. Allantoin concentration in the urine samples was determined by a colorimetric method described in Chen and Gomes (1995) and IAEA (1997). ^{15}N in faeces was determined using a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd, Cheshire, UK) (service provided by UC Davis, University of California) and the remaining samples (plant, urine and soil) were analysed using a Callisto CF-IRMS Stable Isotope Analysis system at UNE using standard procedures (Sharp, 2007). Some of the samples analysed in California were also analysed at UNE and found to give concordant results.

Calculations

Measurement of N retention

Urine samples collected during the six day collection period were analysed for total N and the results for N intake and excretion (faeces and urine) were used to determine N retention in the cattle using the following equation:

N retention (g N/d) = Feed N intake – (Faecal N output + Urinary N output) (3.1)

Measurement of the outflow of microbial N from the rumen

The mean yield of microbial N from the rumen was predicted from the mean daily allantoin excretion in the urine collected from sheep during Days 16-21. Total PD excretion was predicted from allantoin excretion using an equation from Kahn (1996):

Total PD excretion (mmol/day) = (Allantoin excretion in urine (mmol/d) +0.54)/0.89 (3.2)

The yield of total microbial N from the rumen was calculated from the estimated total PD excretion using Equation 3.3 and 3.4 from Chen and Gomes (1995).

$$Y = 0.84X + (0.150\ W^{0.75}\ e^{-0.25X}) \quad (3.3)$$

where, Y = urinary excretion of total PD (mmol/day); $W^{0.75}$ = metabolic body weight (kg); X = the absorbed exogenous purine (mmol/day)

Flow of microbial N (MN, g N /day) into the intestines was estimated from the microbial purine absorbed (X mmol/day):

$$MN\ (g\ N/day) = \frac{X\ (mmol/d)\ x\ 70}{0.116\ x\ 0.83\ x\ 1000} = 0.727X \quad (3.4)$$

where, X is the microbial purine absorbed, digestibility of microbial purines is assumed to be 0.83, the N content of purines is 70 mg N/mmol, the ratio of purine-N : total N in mixed rumen microbes is taken as 11.6:100.

^{15}N enrichment in samples was expressed as atom % ^{15}N in enrichment (APE) as follows:

APE = % ^{15}N abundance in experimental samples – % ^{15}N abundance in reference sample (3.5)

where % ^{15}N abundance in reference samples were 0.3663, 0.3690 and 0.3688 for oats, faeces and urine, respectively).

The percent of N fraction in faeces or urine derived from ^{15}N labelled materials mixed in feeds (%Ndff):

$$\%Ndfm = 100 \times (APE_{faeces/urine}/APE_{mixed\ diet}) \quad (3.6)$$

where, $APE_{faeces/urine}$ = atom % ^{15}N enrichment in faeces or urine, $APE_{mixed\ diet}$ = atom % ^{15}N enrichment in faeces, urine or mulched plant materials applied to pots.

The $\%^{15}N$ recovered in faeces or urine from ^{15}N labelled mixed feeds was calculated as:

$$\%^{15}N\ recovered = 100 \times P(c - b) / f\ (a - b) \quad (3.7)$$

where, P is the total N amount in the faeces or urine; f is the total N amount of in the feeds, a is the abundance of ^{15}N in the labelled feeds; b the abundance of ^{15}N in reference samples; c is the abundance of ^{15}N in the faeces or urine.

Statistical analyses

The statistical significance of treatment effects was determined by a student T-test using statistical package JMP version 10.0.2 (JMP for Mac, 2012, SAS Institute Inc., North Carolina). The significant differences between treatments were calculated for a 95% confidence level and Levene's test was used to test equality of variances. The Kolgomorov-Smirnov test was used to test the normality of the data distribution with all data being normally distributed and therefore no transformations were required). The level of significance was set at $P<0.05$ and trends were noticed if P value <0.1.

3.3. Results

DM intake and digestibility, N retention and liveweight gain

Increasing the amount of oats in the sheep diet from 25% to 75% tended to increase DMI, OMI and NI by approximately 16-17% ($P=0.07$), and significantly increased ($P<0.01$) DDMI (27%), DOMI (27%), MEI (33%), DM digestibility (from 49.6 to 54.3%) and OM digestibility (from 52.4-56.6%). There was no effect of dietary treatment ($P>0.05$) on N digestibility, N excretion (via faeces and urine) nor N retention.

Table 3. 4 Intake of nutrients from straw diets with a low or high percentage of oats (*Avena sativa L.*) fed to sheep *ad libitum*
Values are means and standard error of the means (s.e.m.). DM, Dry matter; OM, Organic matter; DOM, Digestible organic matter; CF, Crude fibre; N, Nitrogen; CP, Crude Protein

Parameters	**25%Oats:75%Hay**		**75%Oats:25%Hay**		***P*-value**
Intake					
DM Intake (g/kg LW.day)	23.16	±1.63	26.89	±0.63	0.077
OM Intake (g/kg LW.day)	21.52	±1.52	25.23	±0.59	0.063
DDMI (g/kg LW.day)	11.48	±0.78	14.6	±0.24	0.009
DOMI (g/kg LW.day)	11.27	±0.76	14.26	±0.22	0.009
N Intake (g/kg LW.day)	0.19	±0.01	0.22	±0.01	0.073
CP Intake (g/kg LW.day)	1.20	±0.08	1.39	±0.03	0.073
ME intake (MJ/kg LW.day)	1.62	±0.11	2.15	±0.05	0.005
Digestibility					
DM (%)	49.61	±1.19	54.32	±0.75	0.008
OM (%)	52.43	±1.14	56.55	±0.75	0.011
N (%)	25.55	±2.32	28.84	±2.04	0.329
N metabolism					
N intake (g/d)	8.85	±0.27	10.54	±0.60	0.044
Faecal N excretion (g/d)	6.59	±0.30	7.51	±0.51	0.173
Urinary N excretion (g/d)	2.11	±0.18	2.15	±0.19	0.867
Total N excretion	8.7	±0.46	9.66	±0.58	0.244
N retention (g/d)	0.16	±0.31	0.87	±0.17	0.086
N retention (%)	1.83	±3.37	8.32	±1.66	0.133

Liveweight gain (g/15 d)	-0.03	±0.92	1.48	±0.46	0.213

Rumen parameters and microbial protein production

There was no effect of dietary treatment (P>0.05) on rumen pH, rumen ammonia-N concentration nor microbial N outflow from the rumen (Table 3.5). Total VFA concentrations and their individual proportions (% of total VFA) did not differ (P>0.05) between the treatments, except for propionate percentage (16 and 20%). The ratios of acetate:propionate also varied significantly, being 4.6 and 3.7 for the 25% and 75% oats diets, respectively.

Table 3. 5 Microbial protein production and the rumen and volatile fatty acid profile of sheep fed *ad libitum* straw diets with a low or high percentage of oats (*Avena sativa L.*)
Values are means and standard error of the means (s.e.m.). PD, Purine derivatives; MCP, Microbial crude protein; Efficiency of microbial crude protein

Variables	**25%Oats:75%Hay**		**75%Oats:25%Hay**		***P*-value**
Rumen pH	6.90	±0.03	6.86	±0.03	0.464
Rumen NH_3-N (mg/L)	27.41	±8.08	38.46	±5.85	0.310
PD excretion (mmol/day)	7.57	±0.44	8.56	±0.47	0.172
MCP supply (g/day)	39.18	±2.69	45.06	±2.71	0.174
EMCP (g MCP/kg DDMI)	73.57	±3.56	65.73	±4.99	0.259
EMCP (g MCP/kg DOMI)	74.91	±3.51	67.28	±5.02	0.248
Total VFA (mmoles/l)	70.01	±2.59	77.11	±2.86	0.115
Acetate (%)	74.62	±2.39	75.92	±2.56	0.723
Propionate (%)	16.28	±0.85	20.43	±0.44	0.005
Iso-butyrate (%)	0.17	±0.00	0.27	±0.05	0.044
Butyrate (%)	7.95	±0.77	11.51	±1.65	0.098
Iso-valerate (%)	0.23	±0.04	0.59	±0.15	0.061
Valerate (%)	0.30	±0.02	0.85	±0.12	0.002
Acetate:propionate ratio	4.60	±0.16	3.72	±0.14	0.006

Nitrogen excretion derived from oat supplementation

The faecal N concentration and output were no different (P>0.05) between the two treatments, however, animals fed the 75%Oats:25%Hay treatment had a higher output of urine (g/day). Nevertheless, as the concentration of N in their urine was significantly lower (P<0.01), the urinary N output for both dietary treatments was similar. Urine pH was significantly higher (P<0.01) for 75%Oats:25%Hay (8.33) than for 25%Oats:75%Hay (6.65).

Table 3. 6 Faecal and urinary DM, N output and N concentration from sheep fed *ad libitum* straw diets with a low or high percentage of oats (*Avena sativa L.*)

Values are means and standard error of the means (s.e.m.)

Parameters	**25%Oats:75%Hay**		**75%Oats:25%Hay**		***P-value***
DM output of faeces (g DM/d)	540.31	±23.20	581.42	±34.28	0.359
Faecal N concentration (%)	1.22	±0.02	1.29	±0.02	0.052
Faecal N output (g N/day)	6.59	±0.30	7.51	±0.51	0.173
Urine output (g/day)	281.66	±29.07	414.43	±66.21	0.116
Urinary N concentration (%)	0.84	±0.03	0.56	±0.05	0.004
Urinary N output (g N/day)	2.11	±0.18	2.15	±0.19	0.867
Urine pH	6.65	±0.39	8.33	±0.07	0.003

The N from oats labelled with ^{15}N was detectable in urine 4 h after the sheep ingested the ^{15}N-enriched oats and after 8 h and 16 h in faeces (Figure 3.2).

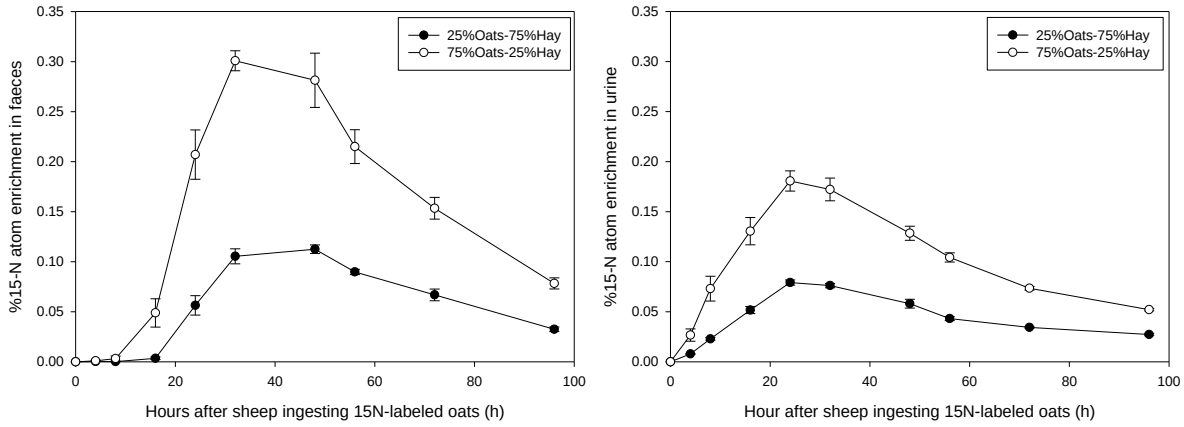

Figure 3. 2 Enrichment (atom % enrichment) in faeces (A) and in urine (B) with time after sheep ingested a meal of straw diets with a low or high percentage of oats (*Avena sativa L.*) labelled with ^{15}N

The cumulative recovery of ^{15}N in faeces (22.9%) and urine (6-7%) after 96 h did not differ between treatments (Figure 3.4A and 3.4B).

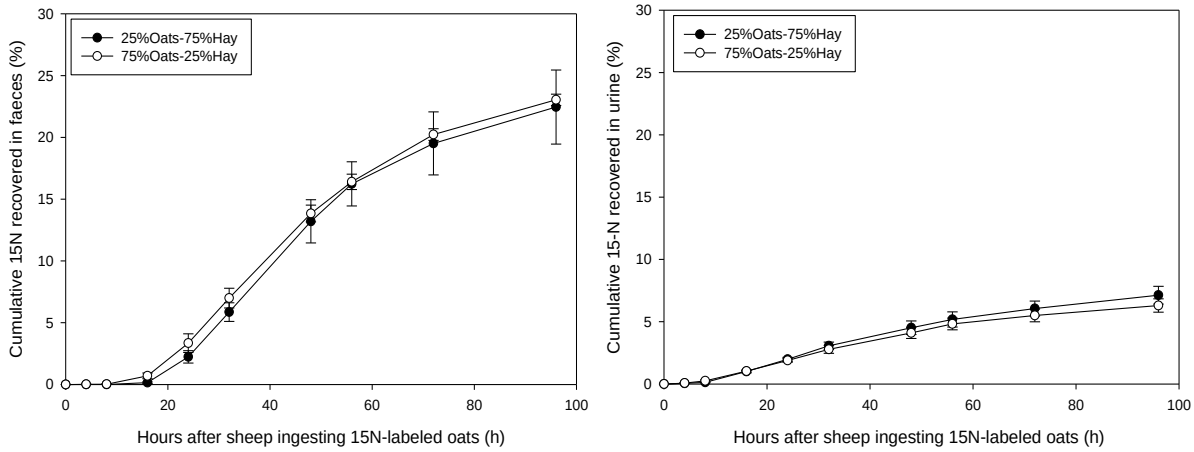

Figure 3. 3 % ^{15}N recovered in faeces (A) and in urine (B) over time (h) after sheep ingested a meal of straw diets with a low or high percentage of oats (*Avena sativa L.*) labelled with ^{15}N

3.4. Discussion

This study has demonstrated that intake of DM, OM, N and CP tended to be improved, while DDMI, DOMI and MEI increased, even though the CP concentration of the basal diet and the supplement did not differ. This improvement was due to higher ME (6% higher in 75%Oats:25%Hay) associated with the higher amount of oats in the diet. The N intake as well as N retained in sheep was also slightly improved, yet despite this, MCP supply and its efficiency were not increased. Finally, the recovery of ^{15}N in faeces and urine did not differ among the treatments - this is discussed further below. However, by tracing ^{15}N from feed to excreta, we gained an insight into N excretion patterns that formed the basis of a subsequent ^{15}N cycling pot experiment (see chapter 4).

The feeding practices of smallholder farmers in the tropics, where the amount of biomass of forages and its availability are targeted rather than the quality of ruminant feeds (Ba et al., 2013; Lisson et al., 2010; Phengsavanh and Frankow‐Lindberg, 2013; Preston and Leng, 1987), can compromise their productivity. These feeding practices, common in south-east Asia (and other areas of the world where similar ruminant nutrition is applied), not only constrain production at the animal level (Arthington and Brown, 2005; Rachmat et al., 1992) but may also limit subsequent crop production due to low rates of mineralisation of N from faeces applied to soil (Delve et al., 2001; Rufino et al., 2006; Snapp et al., 1998).

Hay used in this study contained CP >5%, so poor quality may be related to content of NDF, ADF, ADL and low DMD (see Table 3.3). The low CP concentration (<7%), an amount that would not support CP maintenance requirements in ruminants (McDonald et al., 2011), was similar to that used in other studies that have tested the effect of supplement forages in sheep (Alemu et al., 2014; Gebregiorgis et al., 2012). The quality of the oat forage, however, was poorer than expected, likely a result of the regrowth material used being near maturity. The CP content had declined significantly and NDF, ADF and lignin had increased compared to oat forages from other studies (Assefa and Ledin, 2001; Dias et al., 2011; Salgado et al., 2013; Umunna et al., 1995), meaning our oats were of a lower quality. However, harvesting grass forage after prolonged regrowth does occur in smallholder ruminant farms. With sheep, Archimede *et al.* (2000) found that intake and digestibility of fresh pangola grass (*Digitaria decumbens*) was higher than that of dried pangola grass, as drying affects the quality of forage, especially crude protein. This means that in our study, the lower quality of forage green oats, and hence intake and digestibility, may also have been due to the use of dried chopped oats rather than fresh green oats. Due to the timing of our experiment, oats from a single harvest were utilised in this experiment rather than a continual supply of fresh green oats grown to the same stage of maturity for the duration of the experiment. Our results were

therefore valuable in indicating the importance of using oats at a less mature stage in future experiments.

Despite the lack of differences in N output between the two treatments (*Avena sativa L.*), 75%Oats-25%Hay treatment still increased DM intake and total tract digestibility compared to 25%Oats-75%Hay (Table 3.4); probably due to higher DMD and lower ADL of oats compared to hay (Table 3.3). However, the intake of DM, in both treatments (23 and 27 g/kg LW.day for 25% and 75% oats, respectively), was about 30% lower than the optimal intake of ruminants (normally 3% of LW) (Allen, 1996). As outlined by Morre and Mott (1973), the major factors limiting intake, and also digestibility, are those associated with the rate and extent of forage degradation by microbial and physical factors in the rumen, primarily the amount of cell wall constituents and the extent of lignification. This means that the quality of the feed can be reduced when a species used is at a mature, and hence more lignified, stage of development, (Moore and Mott, 1973), as was the case in our experiment.

Moreover, intake may have been further affected by the size of the rumen pool and mean retention time (MRT) of feed in the rumen of the two diets. For example, Balch and Campling (1962) linked constraints in intake of forages by ruminants to the filling capacity of the forage, as represented by fibre mass. In this case, high fibre mass resulted in low intake when the size of the rumen pool was lower. Although in this study measurements of digestion rate, pool size of the rumen and the MRT were not included, and we are therefore unable to confirm this as an explanation, the high contents of NDF in both diets could further explain the low intake of feeds by our sheep. This is supported by Van Soest (1965), who state that NDF is the best chemical predictor of voluntary DM intake. In addition, in roughage diets such as used in our experiment, the filling capacity of the feed can also be affected by initial particle size, particle fragility and rate and extent of NDF digestion (Fahey, 1994).

In terms of the effect of forage quality on microbial degradation, and hence intake and digestibility, it was evident from our results that intake may have also been constrained by limited microbial digestion of feed in the rumen. The rumen ammonia-N (27 and 38 mg/L for 25% and 75% oats, respectively) in our sheep was lower than the value (50 mg/L) suggested by Satter and Slyter (1974) for optimal microbial activity. Despite the low amount of rumen ammonia-N not affecting N utilisation and retention from either diet, a finding consistent with Slyter et al. (1979), it does indicate that the supply and degradation of rumen degradable nitrogen (RDN) was inadequate from both treatments. As a result, the MCP flow from rumen to lower gut for both treatments (39 and 45 g/day for 25% and 75% oats, respectively) resulted in values that are considered insufficient for animals to retain N in their body (Makkar and Chen, 2004). As outlined by Moore and Mott (1973), these close relationships between lignification and digestibility and between intake and digestibility, become evident

particularly when the major difference in feed quality is due to maturity within a species, as was the case in our study compared to the work of others.

Although DM intake and N retention in the sheep was constrained by a low supply of available N from both diets, the 75%Oats-25%Hay diet did result in higher N intake and a trend towards higher N retention compared to the 25% oats diet. Elliott et al. (1963) and Elliott and Topps (1964) suggest this is most likely due to an increase in available MEI (33% higher for 75% oats), whereby the energy supplied may produce a protein sparing effect, reducing the maintenance protein The interaction of N and energy must therefore be matched to the animal requirements to maximise intake but also subsequent N deposition in ruminants (Schroeder and Titgemeyer, 2008).

In a review of the effects of energy intake on protein deposition, Schroeder and Titgemeyer (2008) found that even though the outcomes could be variable, most evidence seemed to suggest that increases in energy supply led to increased protein deposition, even at limiting levels of the protein supply. Increased energy intake also tended to improve the efficiency of amino acid (AA) utilisation, however, the magnitude of improvement can depend on which AA limits protein deposition (Schroeder and Titgemeyer, 2008). In our experiment, the insignificant increase in MCP production in sheep fed the 75% oats was not an indication of improved amino acid availability in the small intestines relative to digestible energy (P:E) of these sheep. The yield MCP (EMCP as gMCP/kgDOMI) was 75 and 67 for 25% and 75% oats respectively, suggesting that P:E was not a factor that regulated feed intake. However, when MCP synthesis is insufficient, dietary protein not used by the animal is excreted in faeces and urine, as was found in this study.

The proportion of N utilised for animal production or excreted is influenced by the type of livestock and by the concentration and form of N in the diet. In this study, 93-97% of N was excreted via faeces and urine, which was higher than 85-95% of dietary N normally excreted (Whitehead, 1995). Furthermore, the distribution of N excreted in either faeces or urine mainly depends on the concentration of N in the diet. The N excretion in faeces is constant per unit of DM intake (0.8 g N per 100g DM), even though it can change slightly with N concentration in the diet (Blaxter et al., 1971). Changes in dietary N concentration are more so reflected in the amount of N excreted in urine (Barrow and Lambourne, 1962; Betteridge et al., 1986a; Henzell and Ross, 1973; Lantinga et al., 1987). However, as the CP concentration of the two dietary treatments in this study did not differ, so the difference in N excreted in urine was not observed. Contents of NDIN of oat and hay are not measured in this study. Cell wall bound N (NDIN) may be unavailable in the rumen, therefore low ruminal NH_3-N and also contributes to greater faecal-N. While DMD or ME in lower rate of N degradability leads to energy spilling, therefore lowering MCP.

In contrast, a major difference was observed in the pH of urine, with the 75%Oats:25%Hay recording a much higher pH compared to 25%Oats:75%Hay (8.63 and 6.65). An increase in urinary pH from sheep was also evident when NaOH was used to treated a low-quality straw diet (Arndt et al., 1980; Ghasemi et al., 2013). From this, we can infer that the increase in urinary pH with the 75%Oats:25%Hay diet may be in response to increases in the amount of ammonia volatilised, and thus it could be a reason that explains the lower N concentration in urine with this diet. Conversely, the lower pH in urine from the 25%Oats:75%Hay diet may have been in response to reduces amounts of ammonia volatilised as a result of a greater proportion of N being converted into ammonium.

In terms of differences in % ^{15}N abundance, we found there was considerable discrimination between ^{15}N and ^{14}N during digestion and absorption by the animal. This finding was based on measurements of reference samples of oats, faeces and urine, and highlights the importance of using reference samples when calculating enrichment of different samples in a plant-soil-animal system, i.e. forage, faeces or urine. For the time taken for ^{15}N to appear after sheep ingested the ^{15}N-enriched feed, in urine this was between 4 to 8 h and for faeces, between 16 and 24 h. This delay in faecal N excretion represents the interval required for digesta containing small amounts of undigested oats and bacterial residues to travel from the rumen to the rectum, and is consistent with the pattern observed in dairy cattle by Powell and Wu (1999). However, we found that ^{15}N appearance in both urine (by 24 h) and faeces (by 48 h) reached a peak faster than for Powell and Wu (1999) whose peaks were attained at 30 h and 54 h in urine and faeces respectively. The more rapid peak in our study was because we fed the sheep a single ^{15}N-labelled meal that was finished within a few hours, whereas Powell and Wu (1999) fed ^{15}N-labelled forages every 12 hours for 36 hours in an attempt to get enrichment of labelled faeces and urine close to the enrichment of ^{15}N-enriched feeds.

The pattern of ^{15}N derived from feed in the faeces and urine (%Ndff and %Nduf) over the 96h time span after ingesting ^{15}N enriched feeds was different among the treatments for both urines and faeces. Peak ^{15}N concentrations of faeces and urines were 13% and 8% of the ^{15}N concentration respectively, which was much lower than the 41% and 58% in faeces and urine found by Powell and Wu (1999). Again, this difference with other work was most likely due to the poor quality diet used in our experiment, but may also have been a result of the ^{15}N-labelled oats component being only 25% and 75% of the total DM diet, compared to 100% used by Powell and Wu (1999). As shown in Figure 3.1, the ^{15}N in urine and faeces in our study were also further diluted with N in the basal hay.

Of the total ^{15}N ingested by sheep, most was excreted via the faeces (23% compared to 6% for urine), which suggests that rumen degradable N was inefficiently incorporated into rumen microbes and that only a relatively small amount of rumen degradable N was absorbed and subsequently excreted as urea in the urine. Faecal ^{15}N excretion was higher for

75%Oats:25%Hay than for 25%Oats:75%Hay, and because the MCP did not differ between the diets, the extra ^{15}N was most likely derived from undigested oats rather than ^{15}N-labelled bacterial residues. A detailed description of the model, based on a previous model of N transaction in sheep by Nolan (1975), developed for ^{15}N digestion of oats from the two diets in this study is provided in Appendix C.

These amounts of ^{15}N recovered in the faeces and urine after 96 hours were much lower than results reported by Sadik *et al* (Sadik et al., 1990) and Powell and Wu (1999), which ranged between 50-60% for faeces and were 20% for urine. The lower recovery of ^{15}N in our experiment may be the result of slower digestion rates, or a longer retention time of the diets. It is noted that the curve of ^{15}N recovery in faeces and urine in Powell and Wu (1999) remained stable or only gradually accumulated from 96h to 192 h, suggesting that about 20% of ^{15}N was stored in the animal body then slowly recycled and further excreted in faeces and urine. In our study, the ^{15}N recovery curve keeps increasing for both faeces and urine, over 96 hours with almost 70% of ^{15}N feed still traveling in the digestive tract of the sheep. This suggests then that the N excreted in days following the end of our experiment would still be from the ^{15}N-labelled feed component as well as endogenous N.

3.5. Implication of study

The feeding management practice used in this study is representative of feeding practices used by smallholder farmers in South-east Asia, especially Cambodia where improved forages have not been widely adopted in smallholder farming systems. The results from this study suggest that whenever possible, higher amount of grass forage should be added to low-quality straw or hay diets. This could be from either grasses 'cut-and-carried' from natural pastures, or from forage plots planted with improved tropical cultivars, however, it is suggested that the grasses be harvested before the regrowth has reached maturity in order to maintain higher CP and ME concentrations. Even though DM intake and N retention may be improved on a higher oats supplement, the N status of sheep was not improved in our study and hence, the % recovery of ^{15}N in excreta did not differ. This is most likely due to quality of supplemented forage at late harvest and the time of collection was short (4 days) in this study. The use of unlabelled hay N also further diluted ^{15}N in excreta and consequently lowered enrichment of excreta and its recovery.

In terms of future ^{15}N cycling studies, we have learned that collection of excreta from ruminants on low-quality diets may be collected at 48 h for faeces and 24 h for urine after sheep have ingested ^{15}N-labelled feeds. The timing of this collection of faeces after ruminants have been fed an N enriched diet, would also be beneficial for smallholder farmers that normally rely on recycling of animal manure for crop production on their farms.

3.6. Conclusion

This study indicates that even when at maturity, green oats has some potential as a means of improving the intake and digestibility of low quality basal diets for ruminants. The percentage recovery of N from oats was not affected by the quantity of hay ingested, however, higher N concentrations in urine and faeces from the 75%Oats:25%Hay diet indicate that excreta from this diet would be superior as a plant fertiliser or for biogas production. It is recommended that for further improvement of N utilisation and N recovered in excreta could be achieved if other supplements, such as legume, are included. We also recommend a collection period of ^{15}N faeces and urine longer than 96hours to ensure ^{15}N feed still traveling in the digestive tract can be recovered.

CHAPTER 4 USE OF FORAGE OATS AS A MULCH SOURCE FOR PLANT GROWTH WHEN APPLIED DIRECTLY TO SOIL OR AS EXCRETA AFTER INGESTION BY SHEEP

4.1. Introduction

Integration of crop-livestock farming systems form the backbone of agricultural activities of smallholder farmers in Asia (Devendra and Thomas, 2002b). The systems in which animals and crops are mixed together benefit the environment and soil fertility through nutrient cycling (De Haan et al., 1997). The type of crop-livestock system varies in different geographical locations, which leads to the variation of quantity, quality and distribution of animal feed resources throughout the year (Preston and Leng, 1987).

Low quality forages and crop residues, such as rice or wheat straw and maize stover, are abundant and commonly used as basal diet for ruminants in tropical and subtropical countries (Bakrie et al., 1996; Leng et al., 1992). Those feeds are generally low quality and are inefficiently used by ruminants when used as sole diets (Leng, 1990), yet this is a common practice for smallholder farmers almost all year round when green fodders are unavailable (Pezo et al., 2000; Stür et al., 2002). New, high yielding forage cultivars that produce high quality feed can play an important role in these mixed crop-animal farming systems (Horne et al., 2005), and improve income from animals and crops while protecting natural resources (Peters et al., 2001). Nevertheless, the introduction and intensification of forage production by smallholders may pose challenges, for example, extension service, access to seeds, land availability for forage plantation (Nakamanee et al., 2000). It has been shown that when a new forage cultivar is used to supplement a low-quality basal diet, the N status of the animal is improved and, in addition, the recycling of N back to the crop is enhanced, particularly with tropical legumes (Gomez and Kalamani, 2003; Murphy and Colucci, 1999) and tropical grasses (Tikam et al., 2013).

The N in animal manures is an important component of the N cycle in many agricultural systems focused on sustainability (Murwira et al., 1994). The influence of mineralisation-immobilisation processes in soil and losses of N by ammonia volatilisation, denitrification and nitrate leaching, all have a significant impact on the fraction of animal manure N that is available for plant growth. A better understanding of these processes is therefore required in order to improve the utilisation of animal manure N by plants in sustainable integrated systems. However, it is difficult to measure the mineralisation and immobilisation processes separately without using ^{15}N as a tracer (Sørensen and Jensen, 1998). This labeling of animal manure with ^{15}N to measure recovery of the labeled N in soil and plants, allows for

quantification of 1) labeled N losses from the crop-soil system, and 2) immobilisation of inorganic N in soil (Sørensen and Jensen, 1995).

The origin of faecal and endogenous N, and N pools associated with N cycling in ruminants is as outlined in Chapter 3. In order to quantify these pools, two different fractions of faecal N are labeled: a) ^{15}N-labeling herbage and feeding the herbage to animals in order to label the entire faecal-N pool (including undigested feed N, and b) feeding animals with ^{15}N-urea to label the microbial fraction and the endogenous N of the faeces (Powell et al., 2004).

Labelled faeces and urine of sheep have been obtained in numerous experiments by feeding with ^{15}N-labeled feed. For example, Sørensen et al. (1994b) fed a sheep ^{15}N-labelled ryegrass hay for nine days to evaluate the homogeneity of ^{15}N enrichment of different components of faecal N in sheep. The labeled faecal N was divided among two N pools: approximately 60% of easily-decomposable N fraction with similar ^{15}N enrichment to the enrichment of N mineralised, and 40% of the slowly-decomposable N fraction with ^{15}N enrichment similar to that of the feed. Sørensen & Jensen (1998) continued this work, feeding a sheep with ^{15}N-labelled grass to obtain ^{15}N-labelled manure for incorporation into soil used for growing plants. The results show that 61% of faecal N and feed N were recovered in organic form in the topsoil, while 94% of the indigestible feed N remained in the soil. In this study, crop uptake of labeled faecal N in faecal slurry distributed in a sandy and sandy loam soil was also examined. For slurry incorporated into soils, uptake was between 13-25%, and where slurry was distributed on the soil surface only, uptake was between 18-19%.

This isotope technique has led to an increased understanding of the nature of excreta N and the recovery of N in crops. However, the previous studies focused on feeding a labeled diet to one animal for several days where the quality of feed was normally high, and the excreta used in studies of N cycling was mixed into a slurry before application to soil. Studies showing the effect of a single meal of labeled mature forages and different ratios of forage: straw feed, information relevant to ruminant smallholder farmers, remain scarce.

The aim of the following experiment was to determine uptake of ^{15}N by forage maize (*Zea mays L.*) following application of N in mulched hay and the faeces and urine derived from sheep ingesting this same plant material: either low-quality hay with low (25%) or high (75%) levels of supplementation with green oats hay.. It was hypothesised that the fractional uptakes of N from urine, faeces and mulched plant material would be higher in response to higher ratios of oats hay to basal grass hay, and that the fractional release of N from urine would be higher than for faeces and mulched plant materials. It was further hypothesised that the mixing of plant materials or excreta in soil, to simulate ploughing of green manure or ruminant excreta, would reduce the loss of N from urine and increase recovery of N from faeces and mulched plant materials compared to their application to the soil surface only.

4.2. Materials and Methods

Experiment 1 – Production of labeled excreta by sheep

The experimental design and a detailed description of experimental procedures with sheep were described in Chapter 3, Section 3.2.

Establishment of unlabeled and ^{15}N-labeled oats

The forage oats used to supplement the low-quality hay diet of sheep and as the source of soil mulch in this study is a non-tropical C3 species that readily grows in the New England region of NSW. Oats (*Avena sativa L.*) were enriched with ^{15}N fertiliser ($^{15}NH_4{}^{15}NO_3$, 99.23 atom% ^{15}N) on a black, medium clay soil at 'Laureldale' Farm, University of New England (UNE), Armidale, Australia, in October 2010. The amount of fertiliser applied and method of application is described in Chapter 3, Section 3.2. Both ^{15}N-labeled and unlabeled oat plants were harvested three weeks after fertiliser application and air-dried in a glasshouse for seven days. The dried plants were chopped into 2-3cm lengths and stored in chaff bags for feeding to sheep during the experimental period. The DM concentration, N content and atom% ^{15}N excess of the N in soil and different plants parts are provided in Table 1, Chapter 3.

Animals, diets and ^{15}N-labeled oats feeding

Eight rumen-cannulated crossbred (Dorset × Merino) sheep, 3-4 years of age and 47 ± 3.8 kg (mean ± SD) liveweight at the start of experiment were used. Treatment 1 consisted of a low level (25%Oats:75%Hay) of mature oats supplement to hay on a DM basis and treatment 2 a high level (75%Oats:25%Hay). During the labeling period, ^{15}N-labeled oats hay was fed as a single meal at 10:00 h on Day 22. A detailed description of the feeding procedure is given in Chapter 3, Section 3.2.

Collection of ^{15}N-labeled faeces and urine

The sheep were individually housed in metabolism crates to facilitate urine and faeces collection. A glass fibre tray with an outlet at the lowest point was placed underneath the mesh floor of the cage and a urine and faeces separator attached to the outlet. The labeled urine was collected in a narrow-necked bottle, sitting in a bucket filled with ice to reduce N losses due to urease activity and ammonia volatilisation. Urine and faeces were collected over 4 days, at 0, 4, 8, 16, 24, 32, 48, 56, 72, and 96 h after sheep ingested the single ^{15}N-labeled meal on Day 22. The urine bottle was changed at each time of collection and a subsample of 10 mL was acidified with 0.1 mL 18M H_2SO_4. The faeces, which were separated from urine by a mesh screen were collected in a plastic bag. The bag was

changed at the same time urine was collected and a sub-sample taken from the top of the bag (assuming the most recent faeces excretion was located at the point) and stored in separate sample bags. The sub-samples and remaining labeled urine (non-acidified for use in subsequent maize growth study) and faeces were immediately stored in the freezer at -20^{o}C until used for analysis and a subsequent plant growth experiment (Experiment 2).

Quantities of ^{15}N-labeled faeces and urine collected

The labeled oats diets were given to sheep as a single meal of 669 g DM (63.2 and 183.3 mg ^{15}N per animal for 25%Oats:75%Hay and 75%Oats:25%Hay, respectively) to sheep on Day 22 at 10:00 h. All the sheep had finished the meal by 13:00 h after which time the unlabeled diets were resumed. The average amounts of total N ingested during this period from unlabeled and labeled feed were 32.7 and 35 g N for 25%Oats:75%Hay and 75%Oats:25%Hay, respectively.

Experiment 2 – Pot and soil experiment for ^{15}N cycling study

Design of pot experiment

A pot experiment was designed to investigate the recovery of ^{15}N from soil following the addition of three mulches (green manure); oaten chaff, hay with enriched oaten chaff i.e. high (75% oat) and low (25% oat), and two excreta; faeces and urine obtained when sheep ingested the two levels of enriched oaten chaffs. The excreta from these sheep were designated: faeces (F75, F25) and urine (U75, U25). The 8 treatments were: control (soil only), soil with F25, soil with F75, soil with U25, soil with U75, soil with Mulch25, soil with Mulch75, soil with Mulch100. (The Mulch100 treatment was included in the pot experiment, however, was not used as a diet for sheep.) To test for differences between methods of application, treatments were either mixed through the soil or applied to the soil surface. The faeces used for the pot trial were those collected after the 24-48h interval and the urine from the 4-24h interval after sheep ingested labeled oats (Experiment 1).
The treatments were arranged in a 2×8 factorial design (4 replicates of each treatment) in a glasshouse at UNE. In total, 64 pots of maize (var. HyCorn 533) were established for these treatments. The design of the pot experiment is summarised below:

2 levels of oats supplemented diets (25% basal hay and 75% oats)
× 3 materials (faeces, urine and mulched plant materials)
\+ 1 treatment of 100% mulched plant materials (100% Oats)
\+ 1 control
× 2 application methods (mixed with the soil or applied to the soil surface)

× 4 replicates

64 pots

Growing periods for pot experiment

Due to the slow release of N from the faeces and mulched plant materials, three consecutive plantings of maize using the same pots were scheduled. The first planting was harvested after only three weeks as symptoms of N deficiency became obvious due to the immobilisation of N during the early stage of organic material decomposition. The second and third plantings were harvested after 30 days. The detailed schedule for the pot experiment is illustrated in Figure 4.1.

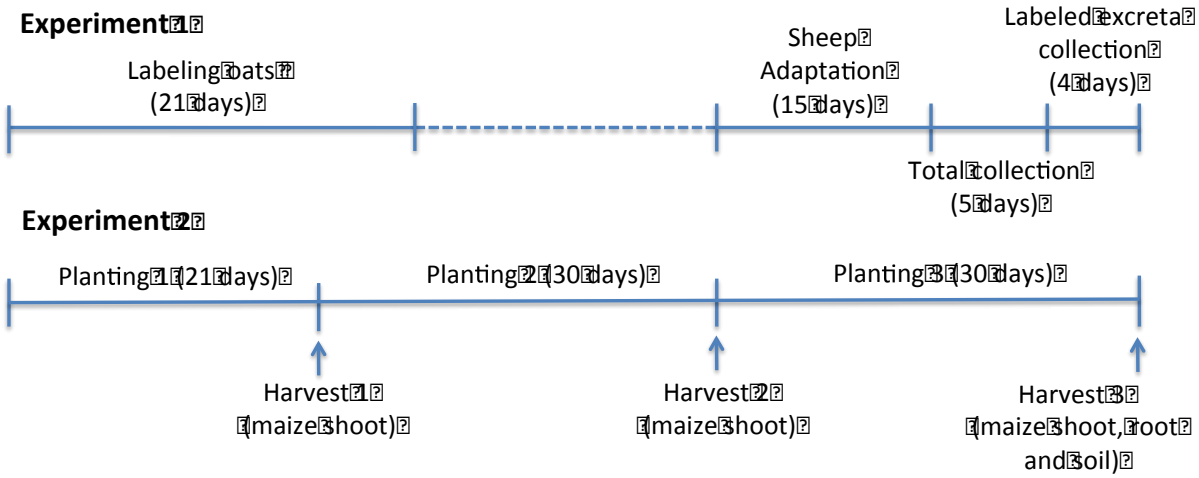

Figure 4. 1 Schematic of programming of labeling oats on the field, feeding labeled oats to sheep and collecting labeled excreta from sheep, and growing maize in pot trail in glasshouse for ^{15}N cycling study

N content and quantities of materials added to pot

The air-dried chopped oats and basal hay (2-3 cm in length) at ratios of 25:75 and 75:25 (from diets fed to sheep) and 100:0 were prepared for the pot experiment. Frozen faeces and urine were thawed to room temperature and immediately applied to pots according to the procedure described in the following section.

In this experiment, the mass of material added to each pot was calculated to contain 300 mg N/kg soil. The N content of each material, atom % ^{15}N excess and amount of materials added to the pots are shown in Table 4.1.

Table 4. 1 N content, atom % ^{15}N excess and amount of materials applied to pots

Parameters	C:N ratio	N (g/kg DM)	*Atom % ^{15}N excess*	Amount added (g DM/pot)
25% Oat-75% hay				
Mulched plant material (Mulch25)	**40**	0.84	0.9882	35.5
Faeces (F25)	**35**	1.12	0.1135	26.7
Urine (U25)	**5**	0.68	0.0517	44.2
75% Oat- 25% hay				
Mulched plant material (Mulch75)	**42**	0.84	2.2777	35.7
Faeces (F75)	**33**	1.14	0.3288	26.4
Urine (U75)	**5**	0.51	0.1236	59.1
100% Oats-0% hay				
Mulched plant material (Mulch100)	**40**	0.84	3.9790	35.8

Preparation of maize seedlings and soils in the pots

Maize (var. HyCorn 533) seeds were germinated on moist blotting paper in an incubator at 25^{o}C for three days prior to planting. Topsoil (sandy loam) from 'Kirby' Farm, UNE, was collected, air-dried and crushed to pass a 5mm sieve to provide the growth medium (Table 4.2). The soil was thoroughly mixed with 114 mg of single superphosphate (SSP) and 44 mg of mono potassium phosphate (MKP) to give 20, 12.5, 12.5 and 22.7 mg per pot of P, K, S and Ca, respectively. The required 64 plastic pots (upper diameter 14 cm, basal diameter 12.5 cm, height 15 cm) were lined with plastic bags (30 cm × 50 cm) and filled with 1 kg of soil (resulting in a soil depth of approximately 12 cm). Prior to the third planting, the soil in each pot was re-supplied with 50 mg of K_2SO_4 and 50 mg of K_2PO_4 as solution to give an additional 10, 10 and 45 mg per pot of P, S and K, respectively.

Planting of the maize seedling and imposition of treatments

For the incorporation application method, approximately 1 kg of dry soil was emptied out of each pot, and pre-weighed green manure, faeces or urine thoroughly mixed into the soil at a constant rate of 300 mg N/pot based on DM and N concentration. The soil was then returned to the pot lined with plastic bag. Treatments without incorporation had the equivalent of 300 mg N/pot applied directly to the soil surface.

Two seedlings were planted near the middle of the pot 4 cm apart then the pot watered to 90% of field capacity (gravimetric field capacity for this soil was 16%). No further water was added to the pot for two days. For the second and third plantings, three seedlings were carefully planted in each pot to avoid disturbing the soil structure.

Table 4. 2 Physico-chemical characteristics of Tenosol, Kirby farm

	Tenosol*
pH (H_2O)	5.44(0.01)
pH ($CaCl_2$)	4.38(0.08)
EC (d S/m)	0.01(0.0)
Total P (mg/kg)	171(6.7)
Colwell P (mg/kg)	5(1.0)
Total N (g/kg)	1.0(0.0)
Total C (g/kg)	12.0(0.1)
C/N	12(0.2)
CEC (cmol/kg)	2.18(0.01)
Soil texture	
Clay (%)	11.3(0.1)
Silt (%)	20.9(0.3)
Sand (%)	67.8(0.2)

* Average from 3 replicates; SE in parentheses
pH (H_2O): soil:water; 1:5; pH ($CaCl_2$): soil:CaCl2 solution: 1:5
Total C and N: oxidative combustion (LECO Corporation, MI, USA)
CEC: based on exchangeable bases and exchange acidity (Rayment and Lyons, 2011)
Particle size: pipet method

Growing conditions and watering

The experiment was conducted in the Glasshouse Complex at UNE. The temperature was set at a constant 30^{o}C to mimic tropical conditions. The pots were watered to water holding capacity (WHC), determined by mass, every 2-3 days.

Management

Maize in the pots treated with urine exhibited ammonia burning on the leaves due to high levels of conversion of urea to ammonia in the root zone (21 day period). In response, some of the maize tops in these treatments curled and did not grow well (Photo 4.1). This ammonia burning was most severe in the surface urine treatments compared to urine mixed in soil. For the second planting, during the first two weeks, ammonia burning was still evident on leaves in some treatments, however, in all urine treatments, the ammonia burning on the top leaves also occurred during the third week, although this occurrence was not as severe as the first planting.

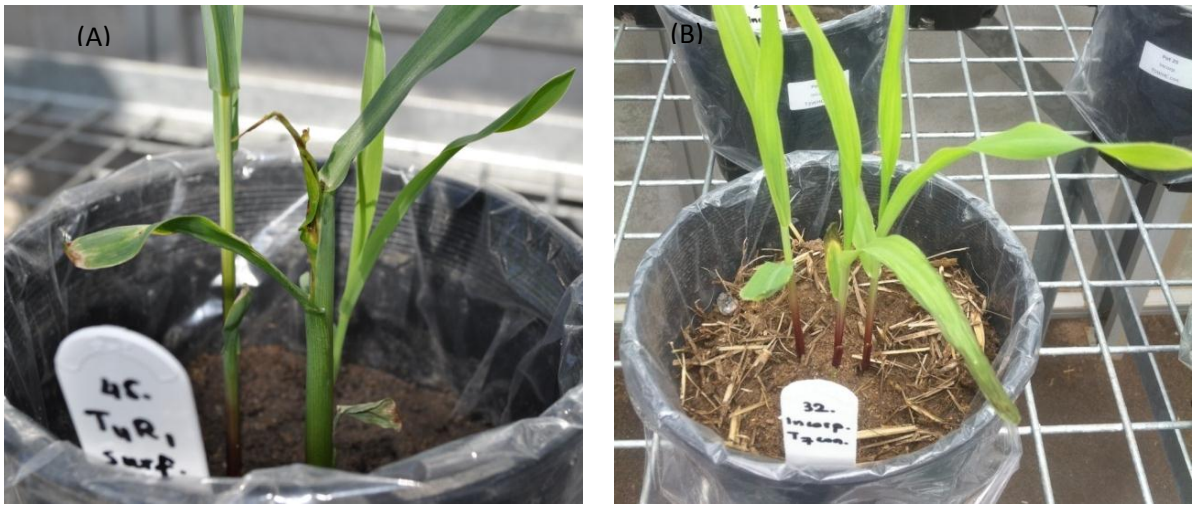

Photo 4. 1 Ammonia burning in some pots with added urine (A) and mulched plant materials (B) during the ^{15}N cycling study. This phenomenon was due to the high level of urea in the root zone in the pot.

Plant biomass harvesting

For the first harvest, the 'above ground' maize plants (also termed 'maize shoots' in this chapter) were cut at the level of the soil surface using scissors and the fresh weights recorded. Samples were dried to a constant weight at 60^{o}C in a fan-forced oven for 48 h to determine DM content prior to milling. This was repeated for the second and third harvests. During the third harvest maize roots were also collected from each pot. These roots were washed with tap water (to remove attached soil) then fresh and dry weights recorded as for shoots.

Sampling procedures and measurements

The dried maize shoots and roots were milled to pass through a 0.5 mm sieve then stored in sample containers prior to analysis for total N and ^{15}N content. For the surface application of mulched hay and faeces treatments, any residue remaining on the surface was only collected at third harvest and wet and dry weights recorded, and samples milled and stored as for shoots and roots.

Six soil cores taken from each pot (using an aluminum pipe with internal diameter 17.5 mm) also at third harvest, were bulked, thoroughly mixed (any obvious grass roots were removed for inclusion in washed-out root) and weighed. These bulked soil samples were dried at 40^{o}C to constant weight for moisture content determination then ground to pass through a 1 mm sieve and stored in sample containers prior to analysis for total N and ^{15}N content.

Chemical analysis

^{15}N in the faeces samples was determined using a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd, Cheshire, UK) (service provided by UC Davis, University of California), and the remaining samples (plant, urine, soil) were analysed using a Callisto CF-IRMS Stable Isotope Analysis system at the UNE using standard procedures (Sharp, 2007). Total N and C content in feeds, faeces and urines were analysed by Dumas combustion using a LECO analyser (TruSpec Carbon and Nitrogen Analyser, LECO Corporation, 3000 Lakeview Avenue St. Joseph, Michigan 49085) (Etheridge et al., 1998).

Calculations

The apparent recovery fraction (ARF) of the applied material N after uptake by maize plants was calculated using the difference method following Harmsen (2003) and IAEA (2001) (Harmsen, 2003; IAEA, 2001):

$$ARF\ (\%) = 100 \times (PN_{Treatment} - PN_{Control})/AN_i \quad (4.1)$$

where, $PN_{Treatment}$ = total N uptake by treatment plants in harvest 1, 2, 3 and roots (mg pot^{-1}), $PN_{Control}$ = total N uptake by control plants in harvest 1, 2, 3 and roots (mg pot^{-1}) and AN_i = total amount of the N applied (mg pot^{-1}). The difference method assumes that the soil provides the same amount of N to all pots and that all N uptake by plants in pots in excess of control N uptake was the result of the treatment.
Total apparent recovery fraction (ARF) in the pot experiment was the sum of ARF from all harvests of plant shoots and roots for each treatment and application method.

^{15}N enrichment in samples was expressed as atom % ^{15}N in excess (APE) as follow:

APE = % ^{15}N abundance in experimental samples – % ^{15}N abundance in reference sample (4.2)

where, % ^{15}N abundance from reference control samples were 0.3663, 0.3690, 0.3688, 0.3699, 0.3689, 0.3716, 0.3692 for oats, faeces, urine, maize top at harvest 1, 2, 3 or roots respectively.

Percent of N fraction in shoots or roots derived from ^{15}N labeled materials applied to pot (%Ndfm):

$$\%Ndfm = 100 \times (APE_{Maize}/APE_{Material}) \quad (4.3)$$

where, APE_{Maize} = atom % ^{15}N enrichment in maize tops at harvest 1, 2, 3 or root, $APE_{Material}$ = atom % ^{15}N enrichment in faeces, urine or mulched plant materials applied to pots.

%^{15}N recovery fraction in maize shoots or roots from ^{15}N-labeled materials:

$$\%^{15}N \text{ recovered} = 100 \times [(P \times APE_{Maize})/(F \times APE_{Material})] \quad (4.4)$$

where, P = the total N amount in the maize shoots at harvest 1, 2, 3 or in roots; F = the total N amount in faeces, urine or mulched plant materials added to pots, APE_{Maize} = atom % ^{15}N enrichment in maize shoots at harvest 1, 2, 3 or roots, $APE_{Material}$ = atom % ^{15}N enrichment in faeces, urine or mulched plant materials applied to pots. The APE was converted to ^{15}N enrichment in mg ^{15}N/mg total N by multiplying by 1.071.

Data from the animal experiment described in Chapter 3 consisting of ^{15}N intake, ^{15}N excretion in faeces and urine and ^{15}N retained in the animal body were used in this chapter to calculate the ^{15}N flow through feed-sheep-soil-plant system. The amount of ^{15}N in each pool was expressed as 'mg'.

Statistical analyses

The statistical significance of treatment effects on each variable was analysed using analysis of variance (ANOVA) in the statistical package JMP version 10.0.2 (JMP for Mac, 2012, SAS Institute Inc., North Carolina) using Fit Model with applied material types and methods of application and its interactions as main effects. The data were log transformed for normal distribution before the analysis. Least square means differences were performed using Turkey HSD test if the ANOVA test of each main effect was significant ($P<0.05$). The data were back transformed before presentation.

4.3. Results

Maize shoot and root DM yield

The DM yield of maize shoots ranged from 0.6 to 2.3 g per pot following Harvest 1, increasing to between 2 to 15 g per pot following Harvest 3 at the end of experiment (Figure 4.2). There was effect of treatment and methods of application and its interaction ($P<0.001$, Table A.2, Appendix A). All the urine-applied treatments had 3- to 4-fold higher ($P<0.05$) DM shoot yield compared to faeces-applied and mulched treatments following Harvest 2, and 3. This was also the case for DM shoot plus root yield following Harvest 3. The DM yield of shoots for U25 (25% oat chaff enriched hay diet), was higher ($P<0.05$) than U75 (75% oat chaff enriched hay diet). There were no differences ($P>0.05$) between the application methods for urine for all harvesting periods.

Methods of application of faeces (to soil surface or mixed through soil) to the pot did not affect (P>0.05) maize DM yield. However, when mulched plant materials were mixed through the soil, DM yield of maize for each of the three mulched treatments was lower (P<0.05) compared to the application of mulches to the soil surface.

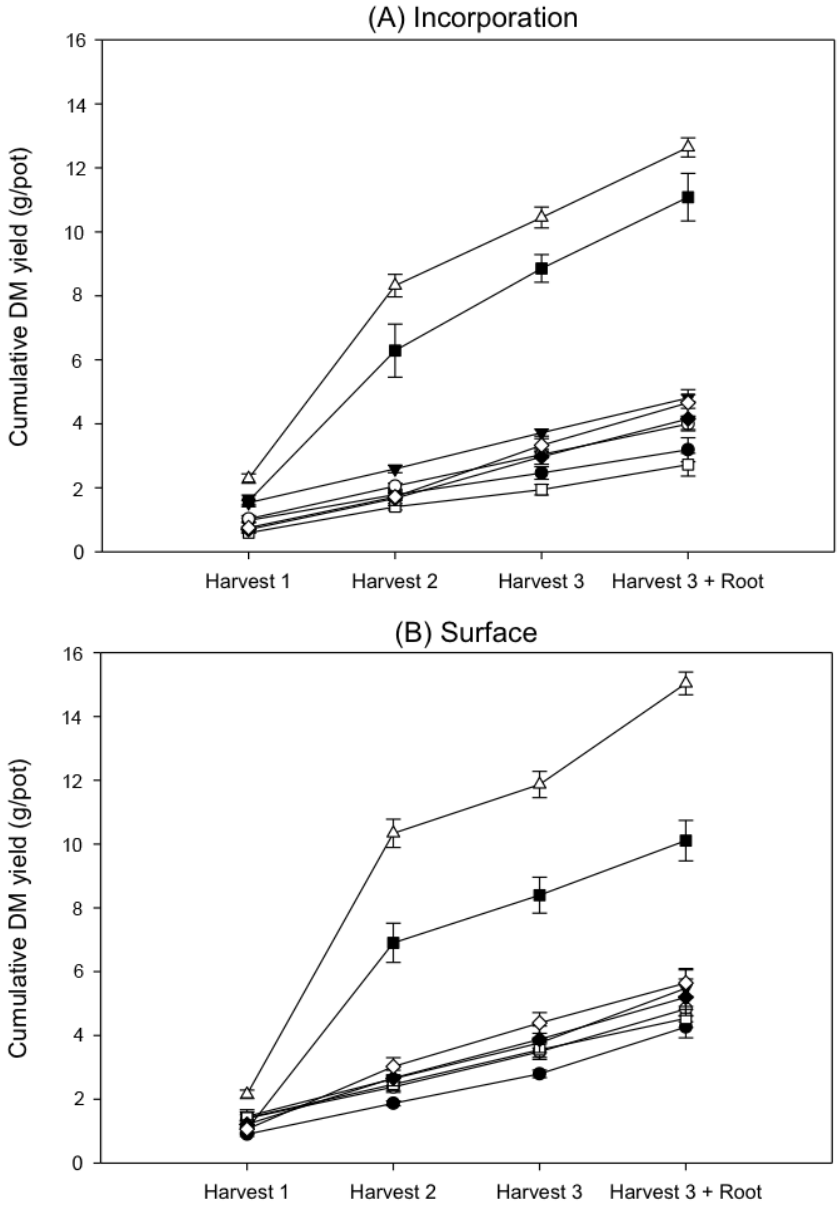

Figure 4. 2 Cumulative DM yield (g/pot) of maize shoots at time of Harvests 1 (21-d planting), 2 (28-d planting) and 3 (28-d planting) and roots from different treatments (Control ●, F25 ○, F75 ▼, U25 △, U75 ■, Mulch 25 □, Mulch 75 ◆, Mulch 100 ◇) mixed through in the soil (A) or applied to the soil surface (B) in pots in a glasshouse study of ^{15}N cycling

Maize N yield and apparent recovery fraction (ARF) of N in shoots and roots

Urine application provided a good source of N for maize to use for shoot and root growth (Figure 4.3). The N yields of maize for urine-applied treatments continually increased from Harvest 1 through Harvest 2 and Harvest 3 (for both shoots and shoots plus roots). Within

the urine-applied treatments, the maize shoot N did not differ (P>0.05) between diets (U25 or U75) nor application methods (to soil surface or mixed through soil) in all harvesting periods. In contrast, for faeces-applied treatments, F75 (75% oat chaff enriched hay diet) provided higher (P<0.05) N yield than F25 (25% oat chaff enriched hay diet). This trend was consistent whether applied to the soil surface or mixed through. None of the mulched plant material treatments differed (P>0.05) in shoot and N yield compared to faeces treatments at the same levels of oats enrichment when applied on the soil surface. Compared to other application methods and treatments, the mulches mixed in soil had the lowest N yields.

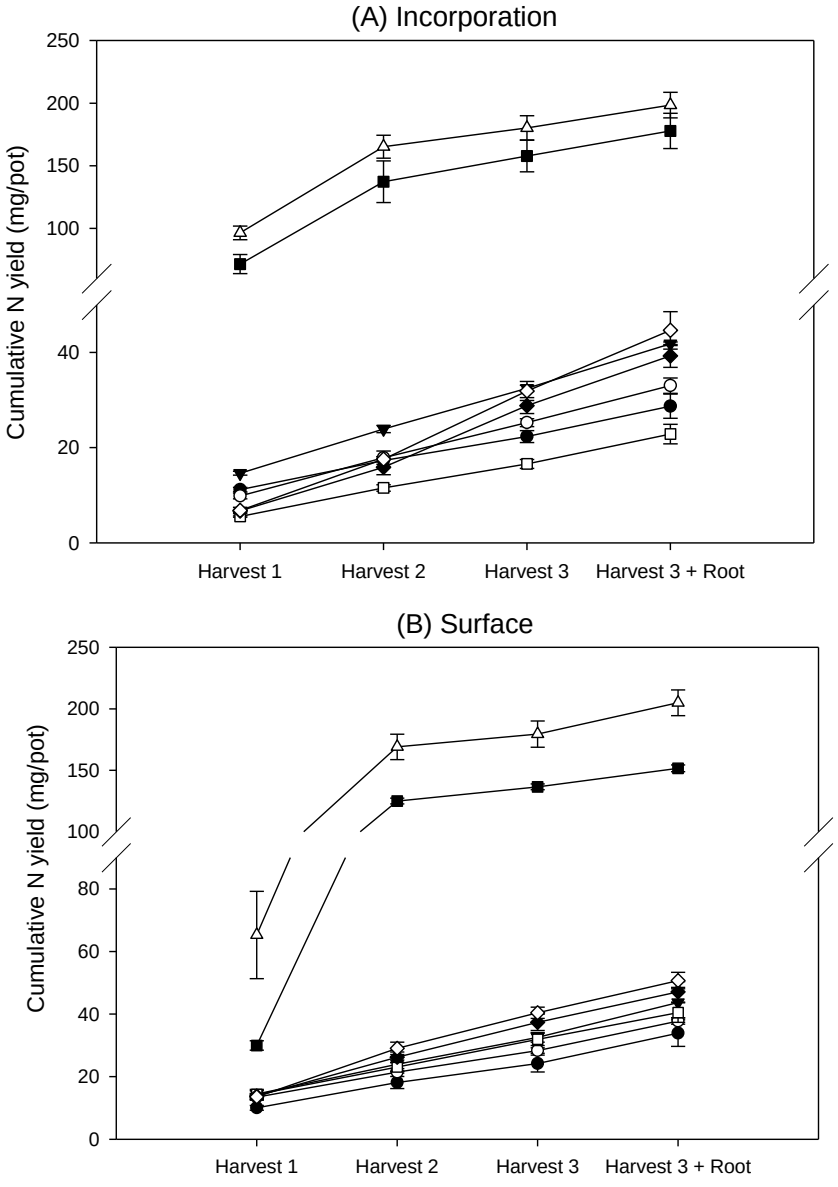

Figure 4. 3 Cumulative N yield (g/pot) of maize shoots during Harvests 1 (21-d planting), 2 (28-d planting) and 3 (28-d planting) and roots from different materials (Control ●, F25 ○, F75 ▼, U25 △, U75 ■, Mulch 25 □, Mulch 75 ◆, Mulch 100 ◇) mixed through in the soil (A) or applied to the soil surface (B) in pots in a glasshouse study of ^{15}N cycling

At Harvest 1, the ARF in urine-applied treatments ranged between 6 to 28%, being higher (P<0.05) when mixed in soil than when applied to the soil surface (Figure 4.4). This recovery

increased up to 50% in Harvest 2 and 3 and 3-5% more was recovered when roots were included. In contrast, the ARF of N in faeces-applied and mulched plant material treatments following Harvest 1 were very low (< 1%), with the. ARF of all mulched treatments mixed through the soil recording negative values (-2 to -1%) - the lowest ($P<0.05$) of all materials and methods of application for all three harvests. At Harvests 2 and 3 (including with roots), the ARF of N from faeces-applied and mulched treatments remained low (< 6%), and although N in the mulched materials mixed through the soil was slowly recovered, it remained negligible (from -2 or -1 to less than 1%).

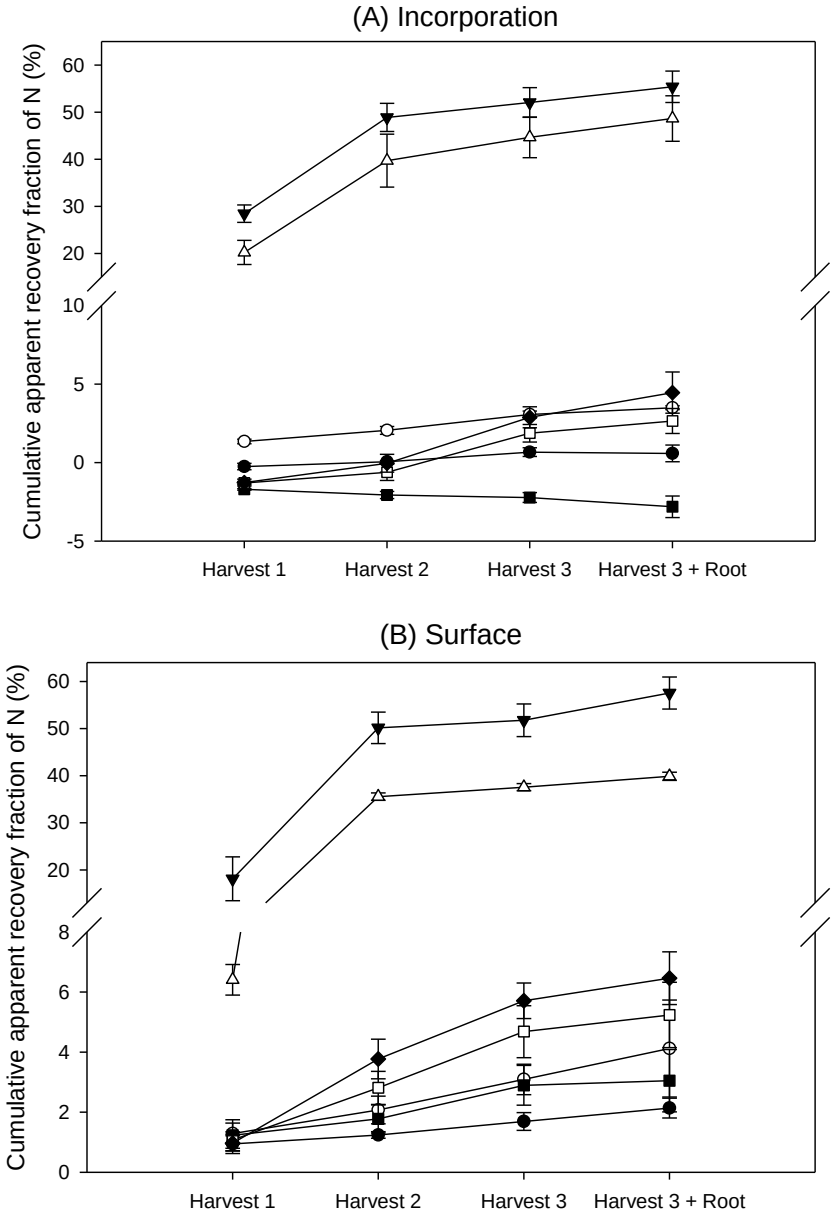

Figure 4. 4 Cumulative percent N recovery in maize shoots during Harvests 1 (21-d planting), 2 (28-d planting) and 3 and roots from different materials (F25 ●, F75 ○, U25 ▼, U75 △, Mulch 25 ■, Mulch 75 □, Mulch 100 ◆) mixed through the soil (A) or applied to the soil surface (B) in pots in a glasshouse experiment using the difference method

Total N, ^{15}N enrichment (APE) and percentage of N derived from materials (%Ndfm)

Total N content of maize shoots was significantly different for all treatments across all plant growth periods (P<0.001). In contrast, the application method alone did not have a significant effect on N content until Harvest 3 (P<0.05) (Figure 4.4). Initially, for Harvests 1 and 2, it was the interaction between treatments and application method that was significant (P<0.01), however, by Harvest 3 this interaction between the two factors was eliminated.

The ^{15}N enrichment (APE) in maize shoots was significantly different for most treatments, including the response to mulched treatments with different oats:basal hay ratios. The effect of application method and its interaction with treatments was also significant (P<0.05) for all the harvests (Figure 4.5).

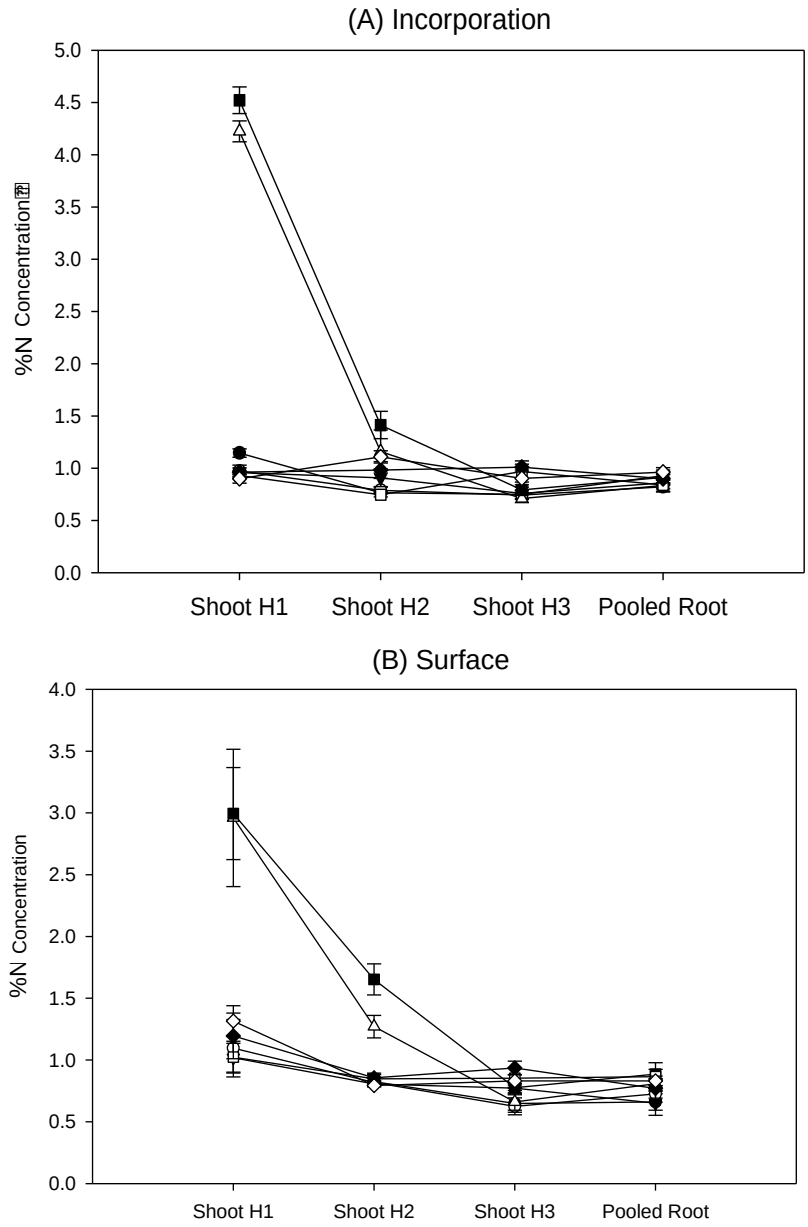

Figure 4. 5 Percent N concentration in maize shoots and roots during Harvests 1 (21-d planting), 2 (28-d planting) and 3 and roots from different materials (Control ●, F25 ○, F75 ▼, U25 △, U75 ■, Mulch 25 □, Mulch 75 ◆, Mulch 100 ◇) mixed through the soil (A) or applied to the soil surface (B) in pots in a glasshouse experiment using the difference method

The percentage of N derived from materials (%Ndfm) was significantly different between treatments and methods of application, and there was also a significant interaction between the two factors at all three harvests (Figure 4.6).

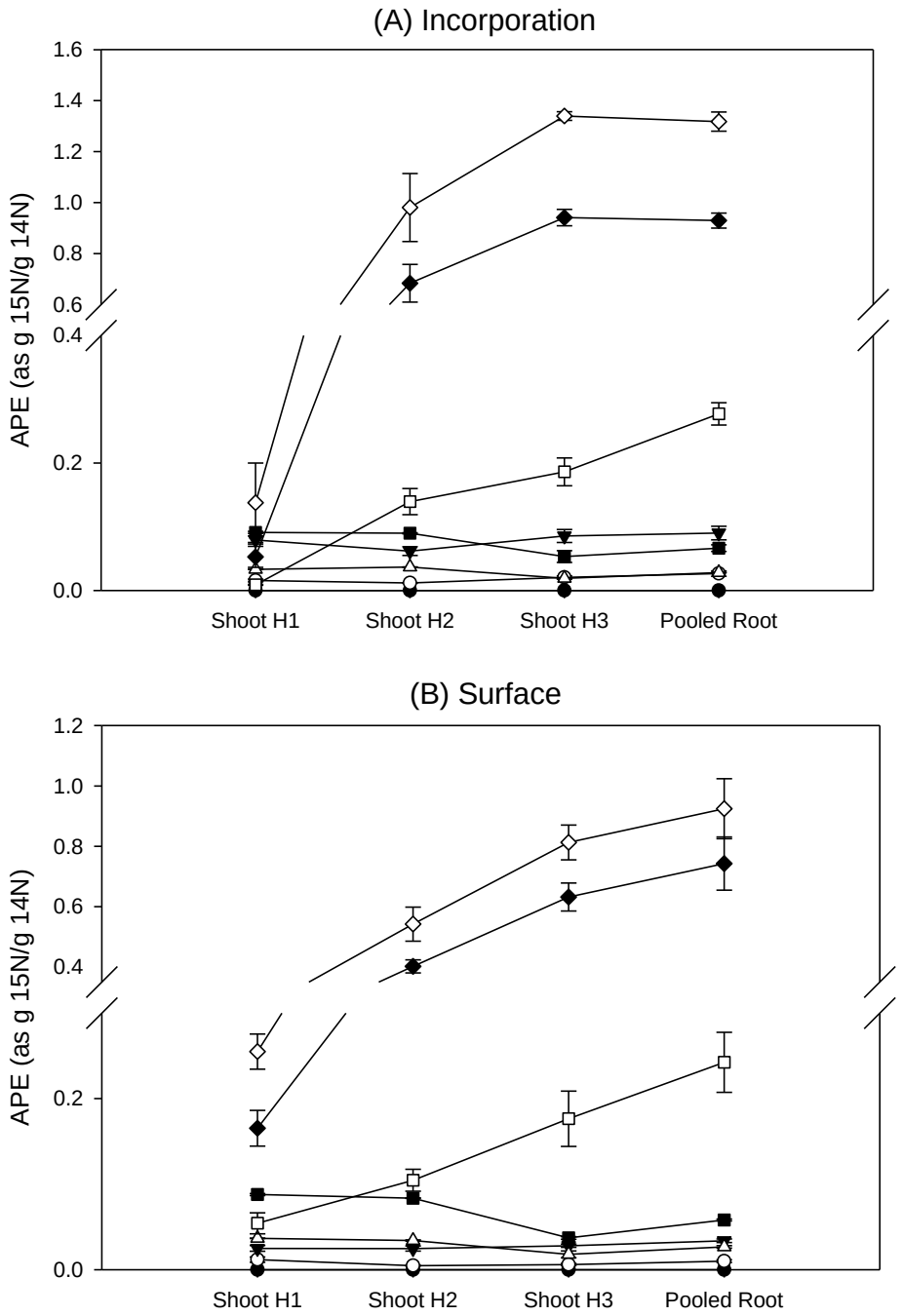

Figure 4. 6 APE in maize shoots and roots during Harvests 1 (21-d planting), 2 (28-d planting) and 3 and roots from different materials (Control ●, F25 ○, F75 ▼, U25 △, U75 ■, Mulch 25 □, Mulch 75 ◆, Mulch 100 ◇) mixed through the soil (A) or applied to the soil surface (B) in pots in a glasshouse experiment using the difference method

%Ndfm of urine-applied treatments were between 60-70% during the first harvest, declined slightly at the second harvest and was reduced ($P<0.05$) to 30-40% at the third harvest. %Ndfm of faeces-applied treatments mixed through the soil were between 13% and 23% at

the first harvest, slightly lower during the second harvest and higher again at the third harvest, with the F75 treatment consistently higher (P>0.05) than F25. This application method was also 5% to 10% higher (P<0.05) than faeces applied on the soil surface.

The %Ndfm of mulched plant materials increased over the three harvest periods (Figure 4.7). During the first harvest, the %Ndfm of mulched treatments mixed through soil (3%) was significantly lower (P<0.05) than for those applied on the soil surface (5%).

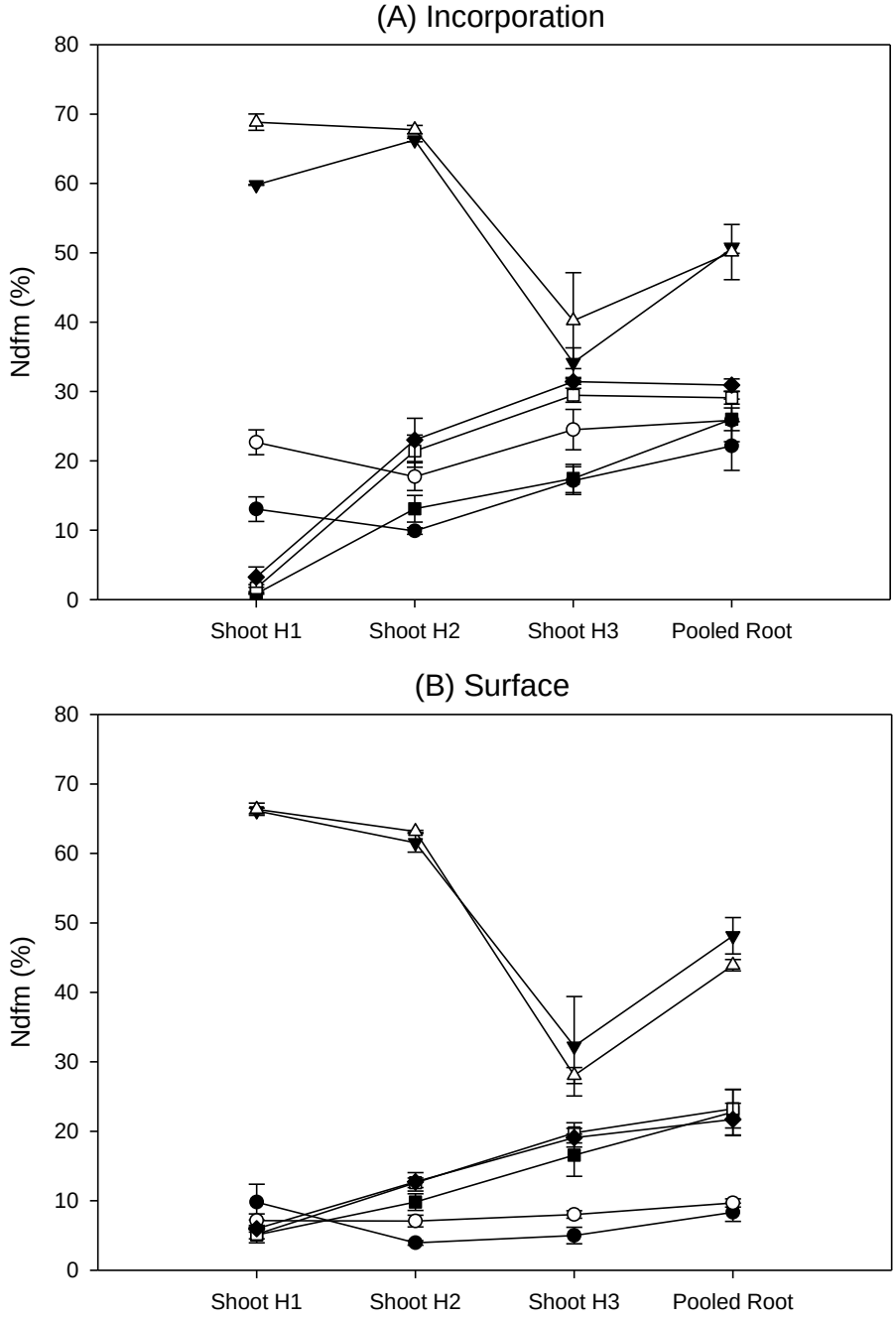

Figure 4. 7 Percentage of N in maize shoots derived from different materials (%Ndfm) (F25 ●, F75 ○, U25 ▼, U75 △, Mulch 25 ■, Mulch 75 □, Mulch 100 ◆) when mixed through in the soil (A) or applied to the soil surface (B) in pots during Harvests 1 (21-d planting), 2 (28-d planting) and 3 (28-d planting) in a ^{15}N cycling glasshouse study

For the soil surface application, there was no difference (P>0.05) between controls and treatments at first harvest, however, an effect was observed (P<0.05) for mulched materials mixed through the soil. During the second and third harvests, Mulch75 and Mulch100 increased the %Ndfm significantly (P<0.05) above than for faeces-applied treatments in the incorporated application method. It increased from 23% to 30% in the second and third harvest, respectively. Mulched materials mixed in the soil provided a higher (P<0.05) %Ndfm than when applied on the soil surface during the second and third harvests.

There was no effect of treatments (P>0.05) on the %N concentration found in roots, however, an affect (P<0.05) was apparent in response to the different application methods (Figure 4.4). The application method also significantly affected (P<0.05) the $\%^{15}N$ enrichments of roots, yet in this case, the treatments and the interaction between treatments and the application method were also significant (P<0.05) (Figure 4.5). For example, the maize roots in F75 pots exhibited higher (P<0.05) $\%^{15}N$ enrichment compared to roots in F25 pots, and the mixing of treatments through the soil returned significantly higher (P<0.05) $\%^{15}N$ enrichment in roots compared to the soil surface application method. Interestingly, of the two maize plant parts, the roots contained higher $\%^{15}N$ than shoots.

For the %Ndfm of roots (Figure 4.6), urine treatments released the highest fraction of ^{15}N (45-50%) into the roots of subsequent maize plants compared to other materials applied to the pots. Mulch25, Mulch75 and Mulch100 did not differ (P>0.05) in %Ndfm in roots for both application methods. For faeces treatments, F25 and F75 both resulted in higher (P<0.05) %Ndfm when mixed through the soil compared to application on the soil surface. This trend was similar to Mulch treatments.

Maize ^{15}N yield and recovery of ^{15}N using the isotope dilution method

The ^{15}N yield differed across treatments and methods of application (for Harvests 1 and 3) and a significant interaction was also evident (P<0.05) (Figure 4.8). Overall, the ^{15}N yield of Mulch75 and Mulch100 increased from Harvest 1 to Harvest 3 and roots contributed a considerable amount (30-40%) to total ^{15}N yield.

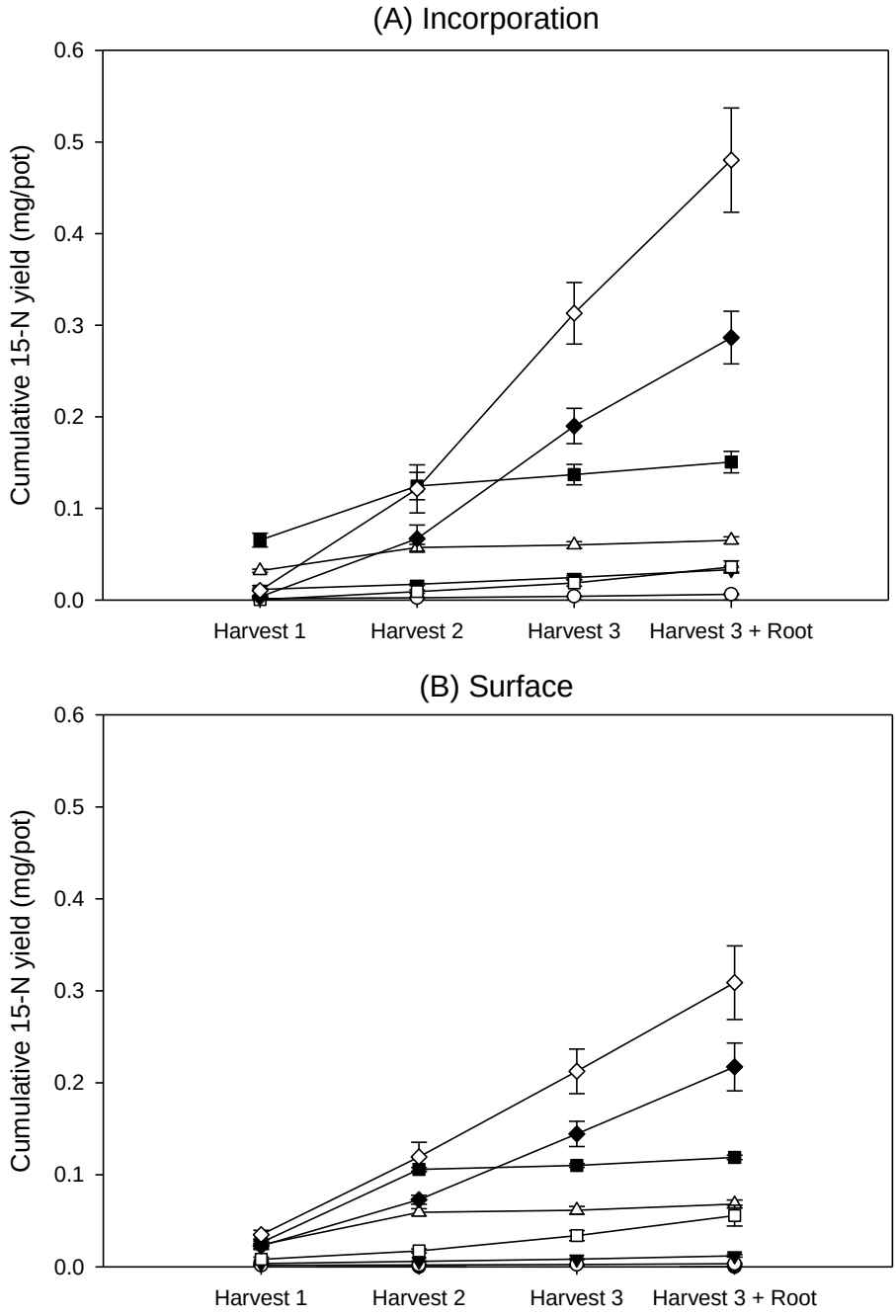

Figure 4. 8 Accumulation of ^{15}N in maize shoots during Harvests 1, 2 and 3 and roots from different materials (Control ●, F25 ○, F75 ▼, U25 △, U75 ■, Mulch 25 □, Mulch 75 ◆, Mulch 100 ◇) mixed through the soil (A) or applied to the soil surface (B) in pots in a glasshouse experiment using the isotope-dilution method.

The capture of ^{15}N from urine in crops was low at Harvest 1 and increased significantly only after Harvest 2 - remaining almost unchanged by the third harvest when roots were included (Figure 4.8). This pattern was similar for both application methods. Of the two treatment, U75 provided higher ($P<0.05$) ^{15}N yield in maize compared to U25. The ^{15}N yield of maize treated with faeces was very low compared to all other treatments, although F75 had higher

(P<0.05) ^{15}N yield than F25 for both application methods. Furthermore, when F75 was mixed through soil it returned higher (P<0.05) ^{15}N yield than when applied on the soil surface, an affect not seen in F25.

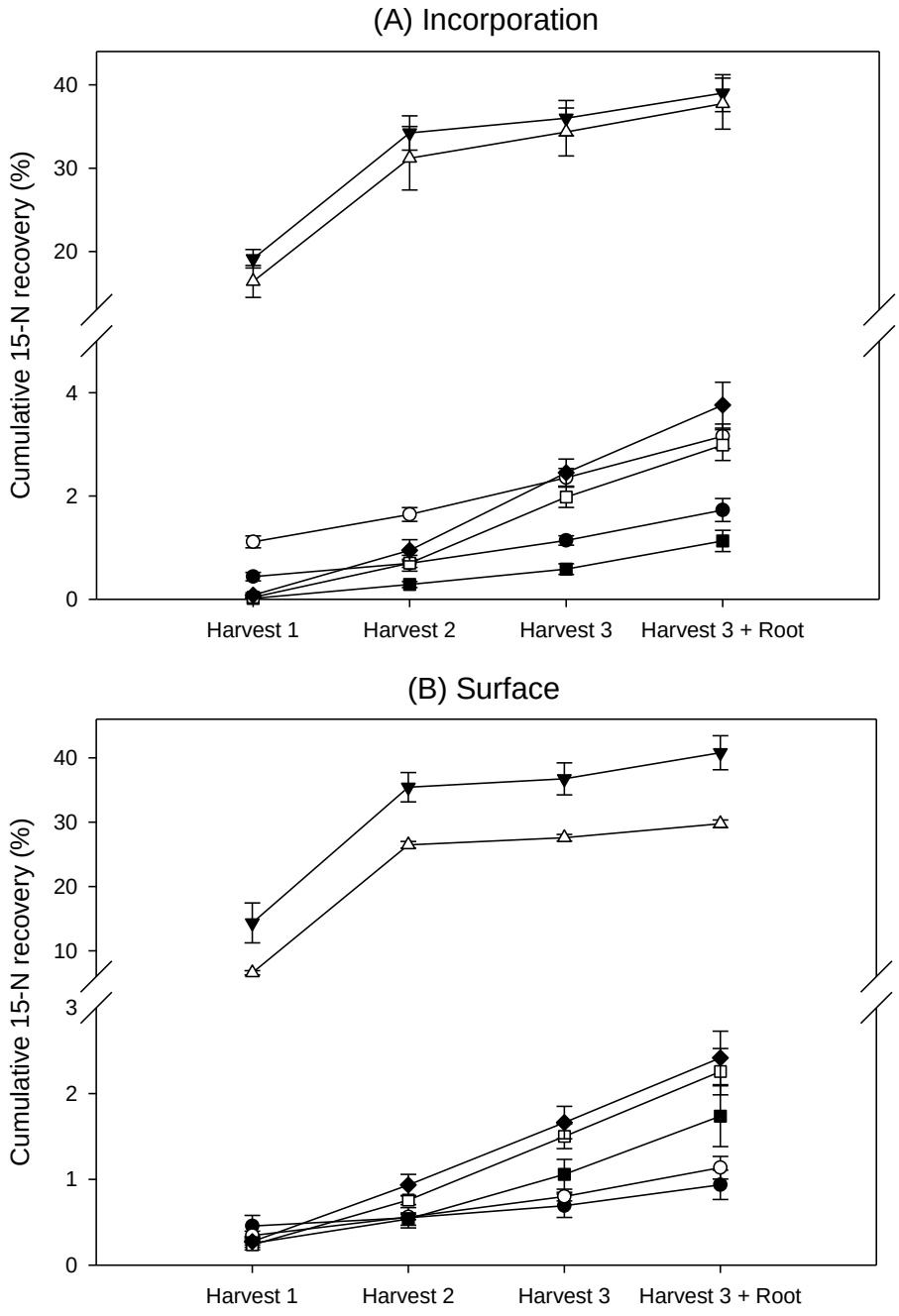

Figure 4. 9 Accumulation of %^{15}N recovery in maize shoots during Harvests 1, 2 and 3 and roots from different materials (F25 ●, F75 ○, U25 ▼, U75 △, Mulch 25 ■, Mulch 75 □, Mulch 100 ◆) mixed through soil (A) or applied to the soil surface (B) in pots in a glasshouse experiment using isotope-dilution method

Overall, the recovery of ^{15}N in maize shoots following both U25 and U75 urine applications increased from less than 20% at the first harvest to between 25-35% at Harvests 2 and 3 and up to 40% with roots included (Figure 4.9), with U75 providing the highest percentages for

both application methods. These were significantly higher than U25 when treatments were applied to the soil surface (P>0.05), however, there was no statistical difference between the two when incorporated into the soil. While incorporation did not result in differences between U25 and U75, it did result in increased recovery of ^{15}N from the U75 treatment (P<0.05) compared to U75 applied at the soil surface.

The recovery of ^{15}N from faeces and mulched treatments increased slightly over the harvests, with values ranging between 1-3% of total ^{15}N applied. The recovery was higher (P<0.05) when materials were mixed through the soil compared to application on the soil surface, and also for mulches with higher ratios of labeled oats to basal hay.

^{15}N flow under controlled conditions in the pot experiment

Figure 4.10 is a schematic developed to describe this integrated ruminant-soil-plant system when ^{15}N-labeled oats, mixed at rate of 25% or 75% with hay (on DM basis) were ingested by sheep, and their ^{15}N-labeled faeces and urine were mixed through soil or applied to the soil surface as a potential source of nutrients for the growth of maize plants. Of 63.2 mg ^{15}N fed to sheep, only 23% and 7% were recovered in faeces and urine, respectively, within a 4-day collection period, and therefore, 70% of the ^{15}N oats remained in the animal body. The recovery of ^{15}N from labelled sheep urine applied to soil in maize plants harvested over a total growth period of 11 weeks was 38% compared with only 1-2% from faeces applied to the soil. Most of the faecal ^{15}N (more than 98%) was still present in the soil. It was calculated that 25% of urine was lost through volatilisation while 37% was stored in soil. Incorporation of faeces through the soil did improve ^{15}N extraction by plants (2%) compared to surface application, where negligible amounts were recorded in plants, approximately 10% was stored in the soil and the remainder was still present in the faeces of the top of the soil.

Figure 4.11 is a schematic showing the passage of ^{15}N from labelled oats (75%Oats:25%Hay) through sheep and soil before being taken up by a crop. 23% and 6% of the ^{15}N in the original feed (183.3mg) was recovered in faeces and urine, respectively, with the remainder still cycling throughout the animal body. When faeces from animals who had ingested the labelled feed was incorporated into soil, ^{15}N recovery of applied faeces was 3 times higher (3% versus 1%) than when applied on the soil surface. Incorporation of their urine through the soil also improved ^{15}N recovery by the maize plants. Surface application of urine from this diet increased volatilisation losses (32%^{15}N applied) and lowered the percentage of applied ^{15}N stored in the soil.

Figure 4.12 is a schematic representation of the flow of amounts of ^{15}N from mulched hay with different ratios of oats:straw, and applied either on the surface or incorporated into the soil in the pot experiment. Incorporating the mulched oats in the soil increased the ^{15}N recovery in maize by 0.5-1% compared to surface application, except for the case of

Mulch25. The higher oats:hay ratio also increased the recovery on ^{15}N in maize - this was consistent for both application methods. For the surface application, the losses of ^{15}N accounted for 1% of ^{15}N applied. The remaining ^{15}N was stored in soil (11-14%) or remained in the mulched plant materials (83-86%).

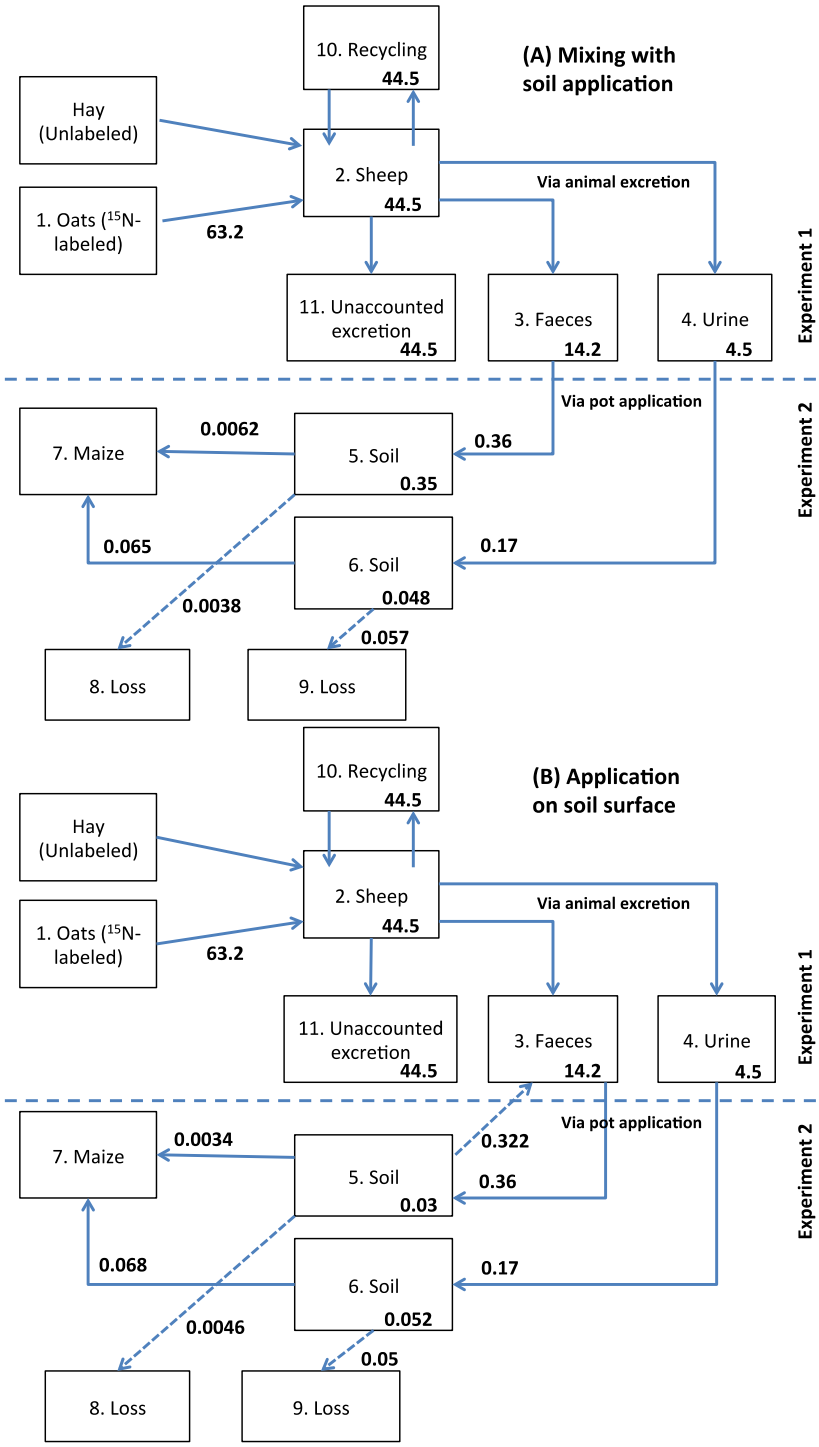

Figure 4. 10 Schematic of ^{15}N flow in a ruminant-soil-plant system when ^{15}N-labeled oats were mixed at rate of 25% with 75% hay (on DM basis) and offered to sheep, and their faeces and urine were applied by mixing with the soil (A) or applied on the soil surface (B) and maize plants were grown in this soil in a glasshouse. The numbers shown are in mg of ^{15}N. The ^{15}N-labeled oats (1) were mixed with hay (unlabeled) and fed to sheep (2) and some of the ^{15}N was excreted via faeces (3) and urine (4). Faeces and urine excreted within 4 days of 15N-labelled oats ingestion were applied to soil (5) and (6) based on N content (300 mgN/pot). The amount of N was recovered into maize (7), which was the sum of three harvests and the loss (8) and (9) through denitrification and volatilisation was calculated. For soil surface application, the ^{15}N in faeces that was not recovered in maize plants remained on the surface and is considered to be giving back from pool (5) to pool (3). Pool (8) and (9) are considered to represent amounts of ^{15}N recycling and traveling through the gut of animals and will be released in the longer term back to the soil. This amount is unaccounted in this study.

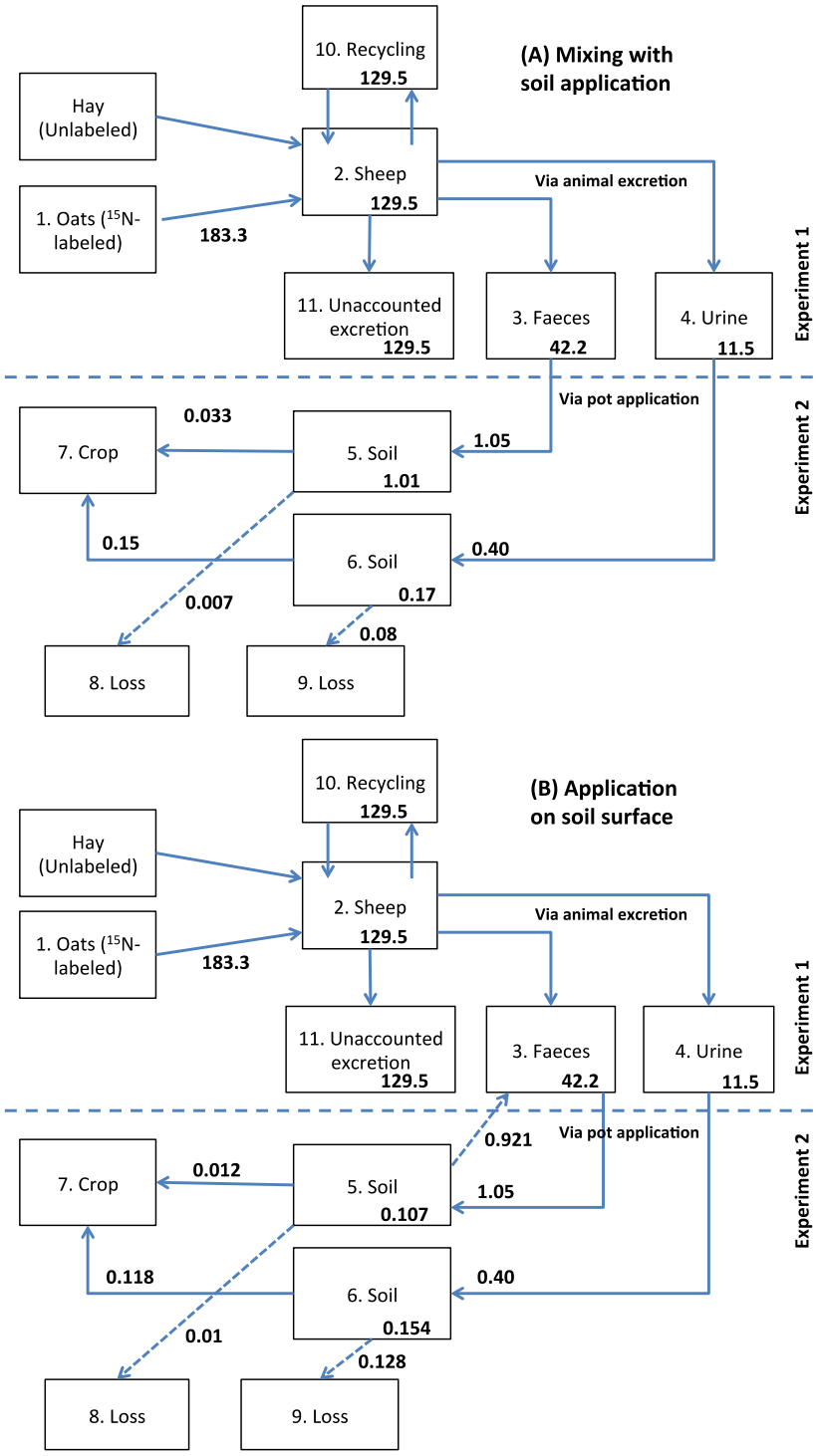

Figure 4. 11 Schematic ^{15}N flow in ruminant-soil-plant system when ^{15}N-labeled oats were mixed at rate of 75% with 25% hay (on DM basis) and offered to sheep, and their faeces and urine were applied by mixing with the soil (A) or putting on the soil surface (B) and growing maize plants in a glasshouse. The numbers shown are in mg of ^{15}N. The ^{15}N-labeled oats (1) were mixed with hay (unlabeled) and feed to sheep (2) and the ^{15}N was excreted via faeces (3) and urine (4). A small portion out of faeces and urine excreted within 4 days were applied to soil (5) and (6) based on N content (300 mgN/pot). The amount of N was recovered into maize (7), which was the sum of three harvests and the loss (8) and (9) through denitrification and volatilisation was calculated. For soil surface application, the ^{15}N in faeces that was not recovered by maize remain on the surface and is considered to be giving back from pool (5) to pool (3). Pool (8) and (9) are considered to be amount of ^{15}N recycling and traveling in digestive tracks of animals and will be excreted within months back to the soil. This amount is unaccounted in this study.

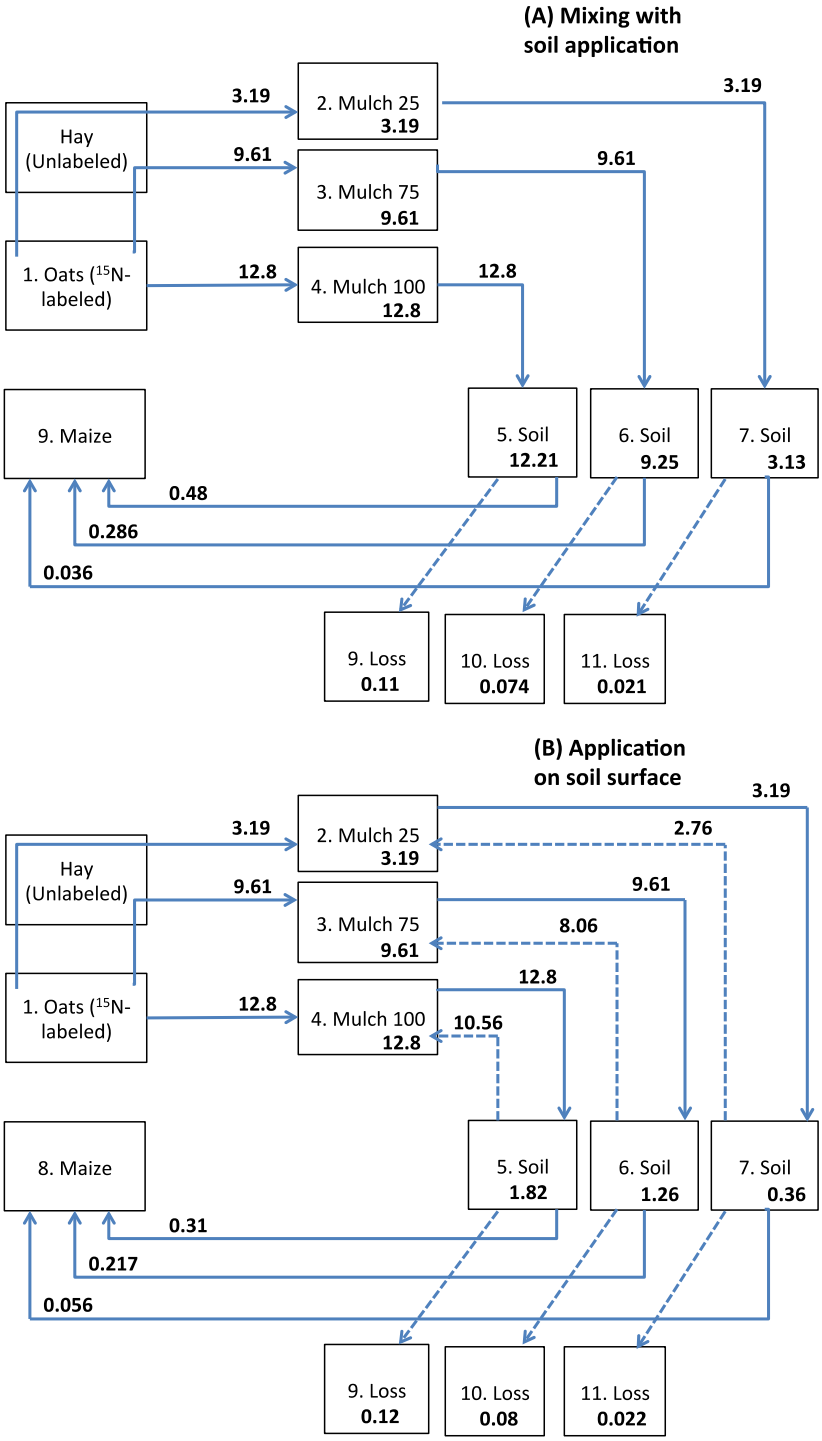

Figure 4. 12 Schematic ^{15}N flow in ruminant-soil-plant system when ^{15}N-labeled oats were mixed at rate of 25% (Mulch 25), 75% (Mulch 75) to hay (on DM basis) and 100% oats were mulched by mixing with the soil (A) or putting on the soil surface (B) and growing maize plants in a glasshouse. The numbers shown are in mg of ^{15}N. The ^{15}N-labeled oats (1) were mixed with hay (unlabeled) to produce mulched plant material (2), (3) and (4), with different oats:hay ratio and were applied to soil (5), (6), (7) based on N content (300 mgN/pot). The amount of N was recovered into maize (8) and the loss (9), (10), (11) through denitrification and volatilisation was calculated. For soil surface application, the ^{15}N from mulched material that was not recovered by maize remained on the surface and was considered to be giving back from pool (5) to pool (4), from pool (6) to pool (3), and from pool (7) to pool (2).

4.4. Discussion

This study has found that direct mulching of oats at different oats:hay ratios gave lower short-term recovery of ^{15}N in a maize crop than faeces or urine from sheep fed the diets of the same ^{15}N-labelled oats:hay ratios, suggesting rumen digestion of oats improves N cycling efficiency. The oats:hay ratio in the diet offered to the sheep affected maize growth for all soil amendments (mulched feed hay and faeces and urine following digestion), such that N uptake by plants following soil amendment with urine was higher than for faeces or mulched

materials. However, the application of fresh urine to the soil can cause problems such as severe ammonia burning and only short-term availability to the plant. Mixing the urine through the soil increased the uptake of N and ^{15}N by the crop compared to the surface application method, especially for the 75%Oats:25%Hay diet, and it is noted that after the 4-day collection period of ^{15}N-labeling excreta, more than 70% of oats ^{15}N were stored in the ruminants. This sink of N would therefore be lost to the system if the animal were sold or slaughtered - the N cycle in the particular farm system ends. In smallholder systems in developing countries such as Southeast Asia, animals can extend this period of N storage and their contribution to subsequent crop production, as cattle are retained for longer compared to intensive systems (Devendra, 1989; Jalaludin, 1989). The use of faeces and mulched hay amendments as a source of organic fertiliser returned very low use efficiency of N for the maize crop, In tropical agroecosystems with nutrient-poor soils resulting from, for example, acceleration of SOM oxidation as a consequence of global warming (Walker, 1999), it is possible then that these two sources of SOM may augment slow release of N as residues humify. Whether these two sources of SOM actually increase total OM in soil and contribute to increased retention of inorganic fertilisers (Seneviratne, 2000) is debatable. The passage of oats through a rumen necessarily removes fixed C from the system (as CO2 and methane), hence the net gain from digestion may be negligible. Therefore, in terms of the whole C cycle, there would be a difference between oats eaten and deposited as faeces compared to oats uneaten and applied as a mulch. This is explored further below.

The mulched plant and faecal materials applied to pots were characterised by relatively low N content. The hay and the oat forages fed to sheep and used as green manure mulch were high in fibre components (NDF > 60%, ADF > 35% and ADL > 7%) and low in DM digestibility (50-54%) (Chapter 3). This explains why rumen microorganisms degrade these materials relatively slowly (Fukushima et al., 1991; Van Soest et al., 1991) and why mineralisation of faeces and mulched plant materials in soil is also relatively slow (Cobo et al., 2002; Delve et al., 2001; Kyvsgaard et al., 2000; Sørensen et al., 2003; Van Kessel and Reeves, 2002). In terms of DM and N yield of maize in the pot experiment, urine treatments were superior (3- or 4-fold) as an iso-nitrogenous organic fertilises for plant growth compared to mulched plant or faeces materials.

Other researchers have also observed poor N recovery from faeces and provide more detail on the N forms in the faeces that may explain our results. Powell et al. (2004), Bosshard (2007) and Hoekstra et al. (2011) have all found uniform labeling of the entire faecal-N pool (including undigested feed N) by feeding ^{15}N-labeled herbage (Powell et al., 2004) difficult to achieve. This is because the NDFN fraction tends to be less enriched than the grass cell contents. Low transfer of N from sheep ingesting ^{15}N ryegrass to crops was also reported by Bosshard et al. (2011) who linked this to three processes: a low content of ammonia and

amino acids in faeces, a low rate of release of N during soil incubation and because faecal protein is in microbially bound forms.

In terms of the methodologies used to recover N, in general, from an agronomic point of view, the difference method (ARF) accurately reflects the overall effect of fertiliser application on crop N uptake, whereas the isotopic dilution method provides meaningful quantities of ^{15}N that can be translated into fertiliser N use efficiency and the N balance in a soil-crop system (Harmsen, 2003). According to literature, the difference method usually results in higher recoveries of N compared to the isotope-dilution technique (Hauck and Bremner, 1976; Jansson, 1971; Jansson and Persson, 1982). In our study, however, recovery of N using the isotope dilution method was both lower and higher than that observed using the difference method.

For example, the ^{15}N recovery in maize measured by the isotope dilution method when compared with the ARF method, was approximately 5% lower for urine and 1-2% lower for faeces and mulched plants applied to the soil surface. This is consistent with the above literature, and further reflection on the circumstances in which the difference method results in higher N recovery including examples from Hoekstra et al. (2011) and Fox et al. (1990). The ^{15}N-labeled slurry of faeces and urine recovered in grass in micro-plots under field conditions was estimated by Hoekstra et al. (2011) to be 15% after 6 weeks using the isotope dilution technique and 21% using the difference method (Hoekstra et al., 2011). Fox et al. (1990) report the difference between the two methods could be up to 20% lower in difference method when there was a high mineralisation rate (11-47% of added N) for legume leaves (alfalfa, cassia, leucaena, stylo, medic or vigna) incorporated in soil (Fox et al., 1990).

In our study, however, there were also instances of the isotopic dilution method resulting in recovery of N exceeding the difference method. These circumstances revolved around extremely low recovery of N from faeces and mulched plant materials mixed through the soil (some treatments pot had lower N uptake than the control pots). In these cases, it's likely the incorporation of faeces or mulched materials caused mineralisation-immobilisation turnover in the soil, and the recovery in the crop is less accurately measured by isotopic dilution (Harmsen, 2003).

By adding the high C:N ratio materials to a low N soil (>40% for plant materials and >30% for faeces in this study), further losses of already low levels of labile N result hence, the N measured by the difference method is lower. Furthermore, of the N actually taken up by the plant, it is likely that a higher proportion of the added N, compared to native N, is accessed. This can be explained by the large proportion of added N now present in the soil which is immobilised, then remineralised - contributing to a higher fraction of the labelled N in plants. This is in complete contrast to results reported by Harmsen (2003). Here, N-deficient soils

(such as those used in this study) recorded lower values of N recovery using the isotopic dilution method, which Harmsen (2003) suggests was because of increased uptake efficiency of soil N in fertilised treatments. It was soils high in available N that recorded higher N recovery values for the isotopic method. Harmsen (2003) proposed this was because the fertiliser N mixes with the soil mineral N pool to allow the plant to derive its N from applied plus soil N (even if there is little or no crop response to applied N) (Harmsen, 2003).

Overall, and regardless of methodology used to apply and recover N, urine amendments resulted in far greater recovery of N in maize plants after three harvests over an 11 week period. The cumulative recovery of ^{15}N in maize shoots and roots was approximately 40% for urine-applied treatments and less than 4% for faeces and mulch treatments. When mixed through soil, the urine N recovery in maize plants did not differ between the diets; however, a difference of approximately 10% between 25oats:75hay and 75oats:25hay was found for all harvests with application on the surface, suggesting that incorporation of urine into the soil reduced the loss of urine N. During the first harvest (21 days after the application of urine to the soil), the recovery of N in maize plant materials was between 10-20% and the recovery increased to 40% in Harvest 2 and remained stable until Harvest 3. The remaining 60% of ^{15}N was accounted for by ^{15}N remaining in the soil and volatilisation losses.

With the recovery of urinary N levelling off after Harvest 2 (Figure 4.8), this suggests that the ^{15}N stored may be expected to be slowly released and become available for uptake by crops in the longer-term. This is in contrast to high and rapid losses due to volatilisation. The loss of 60% of urine ^{15}N after 11 weeks was similar to that recorded by Catchpool and Blair (1990b) who applied urine from sheep fed legume tree leaves to pots in which grass plants were grown. The slight difference in our results (approximately 10% higher losses) may have been due to the different plant species used and their stage of growth. Catchpool and Blair (1990) used three five-month-old grass seedlings per pot whereas in this study two germinated maize seeds were used. The presence of an active and established root system may therefore explain their reduced losses to volatilisation, as these roots could access the available N from a greater portion of the growth medium. This type of variation in volatilisation losses was reported by Watson and Lapins (1969), who compared urine losses to volatilisation in soil with or without pasture plant growth. In this study, 54% and 73% of urine, respectively, was lost to volatilisation 56 days after applying urine to the soil.

Furthermore, volatilisation (NH_3^+) and denitrification (N_2O and N_2), may also explain the large decreases in N derived from urine present in the maize, particularly between Harvests 2 and 3. At these harvest times, %Ndfm in the urine-applied treatment decreased from 65% in Harvests 1 and 2, to 30% in Harvest 3, suggesting an increased N fraction in the maize is drawn from native soil N rather as urine sources of N are lost over time. In a study by de

Klein et al. (2003), the losses of urine N, i.e. N_2O, lasted for up to four months after urine was applied and ranged from between 0.3-2.5% (de Klein et al., 2003).

One can also look to the N and protein contents in the ruminant's diet to explain our results. In the first instance, higher N intakes proportionately increase the rate of urinary N excretion and the urea concentration in the urine (Smits et al., 1995; Topps and Elliot, 1967), and the volatilisation losses of N from urine also appear to be greater when animals are offered a diet with higher N content (Frank et al., 2002; Smits et al., 1995). These trends were supported by the results in this study, with reduced ^{15}N recovery in maize fertilised with urine excreted by sheep fed the high N content 75%Oats:25%Hay, although this was significant only for urine applied on soil surface. It is likely then that these increased losses (the difference between plant ^{15}N uptake plus ^{15}N in soil and ^{15}N applied) were due to the higher level of N supplied in the oats.

In terms of protein, Paul et al. (1998) have found that a lower crude protein in the animal's diet can decrease the fertiliser, or N value, of excreta. In this study, however, crude protein levels for each treatment were intentionally kept at 5% and were lower than the dietary crude protein tested in experiment described above. This was to mimic protein levels fed to smallholder ruminants, and therefore, protein levels were not considered a contributing factor.

While overall, urine amendments resulted in far greater recovery of N in the maize plants, the recovery of N from faeces provide further insights into the cycling of N in a ruminant-soil-plant system. In contrast to the results for urine treatments, the recovery of faecal ^{15}N derived from 75%Oats:25%Hay in the maize plants was higher than for 25%Oats:75%Hay, however, this was only significant when faeces were incorporated in soil. In this case, and again, in contrast to urine treatments, the higher N availability from faeces of sheep fed 75%Oats:25%Hay was congruent with the higher N content of the feed. Catchpool and Blair (1990b) also found different recoveries of faecal ^{15}N from application of faeces derived from different N content feeds; incorporated *Leucaena*-derived faeces and *Gliricidia*-derived faeces. They suggested that the initial N content of the faeces (dependent on the diet fed to the goats producing the faeces) was the main reason for the difference. This same pattern is supported by Seneviratne (2000) who, in their meta-analysis of multiple studies, suggested that with limited N concentration (<2%), (as is the case for faeces), the N released is determined by N concentration of faeces.

Furthermore, by measuring the rate of mineralisation of N from faeces mixed through soil, Barrow (1961) found that the rate of mineralisation over 12 weeks was positively correlated with the initial faecal N content. This is consistent with our %Ndfm findings: the %Ndfm from the faeces fertiliser found in maize suggests that faeces derived from the 75%Oats:25%Hay diet contained more readily available N for use by the maize crop (23%), compared to faeces

derived from 25%Oats-75%Hay (12%) after the first 3 weeks (Harvest 1). Interestingly, the %Ndfm then dropped at Harvest 2, suggesting that N immobilisation by microorganisms had limited the rate of ^{15}N released to the crop, and then increased at Harvest 3. This fluctuation suggests a process of remineralisation of N from the soil microbial pool following immobilisation of ^{15}N from the organic source (Shindo and Nishio, 2005).

The recovery of N in the maize was different when mulched plant materials were either incorporated or added at the soil-surface. Measured with the isotopic method, the incorporated-mulch had higher recovery of ^{15}N than surface-mulch material, and is consistent with other findings that mineralisation/immobilisation is slower when mulch is applied on the soil surface than when it is incorporated into soil (Mulvaney et al., 2008). However, when using the difference method to assess the recovery of the applied material N in the maize plants, the results suggested the release of N from incorporated-mulched materials was lower than for the surface applied mulch; an observation also made by Seneviratne (2000) for residues with comparable contents of C, N, polyphenols and lignin. They observed only net N mineralisation for surface-mulched materials, whereas the soil-incorporated mulch showed net N immobilisation of residues in soil

In our study, the difference method showed that N in mulched plant materials with either 75% or 100% oat proportions, was immobilised (negative AFR) for at least seven weeks over the 11 week experiment, whereas the incorporation of 25%Oats:75%Hay caused immobilisation during all three periods of maize plant growth. Based on evidence from Seneviratne (2000), this may be correlated with the C:N ratio of the materials used. In our study, the higher ratio of oats in mulched materials, while not altering the total N content, did slightly decrease the C:N ratio of the mixture (44, 42, 40 for 25%, 75% and 100% oats respectively). In the meta analysis conducted by Seneviratne (2000), the C:N ratio was the best determinant of N release for mulched plants with a wide range of N concentrations in tropical agriculture. They suggest that immobilisation is due to high levels of C and plant nutrients limiting the enzyme activities of microbial decomposers of N, and provide a correlation graph for C:N ratios and immobilisation/mineralization (Seneviratne, 2000). According to the graph, the C:N ratios of material used in this study (40-45) would most likely correlate with immobilisation. Seneviratne (2000) also show that plant residues with N concentrations lower than 2% generally resulted immobilisation of N in soil due to the substrate-N limitation on the enzyme activities of microbial decomposers. This was evident in a study by Fox et al. (1990), whereby incorporation of legumes (containing <20 g N/kg, or <2%) into soil resulted in net N immobilisation over the first 6 weeks.

Recovery of N from faeces and mulched materials in all pot experiments was very low for all systems tested, being less than 5% over the 11 weeks. This was most likely due to the low N contents in the initial materials applied to the soil and the high C:N ratio. The C:N ratio of

both mulched plant materials (40-45) and faeces(32-35) were higher than the cut-off point for net N mineralisation (25 (Myers et al., 1994) or 27 in tropical areas (Seneviratne, 2000)) and this is reflected in almost 95% of N from faecal and plant materials remaining in the soil at the end of 11 weeks of this study. However, due to its lower C:N ratio, it is expected that over time, the release of N from faeces would be faster than for mulched plant materials.. Importantly, the remaining organic matter and N may, respectively, provide benefits and become available for cropping in the longer term.

To determine the value of the mulched plant materials over the longer term, a study by Fang et al. (2007), was conducted in an area of South-western China where mulch (or 'green manure') is used as an important soil fertiliser on meat-producing farms. Here, green manure crops are grown and incorporated into the soil to improve fertility by increasing the soil concentrations of organic matter and other nutrients. Fang et al. (2007) demonstrated that it took 18 months for mulched grass placed in the soil in litter bags to be fully decomposed, and that annual net N mineralisation was higher in mulched plots than in plots without mulch. They also recorded an improvement in soil nutrients levels with higher applications of mulched materials (Fang et al., 2007). In tests on incubated soils, they found that the majority of the accumulated N in soils with grass mulch applied existed as NO_3-N, a form of N that is very susceptible to being lost under field conditions (Fang et al., 2007). This was reflected in our urine treatments, where N recovery in maize reached up to 35%, however, more than 30% was lost to the air and the other 35% remained in the soil but in a form more susceptible to loss.

4.5. Implications of study

This study has advanced the understanding of N flows through a ruminant-soil-crop ecosystem under conditions mimicking the tropics where low-input ruminant nutrition is commonplace (i.e. straws supplemented with green forages). It also provides estimates of the recovery of N from forages applied directly to the soil or applied after being subjected to digestion and excretion by ruminants. In addition, the effects on volatilisation, immobilisation or mineralisation from different methods of their application (mixed through the soil or applied on the soil surface) have been elucidated. The information can be applied to improve the whole system efficiency of smallholder livestock production in developing countries such as Cambodia, where adoption of forage technologies is becoming more commonplace. For example, green grasses are well integrated with ruminant-crop systems in South-east Asia (Chin, 2002; MacLeod et al., 2007) because of their benefits for animal performance and soil fertility (Horne et al., 2005).

In South-east Asia, lower quality ruminant diets are commonly supplemented with better quality grasses in so-called 'cut-and-carry' systems, and in this study, we have shown that a low quality hay-based sheep diet combined with higher ratios of oats (mimicking the cut-and-carry system) is likely to produce faeces and mulch plants that will release N more rapidly than diets with lower ratios, In our case, a higher ratio of oats was used to improve feed quality, however this could also include other forage supplements.

The collection of faeces and urine from penned animals for use on farm for smallholder systems should, therefore, promote more efficient cycling of N than occurs when animals graze freely; in this case, nutrients are largely applied to the pasture and soil surface and the distribution of nutrients can be uneven. In general, faeces and urine N are likely to be retained more efficiently when ploughed into the soil than when applied to the soil surface and this can result in N being available more slowly for plant growth. However, the losses from different materials (urine, faeces or mulched plant materials) applied to soil are different over time, being faster with urine application than faeces.

In the tropics, the current use of mulched materials releases relatively low N to the soil, however, it can add more soil OM through organic carbon inputs. With global warming likely to accelerate SOM oxidation, and therefore increase the turnover and release of fertiliser N, this may pose serious environmental issues for countries in the tropics. This study provides some further understanding of how the use of oats added to a hay basal diet effects N turnover by crops and the potential role that mulch may have in addressing potential environmental issues. i.e. managing mulch in order to synchronise nutrient release with plant nutrient demand. The synchrony principle suggests that low (i.e. low N and high lignin) and high (i.e. high N and low lignin) quality mulch should be mixed to achieve synchronisation (Myers et al., 1994). Mixing urine with mulch materials may therefore be a way to reduce losses and increase N uptake in crops. The mixtures of oats and hay applied as mulch or as a fertiliser (after it had passed through the animals) in this study, did not vary in N content, however they did improve recovery of N recovery in the maize. To improve this result the addition of legume into the mulch may provide better synchronisation. Using both methods of application (incorporation and application on the soil surface) at the same time may also better synchronise N release and N uptake. Finally, these approaches used to achieve synchrony of nutrients between crop demand and fertiliser supply must also be economical and accepted by smallholder farmers.

4.6. Conclusion

The isotope dilution technique more precisely quantified the fate of ^{15}N of forage from sheep to excreta and from excreta to soil and plants compared to the difference method. In terms of the absolute amount of ^{15}N flow, the diet of 75%Oats:25%Hay brought more N into the

system with more N from this diet captured in sheep excreta and more N from the excreta being retained in the soil and crops after its surface application or incorporation into the soil. Also, by increasing the ratio of oats:hay in mulch incorporated in soil, the soil nutrient supply for plant crops was again improved. However, the mineralisation rate of the organic N from mulch or faeces was quite low and much of the added N was immobilised and unavailable for crop production in the short term. To better understand this process and the small differences in recovery between these two different forms of organic N, longer term studies and the use of grass of higher quality and N content as a feed supplement or as mulch are suggested.

In this study, the digestion, metabolism and excretion of oats by the ruminant animal improved the rate of short-term recovery of ^{15}N in a maize crop compared to the same amount of oats used as green manure. Smallholder farmers would therefore benefit if they use better quality forage to supplement their livestock and collect the excreta for incorporation into the soils they use for crop production. In this regard, improving the quality of grass supplements and introducing legume forages, rather than feeding with grass only, will support higher levels of animal and crop production.