

nn08201.4159

**Chemical characterization of manure in relation
to manure quality as a contribution to a reduced
nitrogen emission to the environment**

Bert van der Stelt

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
Prof. dr. M.J. Kropff
in het openbaar te verdedigen
op vrijdag 30 maart 2007
des namiddags te vier uur in de Aula

1037602

Van der Stelt, B.

Chemical characterization of manure in relation to manure quality as a contribution to a reduced nitrogen emission to the environment

PhD-thesis Wageningen University, the Netherlands, 2007. –With references.
–With summary in English and Dutch.

ISBN 90-8504-630-0

Propositions / Stellingen

1. Door de ontwikkeling van de DMT-mestcel is de gebruiksvriendelijkheid en de toepasbaarheid van de Donnan Membraan Techniek sterk verbeterd.
Dit proefschrift
2. De uitkomsten van ammoniakvervluchtigingsmodellen die geen rekening houden met de pH van de mest dienen kritisch te worden beoordeeld.
Dit proefschrift
3. If a butterfly beating its wings in an Amazonian rain forest can cause a tornado in Texas, than a cow's fart can cause the melting of Arctic sea ice.
Based on E.N. Lorenz' "Butterfly effect"
4. Boerenverstand is een vruchtbare bron voor wetenschappelijk onderzoek.
Winklerprins, A.M.G.A., Sandor, J.A., Local soil knowledge: insights, applications, and challenges. Geoderma 111 (2003) 165-170.
5. De gezelligheid die de omgeving van een gezelligheidsroker ervaart neemt af met het aantal gerookte sigaretten.
6. Afstand is relatief en niet een kwestie van kilometers.
Vrij naar Albert Einstein
7. Voor de wetenschappelijke diepgang en de praktische uitvoering van dit promotieonderzoek waren de bijdragen van zowel de co-promotor als de copro-motor onmisbaar.
8. "In order for the wheel to turn, for life to be lived, impurities are needed...."
Quote by Primo Levi

Stellingen behorende bij het proefschrift van B. van der Stelt: *'Chemical characterization of manure in relation to manure quality as a contribution to a reduced nitrogen emission to the environment'*, Wageningen, 30 maart 2007

Abstract

Van der Stelt, B., 2007. *Chemical characterization of manure in relation to manure quality as a contribution to a reduced nitrogen emission to the environment.*

PhD-thesis, Wageningen University, Wageningen, the Netherlands, 152 pages.

More insight in manure composition, ammonia (NH_3) volatilization, and into relationships between soil organic matter (SOM) content and nitrogen (N) flows in grasslands is needed to improve the nutrient use efficiency of manure as fertilizer for plants and concomitantly to reduce the environmental impact of agricultural activities.

Additives are often added to improve manure quality (e.g. reduce NH_3 volatilization). Laboratory studies showed that additives used in this study did in general not affect manure characteristics or NH_3 volatilization, when manure was incubated at three temperatures and either regular mixed or not mixed. Only when Agri-mest® and Effective Micro-organisms® were both added and the manure was incubated at 4 °C without mixing, NH_3 volatilization was reduced by 34%.

To study manure composition in more detail, a DMT-manure cell was developed, which either measures 'free' dissolved cation or anion concentrations in manure. Dilution studies showed that total nutrient concentrations and monovalent 'free' dissolved cation concentrations decreased proportionately with increasing dilution, whereas 'free' dissolved divalent cation concentrations were buffered upon dilution. The buffering of divalent cation concentrations is probably the result of the release of these ions from organic matter, or it is the result of the dissolution of certain phosphate minerals (e.g. struvite and whitlockite). Only a small part of the total phosphorus (P) content (<1%) and the total sulphur content (<13%) was present in a 'free' dissolved ionic form.

A feeding trial with non-lactating cows showed that the total N and P content and the total ammoniacal nitrogen ($\text{TAN} = \text{NH}_{3,\text{aq}} + \text{NH}_4^+$) content of manure increased when the dietary protein or energy content was raised, whereas the 'free'-to-total ratios of Ca and Mg in the manures were not affected by dietary changes. Ammonia volatilization from manure increased with an increase in dietary protein content and decreased with an increase in dietary energy content, although the TAN content of manures produced from diets with a higher dietary energy content were higher.

A field trial, in combination with incubation studies, was carried out in the VEL-VANLA area to study how N dynamics was affected by SOM content. Incubation studies showed that potential N mineralization rates (measured at 20 °C and 60% water holding capacity) of eighteen grassland soils were related to the initial dissolved organic nitrogen content and to the dissolved organic carbon content of the soil, as measured in 0.01M CaCl₂ extracts. During the growing season, two peaks in total soluble N content were observed, which could not be related to the time of manure application, but were both preceded by a period of draught. Maximum total soluble N and nitrate content were higher in soils with a lower SOM content. This is probably caused by faster water depletion of soils with a lower SOM content during drought periods, which will hamper N uptake by plants.

Concluding, adjusting diets seems to be a more promising way to reduce NH₃ volatilization than the use of manure additives, especially since adjusting the diet will reduce NH₃ volatilization at any time of manure handling (housing, storage, and during manure application), which is not always the case for other NH₃ volatilization reduction techniques. A proper estimation of N mineralization during the growing season can help to improve the nutrient gift (via manure) in order for plants to grow with limited risks to the environment. Dissolved organic N might be a promising parameter to estimate N mineralization.

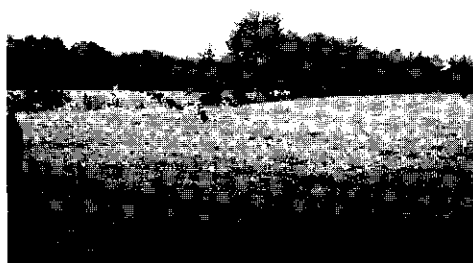
Keywords: manure composition, ammonia volatilization, free ions, Donnan Membrane Technique, manure additives, dietary changes, nitrogen dynamics, grassland soils.

Contents

1. General introduction	9
2. Volatilization of ammonia from manure as affected by manure additives, temperature and mixing	27
3. Measurement of ion speciation in animal slurries using the Donnan Membrane Technique	41
4. Effects of dietary protein and energy levels on cow manure composition and ammonia volatilization	57
5. Measurement of anions using the Donnan Membrane Technique and its application to manure slurry	75
6. Effects of organic matter content on nitrogen dynamics in grassland soils	95
References	117
Summary	131
Samenvatting	137
Dankwoord	143
Levensloop	147

1

General introduction



Agriculture is an important branch in the Netherlands. More than 10% of all economic activities are related to agriculture and agricultural activities cover about 60% of the total surface area of the Netherlands (LNV, 2006). The main sector in Dutch agriculture is dairy farming, which combines plant and livestock (milk) production. Transfer of nutrients between the plant and livestock compartment occurs through animal manure and roughage (Aarts *et al.*, 2000). Dairy farming in the Netherlands is highly intensive and productive, resulting in large quantities of forage and concentrates being imported to the farms. In contrast, relatively small amounts of the imported nutrients are exported from the farm as consumer goods (milk, meat or feed) and so large amounts of nutrients are excreted by the animals, making manure a valuable source of plant nutrients.

Application of manure at higher rates than needed for crop production or out of sync with the uptake of plants can result in a nutrient increase in the soil. When the nutrient binding capacity of the soil is exceeded, nutrients can be lost to the environment by leaching. Losses from manure to the environment can also occur by volatilization during animal housing and storage of manure, during and after application of manure slurry, or during animal grazing.

The most important nutrients related to environmental problems are nitrogen (N) and phosphorus (P). As N cycles through the farm it is transformed into different compounds, which are susceptible for losses to the atmosphere, groundwater and surface waters (Rotz *et al.*, 2005). N losses to the environment contribute to ecosystem fertilization, acidification, eutrophication and climate change. In contrast, P is less susceptible for volatilization and is primarily lost to surface waters by surface runoff, erosion of soil particles and leaching from saturated soils (Borucki Castro *et al.*, 2004), where it can lead to eutrophication problems. At the turn of the millennium, 10% of the climate change problems, 42% of the acidification problems and 80% of the eutrophication problems in the Netherlands could be related to agricultural activities (De Clercq *et al.*, 2001).

During the 1970s, the link between environmental problems and agricultural activities became evident and from that time onwards the government has played an active role in reducing the environmental impact of agricultural activities by implementing several policies and measures (Oenema, 2004).

In this chapter an overview is given of the agricultural developments in Europe, particularly in the Netherlands, during the last centuries. Next, the main policies aimed at reducing nutrient losses from agriculture to the environment are discussed. Subsequently, as N was the main focus of this thesis, the main N flows in dairy farming systems are dealt with. After that, the effects of ammonia

(NH₃) volatilization on the environment and possible reduction measures are discussed. Finally, the objectives and the outline of this thesis are described.

Historical overview

Until the Golden Age era, agriculture in the Netherlands consisted of mixed farming-systems (combined food and livestock production), which primarily worked at a local scale. Coherently with the economic expansion in the 16th and 17th century, agricultural activities specialized in crop production for the industry (e.g. hops) or in dairy farming (De Vries, 1974). During the industrial revolution agricultural production further improved, as cheap energy and transport became available. In 1870, agricultural productivity (per hectare and per head) in the Netherlands was higher than agricultural productivity in most other European countries (van Zanden, 1991).

A cheap supply of wheat and maize from The United States, which lowered the price of cereals, combined with poor harvest yields, high labor wages and high land prices caused an economic crises in European agriculture in the 1880s. Several European governments reacted to this crisis by starting financial support programs for agricultural production (e.g. subsidies and import levies), by stimulating research, education and extension and by subsidizing the reclamation of poor soils (Oenema, 2004). Because of less international competition at the livestock market and an increased demand for livestock products agricultural production shifted towards livestock farming (van Zanden, 1991).

Breakthroughs in fertilizer production, advancing knowledge of soil chemistry, and an increased use of concentrates improved agricultural productivity and land use intensity during the period 1880-1910. Until 1929 income and output levels of agricultural activities increased as technological progress compensated for agricultural prices that deteriorated since the beginning of the 19th century (Smits, 2006).

During the 1930s the economical viability of agriculture worsened rapidly, forcing the Dutch government to intervene. The Dutch government reacted by setting minimum prizes for arable products and ensuring minimum prices for livestock and horticultural goods. Furthermore, a beginning was made with redistribution of agricultural lands, in order to reach scale increases and cost abatements. Agricultural activities shifted from labor-intensive root crops to wheat

and, after 1935, to rye and oats. Mechanization increased, while the amounts of hired labor decreased.

After the second World War agricultural production and intensity strongly increased. Important factors for this increase were the implementation of the Common Agricultural Policy (CAP) of the European union and relatively low prices of inorganic fertilizers and concentrates (Van Keulen *et al.*, 2000). The CAP intended to (Oenema, 2004):

- 1) increase agricultural productivity,
- 2) create a fair standard of living for the agricultural entrepreneurs,
- 3) stabilize markets,
- 4) create stable supply chains, and
- 5) create reasonable consumer prices.

Furthermore, mechanization was stimulated and the use of milking machines and tractors were structurally introduced (Van Horne and Prins, 2002).

Between 1960 and 1980 the number of dairy farms decreased with ca. 60% (Figure 1.1a). In contrast, the total number of dairy cows increased in the same period with 45% and the milk production almost doubled. Furthermore, grassland areas decreased slightly (ca. 10%), whereas maize production increased (Figure 1.1b). Also, the use of concentrates into dairy rations increased (Figure 1.1b), as did the amount of fertilizer N applied to grasslands (Figure 1.1c).

At the end of the 1970s people became aware of the adverse effects of the intensification of agriculture on the environment. Agricultural products (*e.g.* butter, beef, and cereals) were overproduced at the cost of environmental sustainability (Oenema, 2004). To stop overproduction of milk, a quota for milk production was introduced in 1984 for all EU member states (Van Horne and Prins, 2002). The milk quota resulted in a decline in the number of cows (Figure 1.1a) and consequently led to a decrease in the amount of manure produced (Figure 1.1c). Furthermore, the use of fertilizer N decreased, while the use of concentrates leveled off (Figures 1.1b and 1.1c).

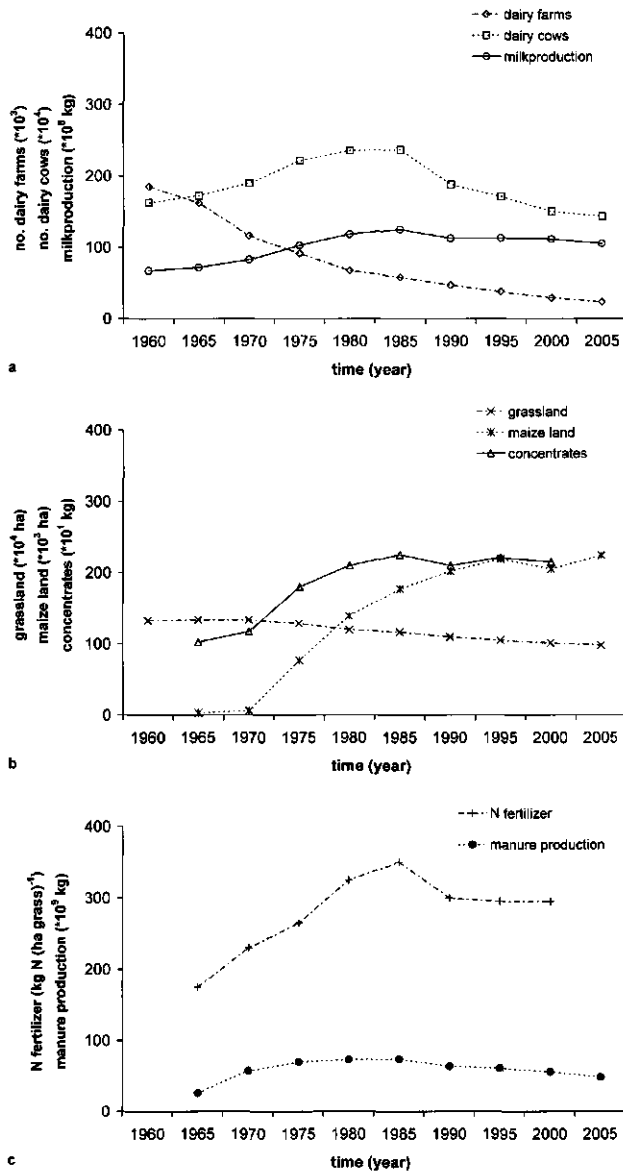


Figure 1.1. Changes in dairy farming system characteristics between 1960 and 2005. Figure 1.1a: —◇— number of farms with dairy cows; —□— number of dairy cows and —○— milk production. Figure 1.1b: —x— grassland; —*— maize land and —△— concentrates. Figure 1.1c: —+— N fertilizer and —●— manure production by cattle. Data are derived from De Clercq *et al.* (2001); Van Horne and Prins (2002); De Hoop *et al.* (2004); and CBS (2006a).

However, nutrient inputs into farming systems remained considerably higher than nutrient outputs via milk and meat. In the period 1983-1986 outputs of N, P and potassium (K) were only 14, 32, and 17% of their inputs, respectively (Van Keulen *et al.*, 2000). From the mid 1980s onwards the Dutch government started to intervene, by regulating both manure production and application (Henkens and Van Keulen, 2001). The introduced regulations have resulted in a gradual decrease in the amount manure produced (Figure 1.1c). Between 1985 and 2002 the amount of dairy manure produced decreased about 27%. Also, manure N and P content decreased between 1985 and 2002 with 29 and 34%, respectively (European Commission, 2005). Furthermore, the number of farms and the number of dairy cows declined, but milk production remained more or less constant (Figure 1.1a). This suggests a better utilization of feed N, since feed requirements per kilogram milk are lower (Aarts *et al.*, 1992).

Manure policies and measures

European policies and measures

To minimize the environmental impact of agricultural activities, several European policies and measures have been introduced, of which the most important are the Agenda 2000, the Water Framework Directive (including the Nitrate Directive) and the EU Air Quality policy.

The action program Agenda 2000 affects nutrient management by concluding agri-environmental commitments with farmers (*e.g.* regulation 1259/99) and by supporting rural development to restore and enhance competitiveness (*e.g.* regulation 1257/99; Oenema, 2004). The program especially aims at less-favored areas and environmental sensitive areas and at agricultural production methods which are designed to protect the environment and to maintain the countryside.

The Water Framework Directive provides a framework for the protection of inland surface waters, transitional waters, coastal waters and groundwater, in order that these waters reach good ecological status by 2015 (De Clercq *et al.*, 2001; Oenema, 2004). To assure that a good ecological status will be reached, the directive imposes water quality standards, emission limits and legislation measures to be taken. Within the Water Framework, the Nitrate Directive (91/676/EC) affects agriculture most, as it's objective is "to decrease water pol-

lution caused or induced by nitrates from agricultural sources and prevent further such pollution" (Oenema *et al.*, 2004). The most important characteristics of the Nitrate Directive are the designation of nitrate vulnerable zones, establishment of action and monitoring programs and a Code of Good Agricultural Practices for these zones (De Clercq *et al.*, 2001). The whole of the Netherlands has been designated as a nitrate-vulnerable zone and the legal standard for NO_3^- concentration in the upper groundwater was set at 50 mg L^{-1} .

The Air Quality Directive (1999/30/EC) aims at reducing gaseous losses (NH_3 , nitrogen oxides (NO_x), sulphur (S) and volatile organic compounds (VOC)) from energy generation, industrial sources, motor vehicles and agriculture, in order to reduce acidification, eutrophication and ground-level ozone (De Clercq *et al.*, 2001). For each individual member state, reduction targets for gaseous losses are set, which have to be reached by 2010. The N reduction targets set for the Netherlands are -43% for NH_3 and -54% for NO_x emissions, compared to their levels in 1990. Furthermore the directive contains measures to control NH_3 emissions from agricultural sources

The manure policy of the Netherlands

In 1984 the Dutch government introduced The *Temporary Act Restriction Pig and Poultry Husbandry*, which can be seen as the first manure policy measure, as it aimed at restricting, both, the use of nutrients and the volume of animal manure produced (Henkens and Van Keulen, 2001). The manure policies and measures implemented by the government since that time can be divided into three phases (Neeteson, 2000; Oenema, 2004).

In the first phase (1984-1990) expansion of livestock production was prohibited and transport of animal manure from areas high in livestock farming to areas high in arable farming was promoted (Neeteson, 2000). Characteristic for this period were the introduction of manure quotas per farm and P based limits for manure application to land (Oenema, 2004).

In the second phase (1991-1997) manure production had to be gradually decreased, which was realized by step-wise decreasing the amounts of P which were allowed to be applied to soils. Furthermore, it was no longer allowed to apply manure to soils during autumn and winter, because of high risk of NO_3^- leaching during that period. Also mandatory guidelines to reduce emission losses of N during storage, handling and application of manure were introduced.

In the third phase (1998 onwards) a transition to more balanced inputs of N and P has to be made. To realize this transition the Dutch government extended and implemented several regulations (Neeteson, 2000), including:

- a ban on spreading animal manure on agricultural land during the winter;
- the obligation to cover storage facilities for animal manure;
- compulsory low-emission methods for the application of animal manure to land;
- manure application limits based on N;
- levies which are applied when the maximum permissible annual N and P surpluses for farms are exceeded.

To record the amounts of N and P entering and leaving the farm, the 'farm-gate' based Mineral Accounting System (MINAS) was introduced. Farmers were allowed to have surpluses of N and P to certain threshold values, which were tightened periodically. Surpluses above these defined thresholds were levied. MINAS effectively decreased N and P surpluses, especially on dairy farming systems (RIVM, 2002). However, in 2003 the Dutch government was forced by the European Court to abandon MINAS and to implement application standards for nutrient losses (e.g. restricting the annual dose of animal manure applied to soils) before 2006 (Oenema, 2004). In contrast to MINAS, application standards are not based on the maximum amount of N (and P) that is allowed to leach to the environment, but are rather based on the N amount required for optimal plant growth. The application standard for N applied via animal manure, including that deposited during grazing, is set at $170 \text{ kg N (ha yr)}^{-1}$ for European countries. However, by proving that with this N amount optimal plant growth conditions were not met for the Dutch situation, because of the long growing seasons and the use of crops with high N uptake, the Netherlands were able to convince the commission of the European Communities to grant the Netherlands a derogation. This derogation allows 'farms with at least 70% grassland' under certain conditions to apply livestock manure (except from veal calves) to a total of $250 \text{ kg N (ha yr)}^{-1}$ (European Commission, 2005). These conditions include 'the establishment of fertilizer plans on a farm by farm basis, the recording of fertilizer practices through fertilizer accounts, periodic soil analysis, green cover in winter after maize, specific provisions on grass ploughing, no manure application before grass ploughing and adjustment of fertilization to take into account the contribution of leguminous crops' (European Commission, 2005). Compared to the year

2002 manure production both in terms of N and P may not increase, which has to be ensured by the government. Furthermore, the request for derogation has to be submitted each year in advance and must be in agreement with the current manure policy (European Commission, 2005).

In order to comply with the manure application standards, while maintaining or even increasing agricultural productivity, the use efficiency of manure needs to be improved. One of the factors determining the use efficiency of manure is manure quality. Insight in the composition of manure and how this composition can be manipulated can help to improve the availability of nutrients to crops and to reduce its potential for nutrient losses to the environment.

N flows in dairy farming systems

Negative effects of N surpluses on the environment were already recognized during the beginning of the twentieth century. However, *i.a.* due to the complexity of the N flows in dairy farming systems (Figure 1.2), the contribution of agriculture to these surpluses was recognized decades later (Oenema, 2004).

The most important inputs into intensively managed dairy farming systems are purchased fertilizers and feeds. About 15-25% of the N fed to lactating cows is used by the animals for maintenance, milk or meat production, while the rest is excreted, either via the feces or via the urinary tract (Aarts *et al.*, 1992). In dairy farming systems, feces and urine are usually stored together as slurry, which additionally may contain some waste water and bedding material.

The main N component in urine is urea ($\text{CO}(\text{NH}_2)_2$), which is rapidly hydrolyzed into carbonate and ammonium (NH_4^+) by the enzyme urease, which is present in feces (Bussink and Oenema, 1998). In manure slurry, NH_4^+ is in equilibrium with dissolved ammonia (NH_3). Furthermore, (dissolved) NH_3 is also in equilibrium with NH_3 present in the air-phase in immediate contact with the slurry surface. Ammonia emissions to the atmosphere can occur, after NH_3 has been transported from this surface boundary layer to the free air phase by convection and diffusion processes (Sommer and Hutchings, 2001). The most important parameters determining NH_3 emissions from manure are pH, temperature, dry matter content and the total ammonical nitrogen content ($\text{TAN} = \text{NH}_4^+ + \text{NH}_{3,\text{aq}}$) of the slurry (Jarvis and Pain, 1990; Sommer *et al.*, 1991).

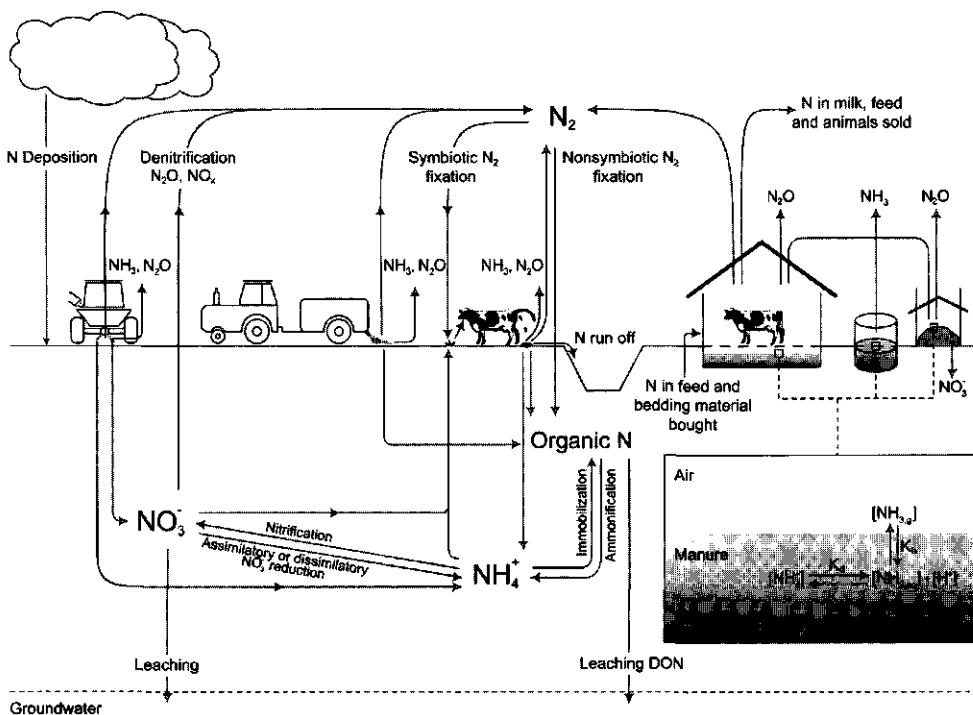


Figure 1.2. Major N flows in dairy farming systems. The box in the lower right corner is adapted from Ni (1999).

When manure is exposed to oxygen (e.g. at the slurry surface), the TAN present in the manure can be transformed into nitrous oxide (N₂O) by micro-organisms (nitrification and denitrification) (Mosier *et al.*, 1998; Oenema *et al.*, 2005). During manure application to agricultural soils up to 30% of the N applied maybe be lost via gaseous N losses to the atmosphere (Rotz, 2004). Deposition of N from the atmosphere to land can contribute to soil acidification and enrichment of sensitive habitats with N. Furthermore, atmospheric NH₃ can also react with atmospheric acids forming (secondary) particles, which can be transported over a large area (Webb *et al.*, 2005). Another part of the N applied to the soil via manure application maybe be lost by run off from the field after rainfall, or by (wind) erosion.

Approximately half of the N present in dairy manure slurries produced in the Netherlands is present as mineral N (Mooij, 1996), which is readily available for plant uptake, after slurry application to soils.

Dissolved organic nitrogen (DON), like intact amino acids, can directly be taken up by plants, although its role in N uptake is probably of minor importance (Jones *et al.*, 2005). Most of the organic N has to be transformed into an inorganic form of N, before plant uptake can occur. After application of manure slurry to the soil, organic N is fragmented by soil faunal communities. The small organic parts obtained are subsequently mineralized by a series of microbial processes, e.g. ammonification and nitrification (Table 1.1).

During ammonification, organic N is transformed into NH_4^+ , with NH_3 as an intermediate. This transformation is regulated by protease and deaminase enzymes, which are produced by heterotrophic micro-organisms (Witehead, 1995). When NH_4^+ is present in excess, it can be nitrified to NO_3^- , or it can be assimilated by micro-organisms (immobilization) to organic N, thereby reducing its availability for plants.

Nitrification of NH_4^+ consists of two steps (Table 1.1). In the first step, nitrifying bacteria belonging to the *Nitrosomonas* genus will oxidize NH_4^+ to nitrite (NO_2^-). In the second step, NO_2^- is oxidized to NO_3^- by bacteria belonging to the *Nitrobacter* genus. Since NO_3^- is very mobile in the soil solution, it is susceptible for leaching. In contrast, NH_4^+ is less susceptible for leaching because it can be adsorbed to either the surface of soil organic matter (Chung and Zasoski, 1994), or to clay particles. Ammonium can also be present in minerals. Griffin *et al.* (2005) found that not only the extent of nitrification of manure NH_4^+ was affected by manure composition, but also the rate of nitrification of rapid and more stable phases during aerobic incubation in the soil.

Nitrate can be taken up by micro-organisms and plants through assimilatory nitrate reduction, when energy is not a limiting factor. In this process, NO_3^- is transformed into NH_4^+ , which is subsequently incorporated into amino acids (Myrold, 1998). Furthermore, NO_3^- can be reduced by dissimilatory processes, of which denitrification is the most important process occurring in soils.

Table 1.1. Microbial processes involved in the transformation of organic N into NO_3^- and N_2 (Myrold, 1998; Amlinger *et al.*, 2003).

Process	Reaction	Micro-organisms
Ammonification	$(\text{humus})\text{-R-NH}_2 + \text{H}_2\text{O} \rightarrow \text{NH}_3 + \text{R-OH}$	Heterotrophic micro organisms
	$\text{NH}_3 + \text{H}_2\text{O} \rightarrow \text{NH}_4^+ + \text{OH}^-$	Heterotrophic micro organisms
Nitrification	$2 \text{NH}_4^+ + 3 \text{O}_2 \rightarrow 2 \text{NO}_2^- + 2 \text{H}_2\text{O} + 4 \text{H}^+$	<i>Nitrosomonas</i> genus
	$2 \text{NO}_2^- + \text{O}_2 \rightarrow 2 \text{NO}_3^-$	<i>Nitrobacter</i> genus
Denitrification	$2 \text{NO}_3^- + 10 \text{e}^- + 12 \text{H}^+ \rightarrow \text{N}_2 + \text{O}$	<i>Pseudomonas</i> and <i>Bacillus</i>

During denitrification, NO_3^- is reduced under anaerobic conditions to N_2 by bacteria primarily belonging to the genera *Pseudomonas* and *Bacillus* (Myrold, 1998). Also N_2O and NO_x can be produced during (nitrification and) denitrification, which can be lost to the air, where they contribute to global warming and tropospheric ozone production (Velthof *et al.*, 2003; Wrage *et al.*, 2004).

NH_3 volatilization from dairy farming systems

About 80-90% of the NH_3 emitted to the atmosphere in Western Europe can be traced back to agriculture, with livestock production, especially dairy farming, as the major source (Bussink and Oenema, 1998). As mentioned previously, NH_3 emissions can lead to acidification after it has been transformed into nitric acid and it can give rise to eutrophication problems. Moreover, NH_3 can react with atmospheric acids, forming particulates (Webb *et al.*, 2005).

The largest NH_3 emissions at dairy farms occur during housing, storage, and application of manure to grassland or arable land and from urine patches on grazed pastures (Smits *et al.*, 2003). To reduce N emissions from the farm changes in feeding strategy, as well as changes in management, are required.

Adjusting feeding regimes to reduce the amount of N excreted by dairy cows seems an efficient way to reduce NH_3 emissions, since it will reduce volatilization at any time when manure is handled. For instance, balancing protein gifts with protein needs of the cow, can reduce NH_3 emissions, because excessive protein N is primarily excreted (as urea) in the urine, where it can readily be transformed into NH_3 , as mentioned above.

Another way to reduce NH_3 emissions is by increasing the productivity of cows. An increase in productivity improves the N use efficiency of the cow, since more N is used for milk and meat production per unit of feed N (Rotz, 2004). Thus, the same production is obtained with a lower N consumption and excretion.

Since the 1980s several measures to reduce NH_3 emissions from dairy farming systems have been introduced in the Netherlands. Differences in N emissions from common housing systems are considerable, varying from (on average) 6% of total N fed in case of a tie housing system to about 50% in case of a feedlot system (Table 1.2; Rotz, 2004). Free stall housing is the most common housing system used in dairy farming and has an average N loss of 16%, which is almost entirely emitted as NH_3 . The free stall system consists of a ma-

nure pit underneath a solid or a slatted floor. Ammonia volatilization from solid floors can be reduced by separating fecal and urinary excretions by means of a small slope of the floor.

To prevent potential losses of nutrients to the environment farmers are not allowed to apply slurry to soils during fall and winter. Since livestock production in that period continues, sufficient storage facility is needed.

Table 1.2. N losses from cattle manure slurry (dry matter content= 10%) from housing facilities, during storage and during manure application to soils^a.

Manure type	Typical loss [% total N]	Range [% total N]	N form lost ^b
Housing			
Tie stall	8	2-35	NH ₃ , N ₂ O, N ₂
Free stall	16	10-20	NH ₃
Bedded pack	35	25-40	NH ₃ , N ₂ O, N ₂
Feedlot	50	40-90	NH ₃ , NO ₃ ⁻ , N ₂ O, N ₂
Storage^c			
Solid heap	20	10-40	NH ₃ , NO ₃ ⁻ , N ₂ O
Solid compost	40	20-50	NH ₃ , NO ₃ ⁻ , N ₂ O
Tank, top loaded	30	20-35	NH ₃
Tank, bottom loaded	8	5-10	NH ₃
Tank, enclosed	4	2-8	NH ₃
Anaerobic lagoon	70	50-99	NH ₃ , N ₂ , N ₂ O
Manure application^d			
Irrigated slurry	30	25-50	NH ₃ , NO ₃ ⁻ , N ₂ O
Broadcast slurry on grassland	25	15-40	NH ₃ , NO ₃ ⁻ , N ₂ O
Broadcast slurry on bare soil	20	10-27	NH ₃ , NO ₃ ⁻ , N ₂ O
Broadcast of solids	20	8-60	NH ₃ , NO ₃ ⁻ , N ₂ O
Band spreading / trailing hose	18	13-26	NH ₃ , NO ₃ ⁻ , N ₂ O
Incorporated within 6 hours	10	6-13	NH ₃ , NO ₃ ⁻ , N ₂ O
Shallow injection of slurry	8	7-12	NH ₃ , NO ₃ ⁻ , N ₂ O
Deep injection of slurry	2	1-5	NH ₃ , NO ₃ ⁻ , N ₂ O
Grazing feces and urine	10	4-20	NH ₃ , NO ₃ ⁻ , N ₂ O

^aAdapted from Rotz (2004).

^bN forms are listed in order of the expected quantity lost, with most of the loss being in the form of NH₃.

^cN losses expressed as a percentage of total N entering storage.

^dNH₃ losses expressed as a percentage of the initial total N applied.

The common manure storing system used at dairy farms is the tank-system, in which fecal and urinary excretions, with or without cleaning water spilled during farming practices, are stored together. The dry matter (DM) content of this so called manure slurry is between 7 and 15% and the slurry is stored under anaerobic conditions. Consequently, N is primarily lost by NH_3 volatilization, and the amount of N lost is mainly determined by the amount of mixing during storage (Rotz, 2004).

When manure is stored as solids (DM > 15%) aerobic decomposition or composting can occur, which can lead to large N losses. On average, N losses from solid heaps and solid compost are respectively 20 and 40% of the total N which enters the storage. Important parameters involved in N loss from stacks are the manure DM content, the Carbon-to-Nitrogen ratio, and the amount of aeration that the manure stack receives (Rotz, 2004).

An effective way to reduce NH_3 losses during storage of manure slurry is by covering the manure storage facility, i.e. a lid will reduce NH_3 emissions with more than 80% (Sommer *et al.*, 1993). In the Netherlands farmers are required by law to cover manure storage facilities (Neeteson, 2000).

About 35 to 50% of all NH_3 emissions to the atmosphere by livestock production can be attributed to land spreading of manure (Webb *et al.*, 2005). Less than 1% of the applied NH_4 is volatilized as NH_3 during the actual manure spreading itself (Sommer and Hutchings, 1995). However, one day after surface-application of slurry, more than 50% of the maximum amount NH_3 which can be lost, is emitted. (Stevens and Laughlin, 1997).

Besides slurry characteristics, NH_3 volatilization during and after land spreading of manure is affected by weather (air temperature, wind speed, precipitation), soil conditions (moisture content, texture, cation exchange capacity, pH, and plant or residue cover), and by the application method (Rotz, 2004).

Several manure spreading techniques have been developed to reduce NH_3 emissions, that are cost-effective, practical and widely applicable (Table 1.2). When manure is applied to soils by either broadcasting or irrigation techniques more than 25% of the total N applied can be lost (Table 1.2). Applying manure slurry in small bands, using trailing hoses, reduces NH_3 volatilization due to a reduced slurry area exposed to the air (Thompson *et al.*, 1990). Circa 30% less NH_3 is emitted when manure is applied via trailing hoses instead of broadcasting (Webb *et al.*, 2005). Incorporation of manure into the soil strongly reduces NH_3 volatilization, although the reducing effect of incorporation rapidly decreases when the time between manure application and incorporation increases (Webb *et*

al., 2005). Injection of manure slurry into the soil results in the lowest (relative) NH_3 losses to the atmosphere (2-8% of the total N applied), and NH_3 losses decrease with increasing injection depth. However deep injection (>5 cm) in grasslands or on arable fields can cause root damage, which may reduce crop growth (Rotz, 2004).

Other measures, like slurry dilution, mechanical separation of slurry, applying water after spreading and optimized timing of manure application can reduce NH_3 emissions. However, the effectiveness of these measures are strongly affected by climatic conditions and management, or the measures are still in development. Some techniques which can reduce NH_3 emissions like acidifying additives or slurry separation are considered to be unrealistic or unproven under practical farming conditions (Webb *et al.*, 2005).

A fraction of the N applied as artificial fertilizers to agricultural soils can be lost to the atmosphere by NH_3 volatilization. In general, it is considered that NH_3 emissions from fertilizers are low (<1%), except for urea based fertilizers and ammoniumsulphate (AS) and diammoniumphosphate (DAP) applied to calcareous (alkaline) soils (Harrison and Webb, 2001).

Also, considerable NH_3 losses can occur during grazing of animals. In the field, feces and urine are normally excreted separately, at different places. N volatilization from fecal patches is low, *e.g.* ca. 5% of the N in feces (Rotz, 2004). In contrast, N loss from urinary patches is normally much higher, since urea in urine is easily hydrolyzed into NH_3 . Depending on weather and soil conditions, N losses from urine can vary between 5 and 66% of the total urinary N content (Rotz, 2004).

Reducing NH_3 emissions by implementing N conserving methods and techniques will improve the nutrient value of manure slurry, but can also lead to higher nutrient losses to other environmental compartments (*e.g.* leaching and denitrification losses).

This thesis

Main objectives

Proper nutrient management in dairy farming systems will supply crops with sufficient nutrients, while nutrient losses to the environment will be limited. For proper nutrient management, each component of the chain: crop-cow-manure-soil has to be matched. Therefore, sound knowledge of the underlying mechanisms is required. In this thesis I have focused on the manure and soil compartment.

The main objectives of the thesis were to gain more insight into manure composition, NH_3 volatilization and to gain more insight into the relationship between organic matter content and N flows in grasslands. A technique was developed to study 'free' nutrient concentrations in manure slurries. Furthermore, it is described how manure composition and NH_3 emissions were affected by feeding strategy and by manure additives. This research has been executed in laboratory experiments, as well as in a field trial, which was carried out within the VEL-VANLA area.

VEL-VANLA

The environmental co-operatives 'Vereniging Eastermar's Lânsdouwe' (VEL) and 'Vereniging Agrarisch Natuur- en Landschapsbeheer Achtkarspelen' (VANLA), located in the northern Frisian Woodlands (the Netherlands), were founded in 1992. The co-operatives aim at integrating environment, nature and landscape, and productivity objectives into their dairy farming systems.

Characteristic for the area is the dense land use pattern of hedges and belts of alder trees, resulting in small-scale and closed landscapes on the higher sandy soils alternated by relatively open areas on the lower peat-clay soils (Figure 1.3; Stuiver *et al.*, 2003). Dairy farming is the most important land use type in this area.

In 1998, a mineral project was launched to explore possibilities to invigorate region-specific farming. The main objective of the project was to increase N use efficiency at the farm level, in order to reduce N emissions to the environment cost-effectively (Wiskerke *et al.*, 2003). A farm system approach was selected (Figure 1.4), which especially focused on the N efficiency of the soil. Main features of the VEL-VANLA approach are I) reducing the amount of artificial fertilizers applied to the soil and II) improving feeding strategy. Lowering protein

content and increasing fiber content of the feed will reduce the amount of N excreted by the animal and will lead to an increase in the organic N fraction (e.g. more N is excreted via the feces), which will lower the susceptibility of the manure for NH_3 volatilization and leaching.

Farmers participating in the project have reduced 'their' N losses from an average surplus of 270 kg N ha^{-1} in 1997 to 172 kg N ha^{-1} , while the average surplus on Dutch dairy farms was approximately 200 kg N ha^{-1} . More than 90% of the farmers involved in the project complied in 2002 with MINAS norms of 2003 (Wiskerke *et al.*, 2003).



Figure 1.3. Characteristic scenery of the VEL-VANLA area. Picture was obtained from Google Earth.

Outline

This thesis presents the main result of the research into manure composition and nitrogen flows in dairy farming systems.

In Chapter 2 it is described how NH_3 volatilization from manure is affected by manure additives, temperature and mixing. After applying the additives to the manure slurries, the slurries were incubated at 3 temperatures (e.g. 4, 20 and 35°C) and half of the samples were regularly mixed.

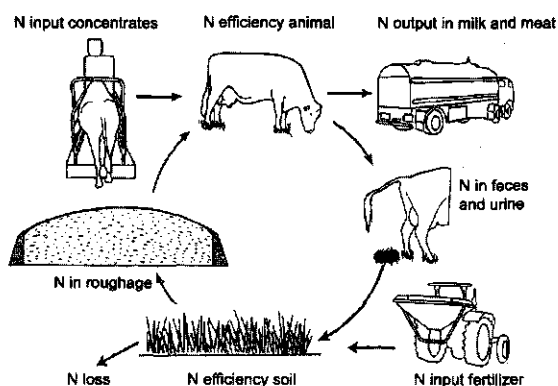


Figure 1.4. The VEL-VANLA strategy as derived from Stuiver *et al.* (2003).

Chapter 3 describes how the Donnan Membrane Technique (DMT) was made applicable to measure 'free' dissolved NH_4^+ and other cation concentrations in manure slurry. The developed DMT-manure cell was further used to study the buffering of 'free' cation concentrations after dilution of manure slurry with distilled water.

Chapter 4 focuses on the relationships between dietary characteristics and manure composition. The slurries studied were obtained from a feeding trial, in which the feeding regimes mainly varied in protein and energy content. Additionally, the slurries were subjected to a NH_3 volatilization experiment.

In Chapter 5 the application of the DMT-manure cell to determine 'free' dissolved anion concentrations and its application to manure slurry are described. First, the DMT-cell was tested in synthetic solutions, after which the cell was used to determine 'free' anion concentrations in three dairy manure slurries.

Chapter 6 deals with the major N flows in grasslands during a growing season. For this experiment, eighteen fields were selected on nine dairy farms within the VEL-VANLA area, covering a broad range of soil organic matter content. Besides field measurements, incubation and modeling studies were performed.

Volatilization of ammonia from manure as affected by manure additives, temperature and mixing



B. van der Stelt, E.J.M. Temminghoff, P.C.J. van Vliet,
and W.H. van Riemsdijk
Department of Soil Quality, Wageningen University, the Netherlands

Bioresource Technology 2007, in press.

Abstract

Ammonia (NH_3) volatilization decreases the N-nutrient value of livestock manure slurries and can lead to soil acidification and eutrophication problems. In this study the effect of three manure additives (Euro Mest-mix® (Mx), Effective Micro-organisms® (EM), and Agri-mest® (Am)) on NH_3 volatilization at three temperatures (4, 20, and 35 °C) was investigated. The manufacturers claim that Mx contains absorbing clay minerals and that applying Am and EM to slurry will reduce nitrogen losses, most likely by enhancing the biodegradation of manure slurry. Furthermore, the effect of mixing slurry on NH_3 volatilization has been investigated. Ammonia volatilization increased with increasing temperature and mixing of the slurries. However, at 35 °C mixing of manure slurry reduced NH_3 emissions compared to non-mixing, which is related to a reduced crust resistance to gaseous transport at higher temperatures for non-mixing. Moreover, mixing introduces oxygen into the anaerobic slurry environment which will slow down microbial activity. The use of additives did not change manure characteristics (pH, dry matter, N_{total} , $\text{N}_{\text{mineral}}$, C/N, and $\text{C/N}_{\text{organic}}$) and did not result in a significant ($p < 0.05$) decrease in NH_3 emissions, except that at 4 °C and no mixing a significant decrease of 34% in NH_3 volatilization was observed, when Am and EM together, were applied to slurry.

Introduction

Most ammonia (NH_3) losses to the atmosphere are related to NH_3 volatilization from livestock manure. In areas with high livestock-intensities more than 70% of the NH_3 emissions can be attributed to farming practices (Sommer and Hutchings, 2001). Apart from a decrease in nutrient value of manure slurry, excessive loss of NH_3 can lead to soil acidification and eutrophication problems. In the atmosphere, NH_3 can react with nitrate and sulphate to form particulates, which can contribute to (regional) acidic depositions (Sommer and Hutchings, 2001). In recent years, several techniques have been developed to reduce NH_3 emissions during livestock housing (e.g. automated scrapers (Phillips *et al.*, 1999)), during storage (e.g. surface covers (Webb *et al.*, 2005)) and during application of slurry to the soil (e.g. trail-hoses or slit-injection (Huijsmans *et al.*, 2001)). In the Netherlands farmers are obliged by law to apply manure to the soil using reduced-emission techniques (Webb *et al.*, 2005). Changing manure composition can also be an effective way to reduce NH_3 emissions (Monteny *et al.*, 2002; Børsting *et al.*, 2003). Main manure characteristics that determine NH_3 volatilization are the total concentration of ammoniacal nitrogen ($\text{TAN} = \text{NH}_4^+ + \text{NH}_3$), pH and dry matter content (Jarvis and Pain, 1990; Sommer and Husted, 1995). Reducing the dry matter content of slurry by diluting slurry with water results in lower NH_3 emissions, due to a lower TAN content and a decrease in pH (Sommer and Hutchings, 2001; Van der Stelt *et al.*, 2005). Changing the diet of the animal can affect manure quality. Külling *et al.* (2001) reported a 68% decrease in NH_3 volatilization from manure slurry (from lactating cows) when the dietary crude protein content decreased 30%. Comparable decreases in NH_3 volatilization with a decrease in the crude protein content of the feed were found by Paul *et al.* (1998) and James *et al.* (1999).

A range of chemical and biological amendments are known to reduce NH_3 volatilization from manure. They can be divided, according to their modes of action, into five groups (McCrory and Hobbs, 2001): (i) Digestive additives are amendments which enhance the biodegradation of manure and consists of microbial strains and/or enzymes. (ii) Acidifying additives lower the pH of manure, which will lead to a shift in the $\text{NH}_3/\text{NH}_4^+$ equilibrium towards a higher NH_4^+ concentration, which concomitantly will lead to reduced NH_3 emissions (Dewes, 1996). (iii) Adsorbing additives, like zeolite (e.g. clinoptilolite) or peat, are involved in the binding of NH_3 , NH_4^+ , or both (Lefcourt and Meisinger, 2001; McCrory and Hobbs, 2001). Other adsorbents bind toxic substances in manure

and thereby improve the conditions for micro-organisms to decompose manure slurry. (iv) Urease inhibitors prevent the breakdown of urea, which is a major source of $\text{NH}_4^+/\text{NH}_{3,\text{aq}}$ in manure (Sommer and Husted, 1995). However, the use of urease inhibitors is momentarily not profitable. (v) Saponins, which are glycosides derived from the yucca plant, are supposed to bind or to convert NH_4^+ .

A number of farmers already use additives like Effective Micro-organisms® (EM), Euro Mest-mix® (Mx), and Agri-mest® (Am). However, little is known about the effectiveness of these additives on NH_3 volatilization from manure. Based on the classification approach of McCrory and Hobbs (2001) EM can be classified as a digestive additive, whereas Mx shows properties of both digestive and adsorbent additives. Am can not be classified into one of the groups as mentioned above, but acts as a kind of catalyst, which enhances the anaerobic fermentation of manure by micro-organisms.

The objective of this research was to study the effects of the additives EM, Mx, and Am on NH_3 volatilization from manure at different temperatures (4, 20, and 35 °C). Also the effect of manure mixing on NH_3 volatilization was studied. Ammonia volatilization is the result of chemical, physical, and (micro)biological processes occurring in manure. All processes involved are temperature dependent. Our hypothesis was that NH_3 volatilization would increase with temperature increase and that the amendments would have more pronounced effects, if any, at higher temperatures. Mixing affects NH_3 volatilization by destroying surface crusts, which can act as diffusion and capillary barriers of gases (Sommer *et al.*, 2003), and by introducing oxygen into the anaerobic slurry environment. We hypothesized that mixing would lead to an increase in NH_3 volatilization.

Methods

Manure additives

Agri-mest® (Am), a protected trademark, consists of two components: Agri-mest_{mineral}® and Agri-mest_{liquid}®. Am is claimed to increase the amount of energy available for anaerobic fermentation of manure by micro-organisms (www.agriton.nl). Effective Micro-organisms® (EM), used in slurry treatments, mainly consists of lactic acid bacteria and yeasts, and smaller numbers of photosynthetic bacteria, actinomycetes, and other types of organisms. EM is claimed to increase the microbial diversity and/or activity in slurry and therefore en-

hances its fermentative decomposition (www.agriton.nl). A combination of both Am and EM is claimed by the suppliers to give the best results on manure quality and is claimed to restrict nitrogen losses, most likely by binding of NH_3 .

According to the manufacturer, Euro Mest-mix® (Mx) mainly consists of a pH buffer and clay minerals (e.g. bentonite). Furthermore, Mx contains cultures of some micro-organisms (not publicly known which) and some unknown supplements to increase the activities of micro-organisms. Mx is claimed to bind toxic substances in slurry and to improve the conditions for micro-organisms to grow. The added bacteria would strengthen this process.

Slurry preparation

Slurry (feces + urine + wastewater) was collected at a dairy farm, located in the "Noordelijke Friese Wouden" (the Netherlands), during the summer period of 2002. EM was obtained as an inactive product, which had to be activated before it could be used. Activating EM was done by mixing 0.75 L EM with 0.75 L sugar solution (molasses) and 15 L demineralized water, after which the mixture was fermented for one week at 22 °C.

The slurry was carefully mixed and divided into five batches (10 kg each; Table 2.1). Two batches received 1 mL of Am-solution (0.01‰). The Am-solution was made by mixing 4 g Agri-mest_{mineral}®, with 2 mL Agri-mest_{liquid}® and 18 mL demineralized water (± 30 °C). The other three batches received 1 mL of demineralized water. According to manufacturers' instructions Am has to be applied to slurry one week before using the slurry. Therefore all batches were incubated at 4 °C for one week. Subsequently, one batch to which Am had been added and one batch to which no Am had been added received 2 mL of the activated EM, the other three batches received 2 mL demineralized water. To one of these batches 20 g of Mx was added. The quantities of the additives applied to the batches were in line with manufactures instructions.

To prevent coagulation of the additives, the amendments were first thoroughly mixed with a small quantity of the slurry and then transferred to the rest of the slurry and again thoroughly mixed.

Table 2.1. Overview of the type and quantity of the additives applied to manure slurry (10 kg each)

Treatment ^a	Euro Mest-mix [g]	Effective Micro-organisms ^b [mL]	Agri-mest ^c [mL]	Demineralized H ₂ O [mL]	
				T = -7 days	T = 0 day
Control	-	-	-	1	2
Mx	20	-	-	1	2
EM	-	2	-	1	-
Am	-	-	1	-	2
Am + EM	-	2	1	-	-

^aManure slurry with or without (=control) additives. Additives: Mx= Euro Mest-mix[®]; EM= Effective Micro-organisms[®]; and Am= Agri-mest[®].

^bEffective Micro-organisms (EM) were first activated by mixing 0.75 L EM with 0.75 L molasses and 15 L demineralized H₂O and incubating the mixture for one week at 22 °C.

^cAgri-mest consisted of a mixture of 4 g Agri-mest_{mineral}[®], 2 mL Agri-mest_{liquid}[®] and 18 mL demineralized water.

NH₃ volatilization experiment

The effect of slurry treatment on NH₃ volatilization was studied using the incubation method described by Velthof *et al.* (2005). In this method transport of NH₃ by air movement was excluded and so NH₃ volatilization occurred in a passive way. Before the start of the incubations the different slurry batches were analyzed for dry matter content, pH, N_{mineral}, N_{organic}, C/N_{total}, and C/N_{organic} (Van Vliet *et al.*, 2005). Per treatment, 18 jars (wide neck bottles; 1 L) were filled with 0.5 ± 0.01 kg of homogenized slurry. A small flask, containing 12.5 mL 3.2 M H₂SO₄, was placed in the manure, using a small ring of synthetic material (Etha-foam, Recticel) for floating (Figure 2.1). The jars were closed with screw tops, divided at random into three groups (6 jars per treatment) and placed at different temperatures (4, 20, and 35 °C). In contact with the H₂SO₄ solution, volatilized NH₃ will be converted into ammonium, which will be trapped inside the acid solution (Velthof *et al.*, 2005). At different times (1, 2, 4, 8, 16, 24, 32, 64, 128, and 223 days after the experiment had started) was the acid solution replaced by new acid solution. Before analysis, the acid solution was diluted with ultra pure water (UPW) to a total volume of 50 mL. The total nitrogen content of the solution was determined by segmented flow analysis (SFA).

The effect of mixing of slurry on NH₃ volatilization was investigated by mixing a part of the slurry samples (three per treatment per day) every even day for the first 32 days of the experiment. During mixing, the acid-containing flask was removed from the jar and closed with a lid. The same procedure, without

mixing, was carried out for the jars, in which crust formation was not disturbed. After 32 days the jars were mixed only once a week.

Statistical analysis

The effects of the different treatments on NH_3 volatilization were tested for significance using the statistical program SPSS (version 12.0; SPSS Inc., 2003). All data were base-10 log transformed to comply with the "equal variances" assumption. A repeated measurement analysis was performed, with fixed factors treatment, temperature, and mixing. Measurement time was selected as the dependent variable.

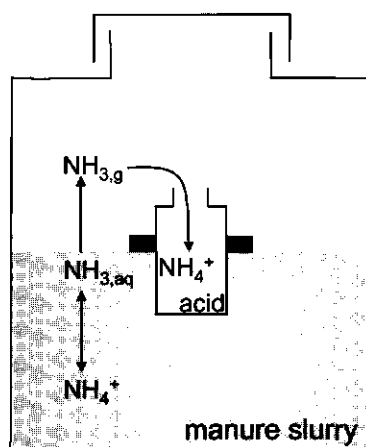


Figure 2.1. Experimental design of the NH_3 volatilization study

Results and discussion

At the time the slurry was collected the cows were only housed indoors during the night. As a consequence, the slurry contained a relatively large amount of wastewater, which led to a dry matter content of the slurry of $6.3 \pm 0.1\%$. Some characteristics of the slurry at the start of the experiment are given in Table 2.2. No differences ($p < 0.05$) between slurry treatments were observed at the beginning (Van Vliet *et al.*, 2005). All measured slurry characteristics were within normal ranges found for slurries produced by dairy cows in the Netherlands (Mooij, 1996). Approximately 45% of the total nitrogen content of

the slurry was present as mineral nitrogen. The cumulative NH_3 volatilization from the different slurry treatments, at different temperatures and either regularly mixed or not mixed, are given in Figure 2.2. Temperature, as well as mixing, affected NH_3 volatilization. Ammonia emissions increased with an increase in temperature ($p < 0.05$), which is in agreement with findings reported by Dewes (1999). An increase in temperature will result in an increase in the $\text{NH}_3/\text{NH}_4^+$ ratio, a decrease in the solubility of NH_3 in the liquid phase of slurry, and will increase the activities of micro-organisms present in slurry.

Table 2.2. Some characteristics of the slurry before the start of the experiment (Van Vliet *et al.*, 2005).

Parameter	Mean ^a	Std ^b
Dry matter [%]	6.3	0.1
pH	7.00	0.05
N_{mineral}^c [g N kg ⁻¹ DM]	18.2	2.5
N_{organic}^d [g N kg ⁻¹ DM]	22.1	1.4
N_{total} [g N kg ⁻¹ DM]	40.3	2.3
%C	36.0	1.4
C/ N_{total}	9.4	0.8
C/ N_{organic}	16.0	1.0

^aMean of three measurements; ^bStandard deviation;

^c $\text{NH}_{3,\text{aq}} + \text{NH}_4^+ + \text{NO}_3^-$; ^dCalculated ($N_{\text{organic}} = N_{\text{total}} - N_{\text{mineral}}$)

Changing the temperature from 4 to 20 °C resulted in a 3.6-5.8 times increase in NH_3 volatilization when no mixing occurred and a 2.6-2.9 times increase when the slurry treatments were regularly mixed. Increasing the incubation temperature to 35 °C resulted in an additional increase in NH_3 emissions by a factor of 5.5-6.0 in case of no mixing and a factor of 3.0-3.5 in case of mixing. Ammonia volatilization was highest during the initial phase (first eight days) of the incubation, when the concentration of easily degradable organic substrates was the highest. Only for the incubation at 35 °C and no mixing the NH_3 volatilization remained more or less constant for more than 200 days. For all process conditions the rate constants were calculated according to the Arrhenius equation (McQuarrie and Simon, 1997):

$$k = Ae^{-E_a/RT} \quad (2.1)$$

where k is the rate constant of the reaction, A is the pre-exponential factor, E_a is the activation energy, and R and T are the molar gas constant and the temperature, respectively. No linear relationships ($p < 0.05$) were observed between the

(log transformed) rate constants of the NH_3 emissions and the (inverse) temperature (Figure 2.3), although the R squared of the regression lines were high, except when slurry enriched with Am and EM was mixed. This suggests that besides chemical and physical processes also (micro)biological processes play an important role in NH_3 volatilization.

Not much is known about micro-organisms present in cattle slurry. According to Zhu (2000) NH_3 is produced during the biodegradation of swine slurry (e.g. decarboxylation of volatile amines), in which anaerobic bacteria, which belong to the genera *Streptococcus*, *Peptostreptococcus*, and *Bacteroides* are involved.

There was no consistent effect of mixing on NH_3 volatilization observed. At 4 and 20 °C NH_3 volatilization was less ($p < 0.05$) in case of no mixing. However, at 35 °C NH_3 volatilization was higher (37%; $p < 0.05$) when no mixing of the slurries occurred (Figure 2.2e-f). These contradicting results show that NH_3 volatilization is affected by mixing in more than one way. We hypothesize that mixing destroys the surface crust and thereby causes the release of entrapped NH_3 to the atmosphere. In contrast, mixing introduces oxygen into the anaerobic slurry environment, thereby limiting the deamination of amino acids by anaerobic bacteria and thus reducing the amount of NH_3 produced (Zhu, 2000). The net result of both processes determines whether mixing increases or decreases NH_3 volatilization.

The reduction in NH_3 volatilization at low temperature (e.g. 4 °C), caused by disturbing the microbial communities by mixing, will be small compared to the release of entrapped NH_3 and so mixing will result in a net increase in NH_3 emissions. At higher temperature the contribution of microbial activity to NH_3 volatilization is much higher (e.g. 35 °C treatments are near the optimal growth temperature of bacteria (≈ 37 °C)) and so NH_3 volatilization will be much more affected by exposure to oxygen. Moreover, the reducing effect of surface crusts on NH_3 emissions decreases at higher temperatures, because crust porosity increases with temperature increase (Husted, 1994; Sommer *et al.*, 2000). Mixing at 35 °C temporarily destroys the pore structure of the crust and therefore it is reasonable to assume that the overpressure of NH_3 in the slurry will be lower, which will result in an effectively less porous crust and thus will lead to a net decrease in NH_3 emissions compared to the slurry samples in which the surface crust stayed intact (no mixing).

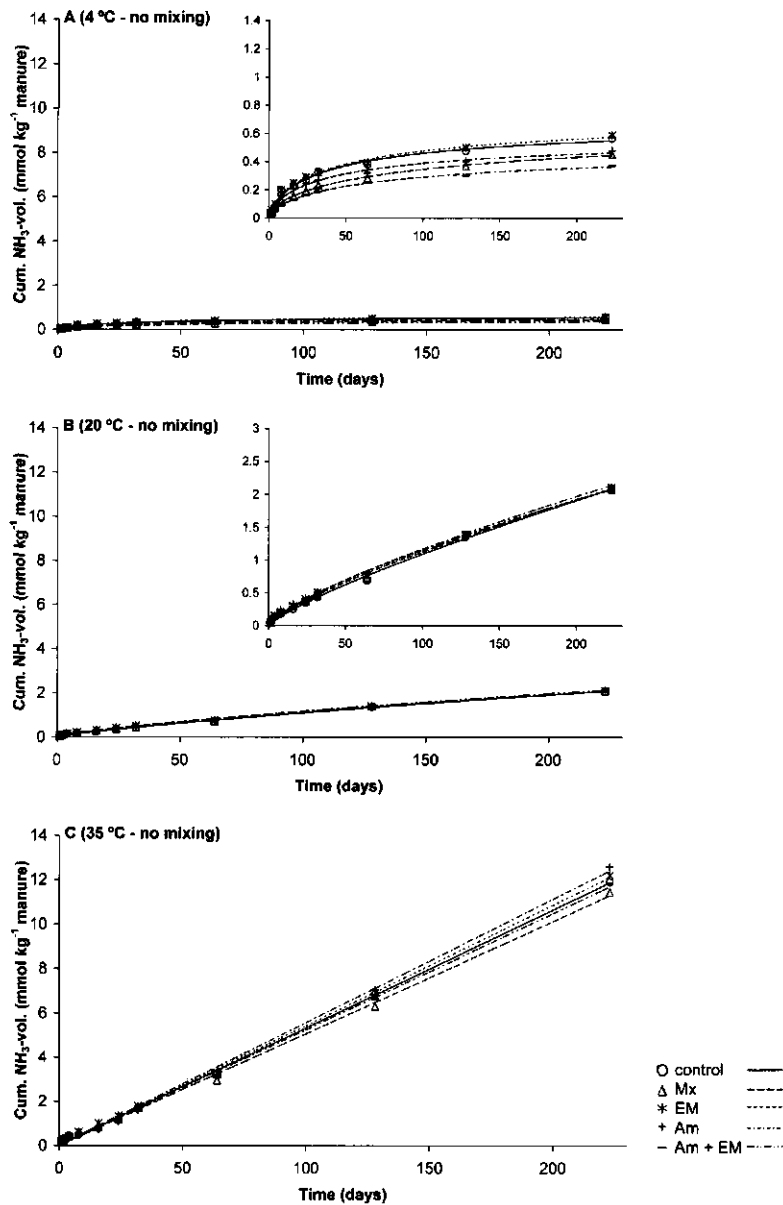


Figure 2.2. Cumulative NH_3 volatilization of the different manure slurry treatments at a) 4 °C and no mixing; b) 20 °C and no mixing; c) 35 °C and no mixing. Additives: Euro Mest-mix® (Mx); Effective Micro-organisms® (EM); and Agri-mest® (Am). Figure is continued on the next page.

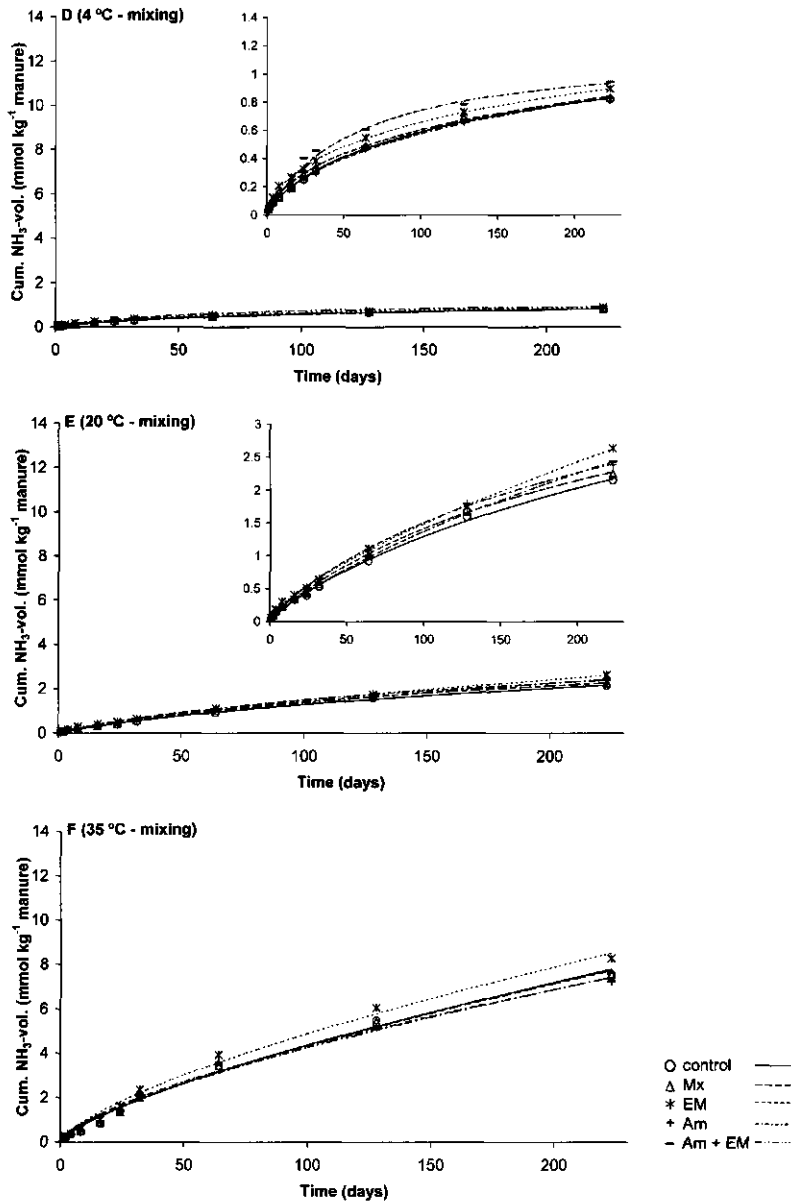
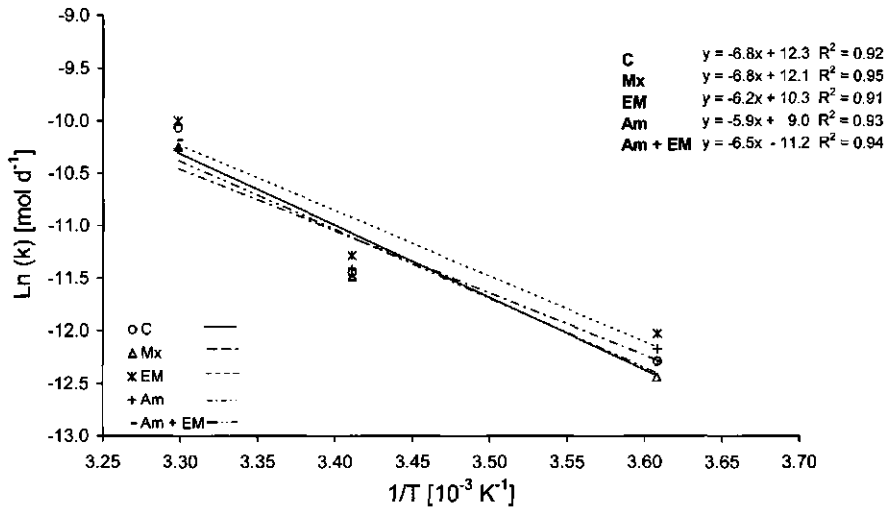


Figure 2.2 (continued from previous page). Cumulative NH_3 volatilization of the different manure slurry treatments at d) 4 °C and mixing; e) 20 °C and mixing; and f) 35 °C and mixing. Additives: Euro Mest-mix[®] (Mx); Effective Micro-organisms[®] (EM); and Agri-mest[®] (Am).

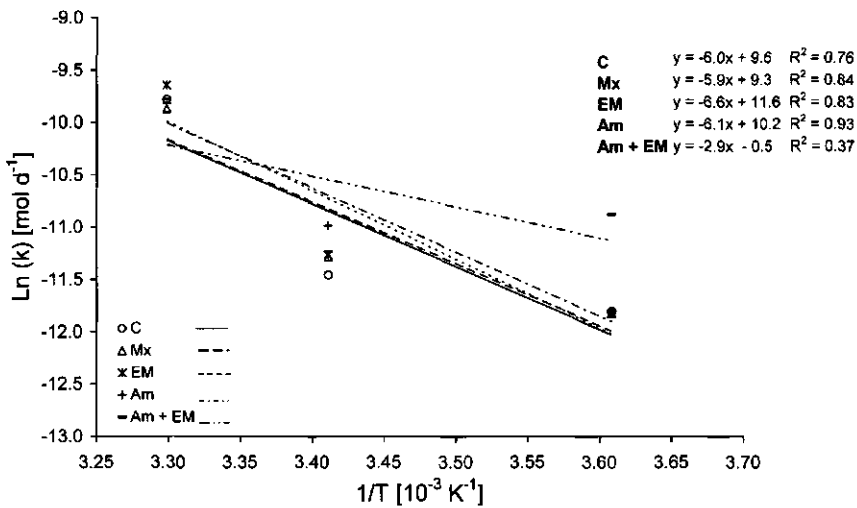
In general, applying additives to slurry did not result in a significant decrease in NH_3 volatilization (Table 2.3). Only at 4 °C and no mixing of the slurry was a decrease ($p < 0.05$) in NH_3 volatilization observed, when a combination of Am and EM had been applied to the slurry. This resulted after 223 days of incubation in a 34% decrease in NH_3 volatilization compared to slurry which did not receive any additive. Although not statistically significant, the effects of amendments on NH_3 volatilization were more pronounced at lower temperatures. There was no consistent effect visible of the manure additives on crust formation (data not shown).

Other chemical amendments, like phosphoric acid or alum ($\text{Al}_2(\text{SO}_4)_3 \cdot 14\text{H}_2\text{O}$) have been shown to decrease NH_3 volatilization. A reduction of 44% was observed when poultry litter received treatments with phosphoric acid and a 62% reduction in NH_3 volatilization was observed when alum was added to poultry manure (Delaune *et al.*, 2004). Both amendments lowered the pH of the poultry litter at the start of the composting period with more than 1 pH-unit. The decrease in pH lowered the $\text{NH}_3/\text{NH}_4^+$ ratio of the litter, which reduced NH_3 emissions. Adding alum to dairy slurry also reduced slurry pH, as was shown by Lefcourt and Meisinger (2001). In contrast to phosphoric acid and alum treatments, no drop in pH was observed after applying Mx, EM and/or Am to slurry, which may (partly) explain the minimal effects of the additives on NH_3 emissions.

The trend in NH_3 volatilization from the EM-treatments suggests a slight increase in NH_3 volatilization when effective microbes are applied to slurry. Only for the incubation at 35 °C and mixing of the slurries, this led to a significant ($p < 0.05$) increase in NH_3 emissions. Since applying EM did not introduce new microbial species into the slurry (Van Vliet *et al.*, 2005), we assume that the addition of EM may have led to a slight increase in the total bacterial population. Comparable findings were reported by Delaune *et al.* (2004), who added a microbial mixture with similar characteristics as EM to composting poultry litter. In contrast, Amon *et al.* (2005) reported a decrease in NH_3 volatilization when EM was applied to cattle slurry. However, Amon *et al.* (2005) noticed no effect or even an increase in NH_3 volatilization when EM was applied to pig slurry with a low DM-content. As noted before, the cattle slurry used in this experiment had a low dry matter content, which might explain the different effects in NH_3 volatilization between the study of Amon *et al.* (2005) and the present study.



a



b

Figure 2.3. Natural logarithm of the rate of NH_3 volatilization versus the (inverse) temperature for the different manure slurry treatments for no mixing (a) and mixing (b) of the samples. Euro Mest-mix[®] (Mx); Effective Micro-organisms[®] (EM); Agri-mest[®] (Am); or a combination of Agri-mest and EM (Am+EM) were added to slurry manure. One treatment received no additive (control (C)).

Table 2.3. Proportional deviation in NH_3 volatilization after 223 days of incubation of four different slurry treatments from the not enriched slurry (control).

Treatment	4 °C; NM ^a	4 °C; M	20 °C; NM	20 °C; M	35 °C; NM	35 °C; M
Mx	0.80 (0.12)	1.02 (0.05)	0.99 (0.03)	1.05 (0.04)	0.96 (0.02)	1.00 (0.05)
EM	1.04 (0.16)	1.09 (0.09)	1.01 (0.04)	1.23 (0.12)	1.03 (0.02)	1.09 (0.04) ^b
Am	0.85 (0.15)	1.01 (0.03)	1.00 (0.04)	1.11 (0.05)	1.06 (0.07)	0.96 (0.05)
Am + EM	0.66 (0.09) ^b	1.15 (0.33)	1.02 (0.02)	1.13 (0.05)	0.99 (0.01)	0.96 (0.02)

The control slurry was given the value 1. The standard deviation is given between parentheses (n=3).

^aNM= no mixing, M= regular mixing during incubation.

^b NH_3 volatilization of the slurry treatment differed ($p<0.05$) from the not enriched slurry.

Conclusions

Additive-enriched manure slurry did not decrease NH_3 volatilization, except for a significantly lower NH_3 emission (34%) after addition of the combination of Am and EM, at 4 °C and no mixing of the slurry.

Applying only EM to slurry increased NH_3 volatilization, although this only led to a significant increase in NH_3 emission when the slurry was incubated at 35 °C and regularly mixed. The increase in NH_3 volatilization when EM was applied to slurry is probably related to the low dry matter content of the slurry. Furthermore, this study indicates that the amount of NH_3 volatilized increases with temperature increase, whereas the effect of mixing on NH_3 volatilization depends on the temperature at which the manure slurry is stored. Since in practice the temperature in manure storage facilities is lower than 20 °C (Velthof *et al.*, 2005), mixing will result in an increase in NH_3 volatilization.

Measurement of ion speciation in animal slurries using the Donnan Membrane Technique



B. van der Stelt, E.J.M. Temminghoff, and W.H. van Riemsdijk
Department of Soil Quality, Wageningen University, the Netherlands

Based on: Analytica Chimica Acta 2005, 552: 135-140

Abstract

The availability of nutrients in animal slurry for plant uptake depends on the total content as well as on the forms in which these nutrients are present in manure slurry. A DMT-manure cell was developed which can help to determine the speciation of nutrients in animal slurries. The cell consists of an acceptor compartment, which is separated from the slurry by two negatively charged cation exchange membranes. The membranes only allow exchange of 'free' cations between the slurry and the acceptor solution. After about 4 days, Donnan equilibrium will be reached between 'free' cation concentrations in the acceptor solution and the 'free' cation concentrations in the animal slurry. The DMT-manure cell has been used to study the effect of dilution of animal slurry (with distilled water) on 'free' ionic species (K^+ , Mg^{2+} , Na^+ , Ca^{2+} , and NH_4^+). Total nutrient concentrations and the 'free' K^+ , Na^+ , and NH_4^+ concentrations decreased proportionately with increasing dilution. In contrast, the 'free' concentrations of Ca^{2+} and Mg^{2+} remained more or less constant upon dilution. The buffering of the 'free' Ca^{2+} and Mg^{2+} concentrations is most probably the result of Ca^{2+} and Mg^{2+} release from organic matter. Also, dissolution of phosphate minerals (struvite and whitlockite), which were likely present in the initial slurry, may have contributed to the buffering of the 'free' Ca^{2+} and Mg^{2+} concentrations.

Introduction

In agriculture animal slurry is being used to supply nutrients to crops and to increase the organic matter content of soils. Excessive use of animal slurry can lead to environmental problems, like soil acidification, eutrophication of surface waters, or metal contamination (Japenga and Harmsen, 1990; Nicholson *et al.*, 1999). To prevent nutrient losses, the additions of nutrients have to be in line with nutrient uptake by plants and the fertility of the soil. Besides total nutrient amounts, also the forms (species) in which nutrients are present in animal slurry determines the potential for nutrient loss to the environment and the availability of the nutrients to crops. Cattle slurry is a mixture of feces, urine and cleaning water (used by farmers). Nutrients in animal slurry can be dissolved in the liquid phase, incorporated in or adsorbed to the solid phase and/or be present in precipitates. The solid phase (mainly) consists of partly digested and non-digested plant material, which has to be mineralized by microorganisms, before nutrients can become available for plant uptake. Nutrients in the liquid phase can be present as 'free' ionic species or as complexes. The ionic forms of the nutrients may determine the potential for binding of the nutrients to solid particles, bio-availability and the tendency of ammonia (NH_3) to volatilize (Jarvis and Pain, 1990). Furthermore, the nutrients in manure may form minerals, as shown by the presence of magnesium (Mg) and calcium (Ca) phosphate minerals (most likely struvite) in the solid fraction of pig manure (Bril and Salomons, 1990). Struvite is stable at a $\text{pH} > 6$ and it may contain 5-15% of the total ammoniacal nitrogen ($\text{TAN} = \text{NH}_4^+ + \text{NH}_{3,\text{aq}}$) in slurry (Bril and Salomons, 1990; Sommer *et al.*, 2003).

To differentiate between the different forms in which nutrients are present in manure is difficult. Even a simple filtration leads to many problems (*e.g.* clogging of the pores) and dialysis membranes cannot be used because they disintegrate in the 'aggressive' medium of the animal slurry. A speciation technique that measures 'free' ionic species, without (hardly) affecting the substrate, is the Donnan Membrane Technique (DMT). The Donnan Membrane Technique has been used to determine speciation of metals in aqueous systems, containing organic and inorganic complexing agents (Temminghoff *et al.*, 2000; Osté *et al.*, 2002), and soil solutions (Weng *et al.*, 2001a; Weng *et al.*, 2001b). Recently, the Donnan Membrane Technique has also been used to study heavy metal uptake by plants (*e.g.* *Lolium perenne*; Kalis *et al.*, 2006a).

The objective of this study was to develop a DMT cell that can be used to characterize speciation of plant nutrients in animal slurry. Dilution studies have been performed to get better insight in the buffering behaviour of various cations.

Experimental

Manure total analysis

Before the animal slurry was analyzed for total nutrient concentrations, the slurry samples were first dried at 70 °C for 3 days and dry matter contents (at 70 °C) were determined. To prevent volatilization of nitrogen compounds during drying, the slurry samples were acidified with 6 M HCl to a pH<4 (Velthof *et al.*, 2005). Before and after acidification, pH was measured by a combined glass-electrode. After drying and grinding of the slurry samples, the samples were digested by microwave with HNO₃, H₂O₂ and HF to determine total Mg, Ca, K, and Na concentrations (Novozamsky *et al.*, 1996). The macro-nutrients K, Mg, Na, and Ca were analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES; Varian, Vista-pro). Total nitrogen and total phosphorus content of the slurries were determined by digesting the dried material with H₂SO₄, salicylic acid, H₂O₂ and selenium (Novozamsky *et al.*, 1983). The digests were analyzed by segmented-flow analysis (SFA; Skalar).

Donnan Membrane Technique

The Donnan Membrane Technique uses the Donnan equilibrium principle to determine 'free' cation concentrations. In this technique, the solution of interest (donor) is separated from an acceptor solution by a negatively charged cation-exchange membrane. Positively charged cations can be transported over the membrane, while transport of negative charged ions and neutral complexes is restricted. Within a few days, (Donnan) equilibrium is reached between 'free' cation concentrations in the donor solution and 'free' cation concentrations in the acceptor solution. The 'free' cation concentrations present in the donor solution can be determined by correcting for differences in ionic strength between the donor and acceptor solution (Helleferich, 1962):

$$(c_{i,d}) = (c_{i,a}) \left(\frac{c_{k,d}}{c_{k,a}} \right)^{z_i} \quad (3.1)$$

where c_i represents the concentration of cation i in the donor (d) or in the acceptor (a) solution, c_k represents the concentration of potassium and z_i is the charge of cation i , respectively.

Potassium is chosen to correct for differences in ionic strength, because it hardly forms any complexes, and thus exists almost completely as 'free' ions (Weng *et al.*, 2001b).

DMT-manure cell

The DMT systems described by Temminghoff *et al.* (2000) and Weng *et al.* (2001b) to determine 'free' ion concentrations in aqueous systems and soil solutions could not be used in animal slurry, because the cells became clogged. Therefore a DMT-manure cell was developed in which an acceptor compartment is separated from the surrounding slurry by two negatively charged cation-exchange membranes (Figure 3.1).

Two Perspex rings keep the membranes in place. The cells are closed with Viton O-rings, nylon screws, and wing-nuts. The acceptor compartment has an inlet and an outlet tip, both placed at one side of the compartment, which can be closed with Luer Lock fittings. The acceptor compartment has a volume of about 5 mL and is filled with an electrolyte solution, which has approximately the same ionic strength as animal slurry.

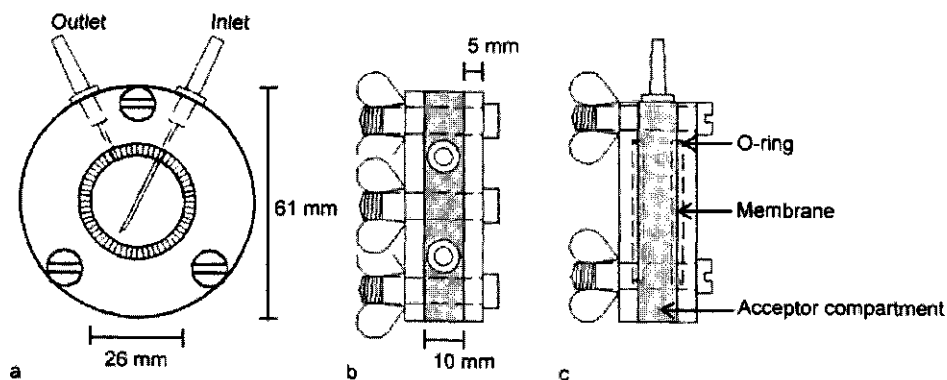


Figure 3.1. Front view (a), top view (b) and side view (c) of the DMT-manure cell. The acceptor compartment is indicated in grey.

The matrix of the two negatively charged membranes (BDH, no. 55165 2U) consists of polystyrene and divinylbenzene with sulphonic acid groups, which are fully deprotonated above pH 2. The membranes have an ion-exchange capacity of 0.8 mmol g⁻¹, are 0.15-0.17 mm thick and have each a surface area of approximately 5.3 cm².

Before using the DMT-manure cell, all materials, except the membranes, were cleaned by successively washing with 0.1 M HCl, ultra pure water (UPW), acceptor solution and UPW. The membranes were prepared by washing with 0.10 M HCl, 0.75 M CaCl₂ and the acceptor solution, respectively. Between each wash step, the membranes were rinsed with UPW.

Equilibrium tests DMT-manure cell

A synthetic solution containing copper was used to test the DMT-manure cell for different process conditions. Copper was chosen since, especially in the case of pig-breeding, copper is added to the feed of animals to suppress bacterial activity in the gut and to increase feed utilization (Nicholson *et al.*, 1999). The use-efficiency of copper by animals is low (<10%) and as a consequence, most copper will be excreted via the manure, which can finally lead to an increase of the copper content of soils (Dach and Starmans, 2005). The synthetic solution consisted of 145 mM KCl, 32 mM MgCl₂, 26 mM NaCl and 16 µM CuCl₂ and had an ionic strength similar to animal slurry. Two 5 L polyethylene containers were filled with the synthetic solution and two DMT-manure cells were placed per container. The composition of the acceptor solution was identical to the synthetic solution, except that no CuCl₂ was added. The synthetic solution in one of the containers was continuously mixed, while the synthetic solution in the other container was not mixed. The acceptor solution of one of the two DMT-manure cells, per container, was circulated by a peristaltic pump (Gilson Minipuls III). Samples of both the donor and the acceptor solution were taken at 0, 1, 2, 5, 8, 12, and 14 days after the experiment had started. Copper was determined by inductively coupled plasma mass spectrometry (ICP-MS; Perking-Elmer, Elan 6000).

Cattle slurry dilution experiment

The animal slurry to which different amounts of distilled water were added was obtained from an experiment in which cows were fed a diet high in crude protein content and high in energy content (Reijs *et al.*, 2007). Dry matter content, pH and ionic strength of the initial cattle slurry were determined. The ionic

strength of the cattle slurry was calculated according to Griffin and Jurinak (1973):

$$I \cong 0.013EC \quad (3.2)$$

Ionic strength (I) is in moles/liter and the electrical conductivity (EC) is in millimhos/cm (at 25 °C), and has been measured using a conductivity meter (Eijkelpamp 18.21 multi-parameter analyser). Cattle slurry was diluted 0, 0.125, 0.25, 0.5, 0.75, and 0.875 times with deionized water and transferred subsequently to polyethylene containers (5 L). The different dilutions are expressed as the dry matter content (DM) of the diluted slurry divided by the dry matter content of the undiluted cattle slurry (DM). After mixing, the slurries were incubated at 4 °C for 2 weeks to adjust the slurries to the changed solid phase/solution ratio. The cattle slurry dilution experiment was performed in a conditioned room (20 °C) for 14 days. In each slurry three cells were immersed. The acceptor solution of the DMT-manure cells contained 0.145 M KCl, 0.032 M MgCl₂, 0.026 M NaCl, and 0.036 M CaCl₂ and was based on average salt levels found in cattle slurry (Mooij, 1996; Sommer *et al.*, 2003). The slurries were continuously mixed to prevent settling of the solid phase. To minimize volatilization of gases, the containers were closed by lids, which had small openings in which the stirrers could be placed. At different times (0, 7, 9 and 14 days), samples, of both the acceptor solution and the cattle slurry, were taken. Per cell, approximately 3 mL acceptor solution was taken using a syringe. Slurry samples (ca. 250 mL) were taken at each time interval for total analysis (in triplicate). The samples of the acceptor solution could be measured directly by ICP-AES (K⁺, Mg²⁺, Na⁺ and Ca²⁺), or by SFA (NH₄⁺). All speciation calculations have been performed using the chemical speciation program Ecosat (Keizer and van Riemsdijk, 2002).

Results and discussions

Equilibrium tests DMT Manure cell

The cell was first tested in a synthetic solution to determine the time needed to obtain Donnan equilibrium for 'free' copper. Next it was tested if mixing of either the acceptor and/or the synthetic solution was necessary. After 4 days, more than 95% of the Donnan equilibrium concentration had been reached for all operating procedures (Figure 3.2). The medium salt concentration (0.4 M)

reduces the absolute value of the Donnan potential of the membrane and thereby increases the time needed to reach equilibrium. Mixing of either the acceptor and/or the synthetic solution hardly affected equilibrium time, indicating that the rate-limiting step to obtain Donnan equilibrium is the transport of cations over the negatively charged membranes. This is in agreement with the kinetic model for the DMT as developed by Weng *et al.* (2005), which shows that the Donnan equilibrium time becomes more dependent on membrane diffusion-controlled transport with increasing ionic strength of the background solution. Since no complexing agent is added to either the synthetic (donor) or the acceptor solution the $C_{\text{acceptor}}/C_{\text{donor}}$ ratio at equilibrium should be equal to 1.

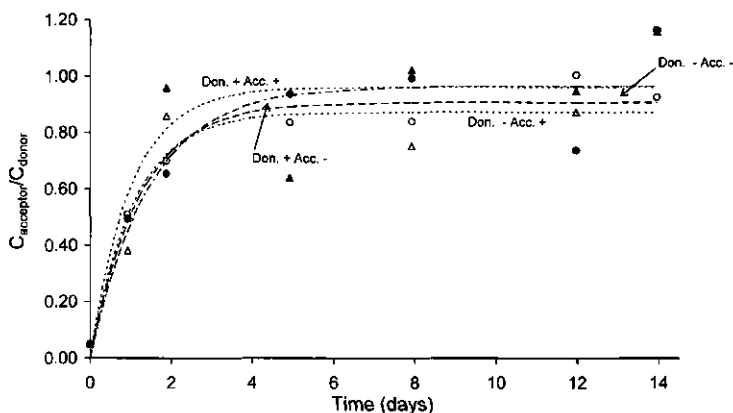


Figure 3.2. Ratio of the copper concentration in the acceptor solution and the copper concentration in the synthetic solution (donor) as a function of time for different operating procedures of the DMT-manure cell. Open symbols represent measurements, in which the synthetic solution was not stirred, while solid symbols represent mixing of the synthetic solution. Circles represent measurements in which the acceptor solution was not mixed and triangles represent measurements in which the acceptor solution was mixed. Curves are based on modeled values.

Cattle slurry dilution

To study the buffering behavior of cations, cattle slurry was diluted with deionized water. The main slurry characteristics are presented in Table 3.1 and are comparable with average values found for cattle slurry (Mooij, 1996). The dry matter content of the different cattle slurries (DM; 70 °C) were in agreement with the calculated dry matter contents, which were determined by multiplying

the DM value (of the initial cattle slurry) with the different dilution ratios. Also, the pH of the slurries decreased with increasing dilution (Table 3.2). During the first 9 days of the DMT-experiment the pH of the different cattle slurries increased with 0.2-1.2 pH-unit. The increase in pH was probably due to chemical and biological processes, which continuously takes place in the slurries (e.g. CO₂-release). CO₂ and water are formed when bicarbonate reacts with a proton and thus will lead to a pH increase (Husted *et al.*, 1991; Ni *et al.*, 2000). The increase in pH will stimulate NH₃ volatilization, which will counteract the pH increase, since protons are released when ammonium is converted into ammonia (Husted *et al.*, 1991; Ni *et al.*, 2000).

Table 3.1. Some characteristics of the (initial) manure slurry used in the experiment.

Characteristic	Mean	
Dry matter [% ww ⁻¹]	10.1	(0.4)
pH	8.32	(0.10)
Ionic strength [M]	0.36	(0.01)
Total Ca [mM]	34	(2)
Total K [mM]	207	(10)
Total Mg [mM]	43	(2)
Total Na [mM]	28	(1)
Total P [mM]	45	(11)
Total N [mM]	448	(43)

The standard deviation is given in parentheses.

Table 3.2. Dry matter content (calculated and measured) and the initial and final pH of the manure slurries used in the DMT-experiment.

DM/DM _i ratio	Dry matter content ^a [% ww ⁻¹]		pH	
	Calculated	Measured	Initial	Final
1.00	10.10	10.10 (0.35)	8.32	9.30 (0.10)
0.87	8.83	8.82 (0.43)	8.26	9.40 (0.06)
0.73	7.57	7.38 (0.49)	8.24	9.18 (0.10)
0.51	5.05	5.10 (0.29)	8.16	9.08 (0.07)
0.26	2.52	2.64 (0.16)	7.93	8.22 (0.23)
0.12	1.26	1.19 (0.08)	7.43	8.20 (0.07)

The different slurries are given as the ratio of the dry matter content of the slurry compared to the initial manure slurry (DM/DM_i). The standard deviation is given in parentheses.

^aDetermined at 70 °C.

For both potassium and sodium the 'free' metal concentrations are nearly identical to the total metal concentrations and their content decreases proportionately with increasing water content (Figure 3.3).

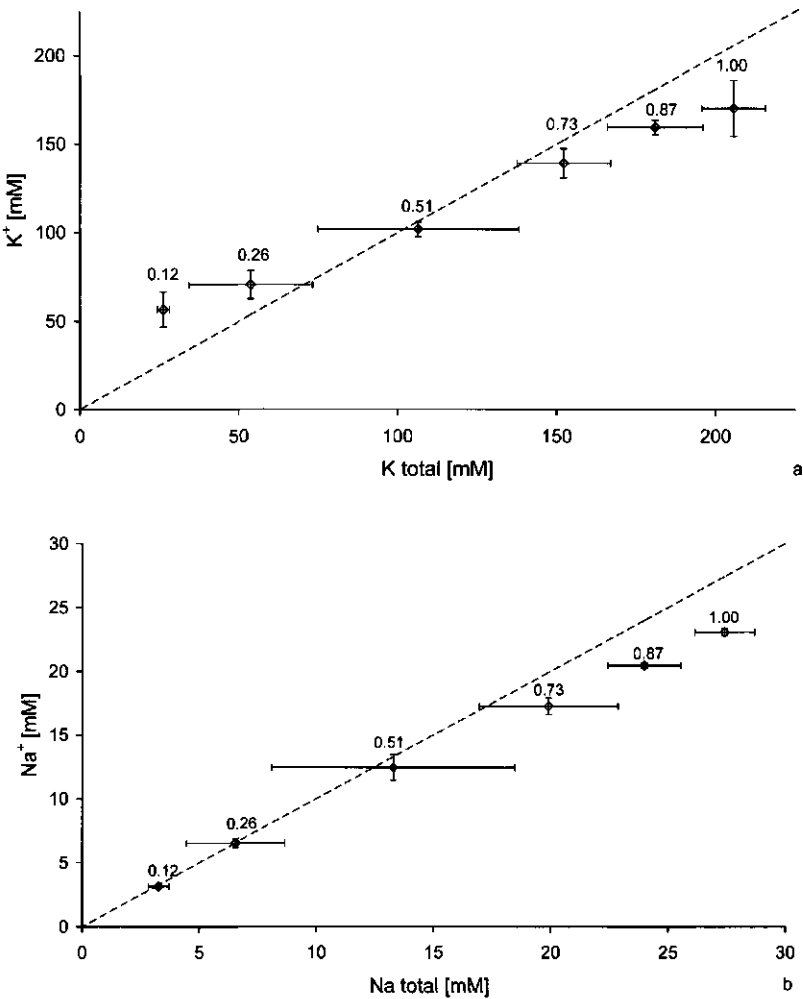


Figure 3.3. The 'free' nutrient concentration (measured by the DMT-manure cell) compared to the total nutrient concentration (determined by microwave-assisted acid digestion) for potassium (a) and sodium (b) in manure slurry. The value above each measurement represents the dilution of the slurry, as compared to the initial cattle slurry, which is expressed as the fraction DM/DM_i . The dashed line represents the 1:1 line.

This confirms that both potassium and sodium are present in cattle slurry almost completely as 'free' ions (>90% on average for both K and Na). The standard deviation in total K and total Na as determined by acid digestions is much larger than the standard deviation in the 'free' K as determined by DMT analysis (Figure 3.3), which may be due to the more heterogeneous composition of the cattle slurry as compared to the (clear) acceptor solution.

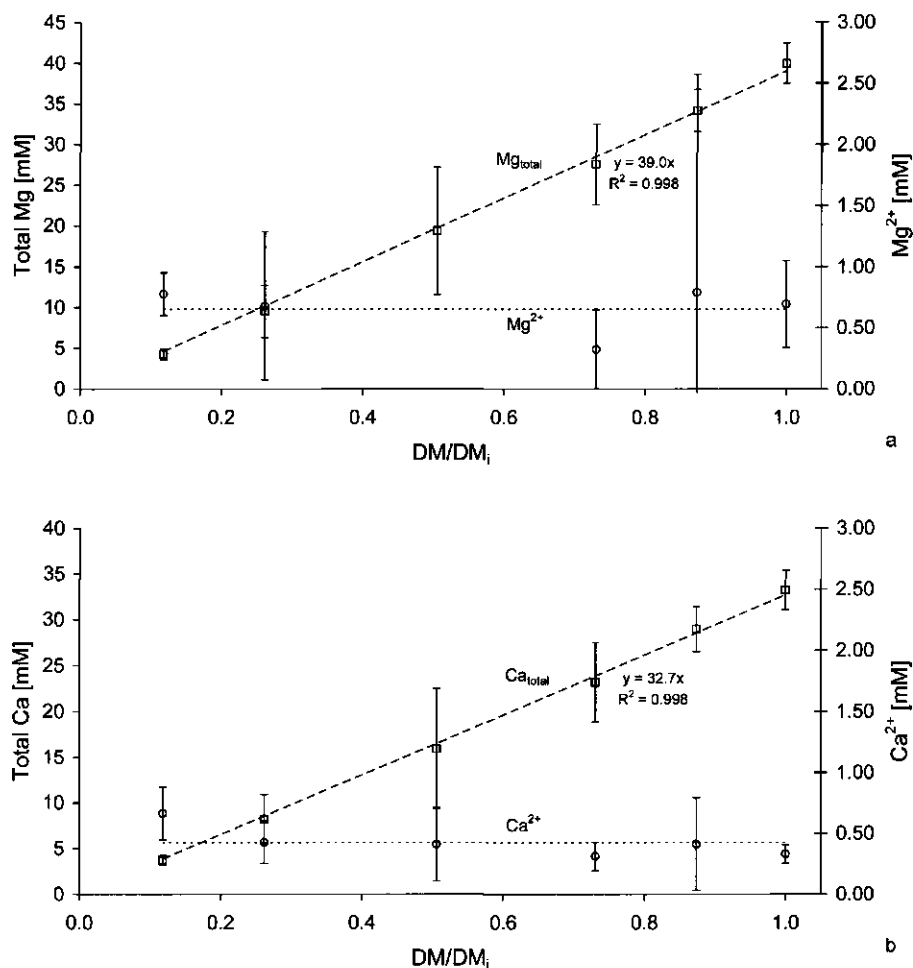
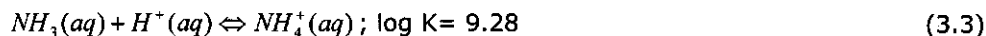


Figure 3.4. Total (□) and free (○) nutrient concentrations of magnesium (a) and calcium (b) at different cattle slurry dry matter ratios (DM/DM_i). The dashed line (---) represents the regression of the total 'free' nutrient concentration on the dry matter ratio. The dotted line (---) represents the average 'free' nutrient concentration on the dry matter ratio.

Total calcium and total magnesium concentrations decreased almost linearly with increased dilution, whereas the 'free' calcium and 'free' magnesium concentrations remained more or less constant (Figure 3.4). The percentage 'free' calcium and 'free' magnesium increased from 1 and 2% in the initial cattle slurry to 17 and 20%, respectively, when cattle slurry was diluted to a DM/DM_i ratio of 0.12.

Maintaining 'free' ionic concentrations at a constant level upon dilution implies a buffering mechanism. The decrease of Ca²⁺ and Mg²⁺, as a response to the dilution with water, are most probably compensated for by calcium and magnesium release from organic matter or by dissolution of minerals (see 'Minerals in cattle slurry'). Calcium and magnesium binding to natural organic matter occurs predominantly to low affinity sites (*e.g.* carboxylic groups) and in the Donnan phase of organic matter (Kinniburgh *et al.*, 1999). This is in agreement with Bril and Salomons (1990), who showed that most calcium and magnesium in the solution phase is adsorbed to organic matter. Adding water to animal slurry will decrease the ionic strength of the slurry, which will lead to a decrease in cation concentrations in the Donnan phase, and desorption from organic matter buffers the 'free' ionic concentrations in animal slurry.

In line with the total nitrogen content, NH₄⁺ concentrations decreased proportionately with increasing dilution (Figure 3.5). The percentage NH₄⁺ increased from ca. 36% in the initial cattle slurry to 44% in cattle slurry with a DM/DM_i ratio of 0.12. Besides 'free' NH₄⁺, the mineral nitrogen fraction in cattle slurry mainly consists of NH_{3,aq}. The ammonia concentrations in the cattle slurries were calculated using the measured NH₄⁺ concentrations, the pH of the slurries and the equilibrium condition:



Both, the 'free' NH₄⁺ concentration as well as the NH_{3,aq} concentration decreased with increasing dilution. However, at DM/DM_i ratios of 0.87 and below 0.26 the ammonia concentration appears to be somewhat buffered. At DM/DM_i 0.87, pH was slightly higher compared to the pH of the initial cattle slurry, which led to a (small) increase of NH_{3,aq} and therefore counteracted to some degree the NH₄⁺ decrease. At a DM/DM_i ratio lower than 0.26, ammonia volatilization was hardly affected anymore by the dry matter content of the slurry. This is in agreement with Sommer and Olesen (1991), who showed for surface-applied cattle slurry that ammonia volatilization is not dependent on dry matter concentrations below 4% (DM/DM_i < 0.26). The TAN (NH₄⁺ + NH_{3,aq}) slightly decreased

from about 63% (of the total nitrogen content) at a DM/DM_i ratio of 1 to about 46% at a DM/DM_i ratio lower than 0.26. Because of the decrease in slurry pH upon dilution, the fraction $NH_{3,aq}$ of TAN strongly decreased. At a DM/DM_i ratio of 1.0, the fraction of ammonia is about 42% of TAN, whereas at a DM/DM_i ratio lower than 0.26, this is only 6%.

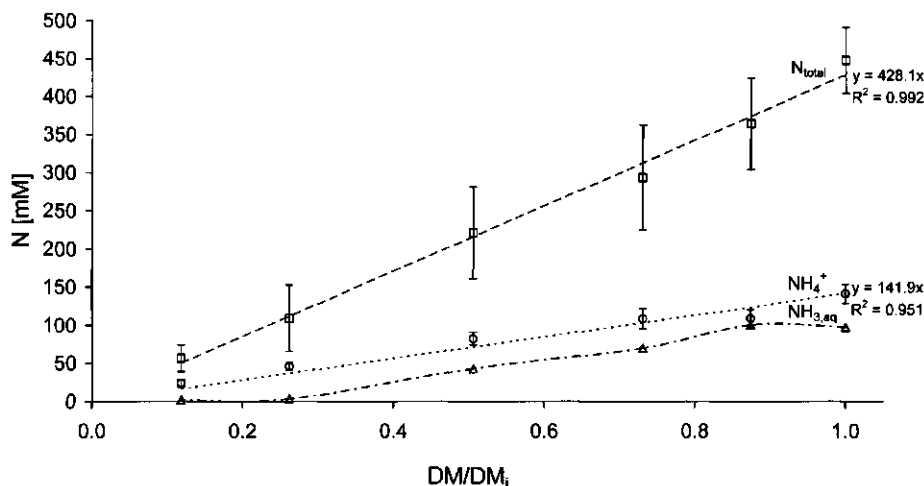


Figure 3.5. Total nitrogen (□), ammonium (○) and ammonia (Δ) concentrations in cattle slurries at different slurry dry matter ratios (DM/DM_i). The dashed line (—) represents the regression of the total nitrogen concentration on the dry matter ratio. The dotted line (---) represents the regression of the ammonium concentration on the dry matter ratio. The dashed-dotted line (— · —) represents the decrease of ammonia upon dilution.

Minerals in cattle slurry

Besides organic matter, also dissolution of minerals can contribute to the buffering of 'free' nutrient concentrations. In order to estimate whether buffering by minerals played a role in this experiment, we calculated the inorganic phosphate concentrations required for the most likely Ca and Mg phosphate minerals to be present in animal slurry (Table 3.3).

The required concentration of total dissolved inorganic P for struvite formation accounts for less than 1% of the total phosphorus concentration (Table 3.1) present in the cattle slurry. In contrast, Sharpley and Moyer (2000) reported that about 63% of the total P present in dairy manures consists of inorganic phosphate, of which more than 80% is water extractable. It is therefore likely that

struvite may have been present in the initial cattle slurry. However, the 'free' NH_4^+ concentration decreased linearly with dilution, which suggests that dissolution of struvite hardly occurred. Since the cattle slurry is even supersaturated with respect to struvite at the highest dilution, the effect of buffering of the 'free' Mg^{2+} and 'free' NH_4^+ concentrations by dissolution of struvite is negligible. The dissolved inorganic P concentrations required for formation of calcium phosphates varied widely. For brushite and monetite, ca. 30 and 14% of the total P content had to be present as dissolved inorganic phosphate for mineral formation to occur. In contrast, $<1\%$ of the total P content in the slurry was required for precipitation of whitlockite. Therefore, whitlockite is the most likely calcium phosphate mineral to have been present in the cattle slurry and it could have contributed to the buffering of the 'free' calcium concentration upon dilution. However, more research on cations and anions is required to get more insight in precipitation processes in manure.

Table 3.3. Minimal total dissolved inorganic phosphate concentrations required for the formation of different minerals, as calculated by Ecosat, using $[\text{NH}_4^+] = 145 \text{ mM}$; $[\text{Ca}^{2+}] = 0.33 \text{ mM}$; $[\text{Mg}^{2+}] = 0.69 \text{ mM}$ and pH 9.3.

Mineral	Molecular formula	Log K° *	$P_{i, \text{sol.}} [\text{mM}]$
Struvite	$\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$	-13.15	0.02
Brushite	$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	-18.92	13.22
Monetite	CaHPO_4	-19.25	5.96
Whitlockite	$\text{Ca}_3(\text{PO}_4)_2$	-28.92	0.04

*As given in Lindsay (1979).

Conclusions

There is no alternative main stream technique that can analyze simultaneously the 'free' cation concentrations in animal slurries. By allowing direct contact between animal slurry and the negatively charged cation membranes, the Donnan Membrane Technique has been made applicable for characterizing animal slurries. Since no mixing of either the acceptor solution or the slurry is required, it is (in principle) possible to use the DMT-manure cell for in situ measurements in manure storage facilities. Only the total nutrient concentrations and the (clear) acceptor solutions have to be analyzed in the laboratory to determine the 'free' cation concentrations in animal manures. A major advantage is that the final

analysis of the acceptor solution is done on a simple salt solution, instead of the highly complex manure sample.

We observed that the 'free' divalent cation concentrations of Mg and Ca in cattle slurry were buffered upon dilution with water, whereas monovalent cation concentrations, like K or Na, decreased proportional with increasing dilution. Buffering of the 'free' cation concentrations of Mg and Ca is most likely the result of nutrient release from these ions bound to organic matter. Dissolution of struvite will not have contributed much to the buffering of the 'free' Mg concentration, since the slurry was oversaturated for struvite, even at the highest dilution. Other positively charged nutrients, like iron or (trace) transition metals (e.g. copper or zinc), can (in principle) also be measured with this technique.

Effects of dietary protein and energy levels on cow manure composition and ammonia volatilization



B. van der Stelt¹, P.C.J. van Vliet¹, J.W. Reijs², E.J.M. Temminghoff¹,
and W.H. van Riemsdijk¹

¹Department of Soil Quality, Wageningen University, the Netherlands

²Department of Animal Sciences, Wageningen University, the Netherlands

Submitted

Abstract

Adjusting dietary composition is considered to be an effective way to reduce nutrient losses to the environment. The effects of various dietary protein and energy levels on manure composition (Ca, Mg, K, Na, N, P, and pH) were studied by determining total and direct available ('free') nutrient concentrations in eight slurries obtained from a feeding trial. Furthermore, the effects of dietary changes on ammonia (NH_3) volatilization from manure slurries were studied. Raising the crude protein content (CP) of the feed ($108\text{--}190\text{ g kg}^{-1}\text{ DM}$) resulted in an average increase in total N and P content of the slurries of 56 and 48%. Feeding the cows more energy ($5050\text{--}6840\text{ kJ kg}^{-1}\text{ DM}$) increased total N and P content of the slurries on average with 27 and 39%. Total ammoniacal nitrogen (TAN) amounted to 52–77% of the total N content present in manure slurries. A low protein content, or a low energy content of the diets reduced TAN concentrations in the slurries with 43% (CP) or 25% (energy). Changes in the protein content or the energy content of the feed did not significantly affect the 'free'-to-total ratios of Na, Ca and Mg content of the slurries. In agreement with the calculated $\text{NH}_{3,\text{aq}}$ content, NH_3 volatilization from manure slurries was much higher (on average 10 times higher) when the cows were fed higher levels of CP content. Although the TAN content of the slurries were higher when cows were fed energy richer diets, NH_3 volatilization from the slurries were lower.

Introduction

In the last decades agriculture, especially in the Netherlands, has become more intensive and more productive. To ensure high crop yields farmers apply large amounts of manure slurry (which is a mixture of feces, urine, bedding material, and waste water) to their lands. In dairy farming, nitrogen (N) and phosphorus (P) use-efficiencies of cows vary between 15 and 30% (Aarts *et al.*, 1992) and as a consequence most of the N and P present in the feed will be excreted by the animal. Excessive land application of slurry can result in losses of these nutrients to the environment, where they can contribute to eutrophication and acidification problems (Tilman *et al.*, 2001). Applying manure slurry to soils has also led to increased K, Ca, and Mg concentrations in topsoils and nitrate-N, Ca, and Mg concentrations in sub-soils (Edmeades, 2003). To restrict nutrient losses to the environment, while maintaining productivity, several measures can be undertaken, of which manipulating the diets of animals is considered to be one of the most promising techniques (Phillips *et al.*, 1999). The effect of dietary changes on N and P speciation and ammonia (NH₃) volatilization is already studied extensively, although in many cases NH₃ volatilization is estimated by using different model calculations. For other nutrients, information is mainly restricted to estimations of total nutrient concentrations based on DM intake, nutrient concentrations of the feed and the length of the dry period of the cow (ASAE, 2005; Nennich *et al.*, 2005).

Changing the crude protein (CP) content and/or the digestibility of the feed will affect urinary N excretion more than fecal excretion of N, resulting in a different N content, as well as a different N composition of the manure slurry (Kebreab *et al.*, 2002). Lowering the CP content of diets to lower levels than currently used, will not only decrease the amount of N excreted by dairy cattle, but will also reduce the short-term N availability of manure, making it less susceptible for leaching and volatilization (Paul *et al.*, 1998). Sørensen *et al.* (2003) observed an increase in the concentration of N in feces dry matter when the digestibility of the diets increased. Nitrogen utilization efficiency increases when lactating dairy cows are fed grass silage diets enriched with a low degradable starch source instead of a high degradable starch source (Castillo *et al.* 2001).

Excessive N is mainly excreted by the cow as urea via the urinary tract (Vérité and Delaby, 2000; Børsting *et al.*, 2003). After contact with the enzyme urease, which is present in feces, urea is rapidly degraded into NH₃, which is susceptible for volatilization. Ammonia is considered an important source of acidify-

ing gaseous N emissions. Furthermore, NH_3 can act as a N-source in low-nutrient ecosystems and can react with atmospheric acids to form particulates, which can be spread over a wide area (Sommer and Hutchings, 2001; Webb *et al.*, 2005). In Europe, about 75% of the NH_3 emitted to the atmosphere can be attributed to livestock production, and measures restricting NH_3 emissions from the livestock sector are considered to be the most effective approach to reduce the impact of NH_3 on the environment (Webb *et al.*, 2005). Besides the total ammoniacal nitrogen concentration ($\text{TAN} = \text{NH}_4^+ + \text{NH}_{3,\text{aq}}$) in manure slurry, the pH and the dry matter content of the slurry are the most important parameters determining the NH_3 volatilization potential (Jarvis and Pain, 1990, Sommer *et al.*, 2003). Paul *et al.* (1998) observed in two feeding trials decreases in NH_3 emissions from manure slurry when the dietary CP content decreased. In one experiment, CP levels were lowered from 16.4 to 12.3%, which reduced NH_3 emissions by 40%, while in the other experiment CP levels were reduced from 18.3 to 15.3%, which led to a reduction in NH_3 emissions of 20%. Similar decreases in NH_3 emissions with decreasing protein content of the feeds were reported by James *et al.* (1999) and Külling *et al.* (2001).

The aim of this research was to investigate how manure composition was affected by dietary changes. The slurries were produced in a feeding trial in which non-lactating cows were fed specific diets, which varied in protein and/or energy content (Reijs *et al.*, 2007). The effect of feeding strategy on manure composition was studied by measuring total, as well as direct available ('free') nutrient concentrations (N, P, K, Ca, Mg, and Na) in different manure slurries. Also the effects of diet composition and manure characteristics on NH_3 volatilization were investigated.

Materials and Methods

Feeding trial

To study the effect of diet composition on manure slurry quality a feeding trial was carried out at experimental farm "De Meenthoeve" (Rhenen, the Netherlands) from January till March 2003. The feeding trial was described in more detail by Reijs *et al.* (2007). In the trial eight pairs of non-pregnant, non-lactating Holstein Friesian cows were fed high or low protein levels (190 or 108 g

kg⁻¹ DM) in combination with high or low energy levels (6840 or 5050 kJ kg⁻¹ DM). The eight feeding regimes (Table 4.1) can be categorized into four groups:

- (I) high in protein and high in energy content (HPHE);
- (II) high protein content with a low energy content (HPLE);
- (III) low protein content with a high energy content (LPHE);
- (IV) low protein content and a low energy content (LPLE).

The feeding regimes were created by combinations of four different forages, *i.e.* high digestible grass silage, maize silage, low digestible grass silage, and straw and three concentrate ingredients, *i.e.* soybean meal, maize, and beet pulp. For each group two different feeding regimes were chosen, which are denoted by the letters a or b (in subscript) following the groups' code (Table 4.1). Compared to daily practices, extreme differences in the amounts of protein and energy fed to the non-lactating cows were chosen to obtain maximum differences in manure composition.

Table 4.1a. Main characteristics of the diets: feed composition, dry matter intake and C/N ratios of the diets and the (mean) body weights of the cows at the start of the experiment.

Code	Forages ¹ Composition	Intake [kg DM d ⁻¹]	Concentrates ² Composition	Intake [kg DM d ⁻¹]	C/N ³ -	Bw ⁴ [kg]
HPHE _a	60% MS + 40% HDGS	12.3	90% S + 10% M	3.6	15.0	556 (53)
HPHE _b	100% HDGS	9.3	40% S + 60% M	3.4	13.0	557 (35)
HPLE _a	100% LDGS	7.4	100% S	2.8	14.3	555 (29)
HPLE _b	100% STR	4.7	75% S + 25% M	4.1	15.2	565 (81)
LPHE _a	60% MS + 40% HDGS	10.2	55% M + 45% B	3.1	22.9	559 (61)
LPHE _b	100% MS	11.9	43% S + 21% M + 36% B	2.4	27.9	562 (10)
LPLE _a	100% LDGS	7.2	19% S + 81% B	2.6	22.5	482 (99)
LPLE _b	100% STR	3.9	21% S + 21% M + 58% B	4.0	26.3	563 (91)

¹Forages: MS= Maize Silage; HDGS= High Digestible Grass Silage; LDGS= Low Digestible Grass silage; STR= Straw.

²Concentrates: S= Soybean meal; M= Maize; B= Beet pulp.

³Total Carbon-to-Nitrogen ratios of the diets.

⁴Body weight; standard deviation is given in parentheses.

Nutrient intake by the animals was estimated using standard feeding values (CVB, 2003). The total dry matter intake of the animals (forage + concentrates) ranged from 1.4% (LPLE_b) to 2.9% (HPHE_a and HPHE_b) of the mean body weight, which is similar to the range reported for non-lactating cows in the standard of the American Society of Agricultural Engineers (0.7-2.2%; ASAE, 2005). During

the trial the cows were housed in a tie-stall barn. Eleven weeks after the start of the experiment (including a three week adaptation period) bedding-free manure slurries, consisting of urine and fecal mixtures, were collected. To minimize NH_3 volatilization, accumulated manure slurries were mixed just before subsampling and subsequently transferred to 25 L barrels, closed and stored for 5 months at 4 °C. In those cases that the viscosity of the slurries prevented pumping, water was added (Table 4.3). The total and 'free' nutrient (N, P, K, Na, Ca, and Mg) concentrations of the different slurries were determined following the procedure described by Van der Stelt *et al.* (2005).

Table 4.1b. Main characteristics of the diets: Dietary characteristics and mean nutrient content of the diets.

Code	Dietary characteristics ¹ [kg^{-1} DM]					Mean nutrient content [g kg^{-1} DM]					
	NEm [kJ]	CP [g]	NDF [g]	ADF [g]	Dig. OM [%]	K	Na	Ca	Mg	P	N
HPHE _a	6800	184	379	216	73	22.8	1.0	4.0	2.5	4.1	29.4
HPHE _b	6800	199	405	247	80	22.7	1.6	3.6	2.1	3.6	31.8
HPLE _a	5300	188	483	324	68	24.7	3.0	5.4	3.3	4.4	30.1
HPLE _b	4845	189	510	335	60	18.0	1.3	2.7	2.6	3.7	30.2
LPHE _a	6870	114	397	239	73	19.0	1.2	4.4	2.1	3.0	18.2
LPHE _b	6880	101	376	232	73	14.8	0.4	3.7	2.0	2.7	16.2
LPLE _a	5170	110	539	350	64	21.5	3.4	6.9	3.0	3.0	17.6
LPLE _b	4900	105	556	350	64	14.1	1.8	4.8	2.4	2.1	16.8

¹Dietary characteristics: NEm = Net Energy for maintenance; CP= Crude Protein;

Dig. OM= Digested Organic Matter; NDF= Neutral Detergent Fiber; ADF= Acid Detergent Fiber.

Manure analyses

At the start of the experiment pH, dry matter content (DM at 105 °C) and electric conductivity (EC) of the different manure slurries were determined. pH was measured with a combined glass-calomel electrode and EC with a multi-parameter analyzer (Eijkelpamp; type 18.2). The measured conductivities were converted into the ionic strength (I) of the manure slurries, using the empiric relationship $I = 0.013 \cdot \text{EC}$, as described by Griffin and Jurinak (1973). I is expressed in moles L^{-1} and EC is expressed in millimhos cm^{-1} (at 25 °C).

Before the slurry samples were analyzed for total nutrient concentrations, the samples were first dried at 70 °C. After drying for 3 days the samples were ground (<1mm) using a Culatti MFC-type Micro Hammer Mill.

Next, the slurry samples were digested by microwave digestion with HNO_3 , H_2O_2 , and HF (Novozamsky *et al.*, 1996) to determine total nutrient concentrations of Mg, Ca, K, and Na. Furthermore, the dried and ground manure slurry samples were digested by H_2SO_4 , salicylic acid, H_2O_2 , and selenium (Novozamsky *et al.*, 1983) to determine total P concentrations in the manure samples. K, Mg, Na, and Ca concentrations were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES; Varian, Vista-pro). Total P was measured by segmented-flow analysis (SFA; Skalar). Total C was measured in the dried slurry samples on a CHN-analyzer (Fisons Instruments EA 1108), while total N was measured in fresh slurry samples using the Kjeldahl method (ISO5983, 1979). Carbon losses during drying were minimized by using an air forced drying oven. The C/N ratios of the slurries were calculated from the total C and total N data.

Determination of 'free' nutrient concentrations

The DMT-manure cell consists of an acceptor solution which is separated from manure slurry by two negatively charged cation exchange membranes. The membranes allow transport of positively charged cations, while transport of neutral and negative ions is restricted. Within 5 days (Donnan) equilibrium is reached between 'free' cation concentrations in manure slurry and 'free' cation concentrations in the acceptor solution (Van der Stelt *et al.*, 2005).

At the start of the experiment each manure slurry was homogenized and 10 kg slurry was transferred to a plastic container, after which three DMT-cells were immersed in each slurry. The containers were closed with lids, which contained a small opening in which a stirrer was placed. During the experiment the slurries were continuously mixed to prevent settling of the solid phase. At different times (0, 7, 10, and 14 days) samples were taken of both the acceptor solution (3 mL) and the manure slurry (ca. 250 mL).

No pre-treatments were required to measure 'free' nutrient concentrations in the sampled acceptor solutions. 'Free' K, Ca, Mg, and Na concentrations were determined by ICP-AES. Sub-samples of the acceptor solution were taken, diluted two hundred times with ultra pure water and measured by SFA to determine the 'free' ammonium (NH_4^+) concentration present in manure slurry. The measured K data were, in accordance with Van der Stelt *et al.* (2005), used to correct for differences in ionic strength between acceptor solution and manure slurries. The ammonia ($\text{NH}_{3,\text{aq}}$) concentrations and the TAN concentrations of the slurries were calculated by the speciation program Ecosat (Keizer and van Riemsdijk, 2002),

using the equilibrium reactions of the different species in combination with the measured pH and NH_4^+ concentrations. Since nutrient concentrations were rather constant during the experiment, the mean nutrients concentrations have been given.

Ammonia volatilization

Volatilization of NH_3 from the different manure slurries was measured using a passive acid trap, which was developed by Velthof *et al.* (2005). 0.5 ± 0.01 kg of (fresh) homogenized manure slurry was transferred to a plastic jar (wide neck bottle square; 1 L). A small container with 12.5 mL of 3.2 M H_2SO_4 was placed in each jar to trap volatilized ammonia. The container was kept floating using a small ring of etha-foam. After closing the jars with screw tops the jars were randomly placed in an incubator. The jars were incubated at 12 °C, which is within temperature boundaries (10-15 °C) found in manure slurry storage facilities during autumn (Sommer *et al.*, 2000). After 0, 4, 8, 16, 24, and 32 days the acid containing flasks were replaced. The acid solution was diluted to a total volume of 50 mL with ultra pure water. Total N concentrations in the samples were determined by SFA. The experiment was performed in fourfold. The data were described by an equation given by Groot *et al.* (1996):

$$G = \frac{A}{1 + \left(\frac{B}{t}\right)^C} \quad (4.1)$$

G represents the (relative) amount of NH_3 volatilized at time t (days) after the start of the incubation. A represents the (relative) asymptotic gas production ($\text{mmol NH}_3 (\text{mol TAN})^{-1}$) and B denotes the time (days) required for half of the amount of gas produced to be formed. The sharpness of the switching characteristic of the profile is denoted by the constant C.

Statistical Analysis

The SPSS program was used for statistical analysis of the data (SPSS Inc., 2003). Differences in manure composition caused by different feeding strategies were analyzed by analysis of variance. Correlations were made between feed characteristics and the chemical composition of manures. Analyses of the effects of feed characteristics on ammonia volatilization were performed using repeated measurement analyses. When necessary to acquire equality of variances data were transformed.

Results and discussion

Manure characteristics

The composition of the different manure slurries expressed on DM basis is given in Table 4.2. The measured nutrient contents were compared with average nutrient contents for lactating cows, as data about the chemical composition of manure slurry produced by dry cows were not available.

The DM content of the manure slurries ranged from 9.3% (HPHE_a) to 13.5% (HPLE_a). Similar DM content can be calculated from data reported by Holter and Urban (1992) for slurries produced by (pregnant) dry cows (DM 13.4 and 14.9%, respectively), assuming a DM content of urine of 3.5% (Oenema *et al.*, 2000). The DM content of the manure slurries was on average 15% higher ($p < 0.05$) when the CP content of the feeds of the animals were lower (e.g. 108 or 190 g kg⁻¹ DM). At higher protein content more N will be excreted via the urinary tract, thereby likely increasing the volume of urinary water required for diluting the N metabolites (Valadares *et al.*, 1999; Broderick, 2003), and hence will result in lower DM content of the slurries. Both, the lower C content and the higher N content of the slurries, when cows were fed diets with higher protein content, resulted in lower C/N ratios (38% on average) of the manures (Table 4.2).

Raising the energy content from 5050 to 6840 kJ kg⁻¹ DM (and simultaneously improving the digestibility of the feed (Table 4.1)) reduced the DM content of the manure slurries. This seems in agreement with results reported by Broderick (2003), who observed a decrease in fecal DM quantity, when the digestibility of the feed increased. Moreover, at higher energy content of the feed, more feed (and N) was taken in by the animals in this experiment, adding to the excess of N (see next section). Excess N will mostly be excreted via the urinary tract, which will lower the DM content of the slurry, as was previously explained. On average the DM contents of the slurries were 14% lower when the energy contents of the feeding regimes were 35% higher. The C/N-ratios of the manure slurries were on average 17% lower when the digestibility of the organic matter was ca. 17% higher.

Total nutrient content

The total K content of the slurries ranged from 26-80 g kg⁻¹ DM (Table 4.2) and were, except for the straw-based feeding regimes (LPLE_b and HPLE_b),

similar to normal K content found in dairy slurries produced in the Netherlands (± 59 g kg DM⁻¹; Blgg, 2005). The amounts of K consumed by cows that were fed straw-based feeding regimes were approximately half of the amounts of K consumed by the other cows. Subsequently, K content in the LPLE_b and HPLE_b-slurries were approximately 50% lower than in the other slurries. The total Na content of the slurries varied between 1.8 and 6.3 g kg DM⁻¹ (Table 4.2). The Na content of all tested slurries were lower than the mean Na content found in dairy slurries (± 7 g kg DM⁻¹; Blgg, 2005). This suggests that the feeding regimes contained low amounts of Na. Nevertheless, minimum daily Na intakes required by cows (7 g day⁻¹; CVB, 2003) were met for all feeding regimes (Table 4.1), except for the LPHE_b-treatment, from which slightly less Na was taken up (6 g day⁻¹) than was required.

The Ca content of the slurries varied considerably between the different diets. The lowest total Ca content was found for the HPHE_a slurry (8.6 g kg DM⁻¹) and the highest Ca content was found for slurry LPLE_b (14.1 g kg DM⁻¹). However the difference in mean daily amounts of Ca excreted were almost equal for these two extremes (e.g. 43 g (cow day)⁻¹ for HPHE_a and 44 g (cow day)⁻¹ for LPLE_b), which shows that besides the content also the absolute amount of manure excreted is important. For all feeding regimes the mean daily Ca excretion was 44 ± 7 g (cow day)⁻¹.

Table 4.2. Properties and chemical composition of the manure slurries.

Code ¹	Nutrients [g kg DM ⁻¹]													
	DM ²	Total C ²	C/N ²	K	Na	Ca		Mg		P		N-components		
	[%]	[%DM ⁻¹]	[-]	Total	Total	Free	Total	Free	Total	Free	Total	Total	Tan ³	N _{org} ⁴
HPHE _a	9.3 ^a	35.8 ^{bc}	4.6 ^a	68.5 ^c	4.2 ^e	3.4 ^{bc}	8.6 ^a	0.3 ^a	7.7 ^d	1.0 ^b	10.3 ^a	77.2 ^b	51.0 ^c	26.2 ^a
HPHE _b	10.2 ^{ab}	34.6 ^b	4.6 ^c	80.4 ^b	6.3 ^d	5.2 ^c	12.9 ^c	0.1 ^c	9.4 ^e	0.04 ^b	14.0 ^f	75.6 ^b	53.6 ^c	22.0 ^a
HPLE _a	13.5 ^c	31.0 ^a	5.4 ^a	53.7 ^d	3.2 ^d	2.6 ^{ab}	8.9 ^a	0.7 ^a	7.0 ^{bcd}	1.2 ^a	9.0 ^d	57.7 ^a	32.3 ^b	25.4 ^a
HPLE _b	11.0 ^a	39.6 ^a	6.1 ^a	33.0 ^b	2.8 ^c	2.3 ^{ab}	9.0 ^a	0.1 ^b	5.9 ^a	0.3 ^{ab}	7.2 ^{abc}	64.6 ^f	49.5 ^c	15.1 ^a
LPHE _a	12.2 ^b	36.7 ^{bcd}	7.0 ^b	62.0 ^a	4.9 ^f	3.6 ^{bc}	11.3 ^b	1.1 ^a	7.3 ^{bcd}	1.2 ^b	7.7 ^c	52.3 ^d	31.1 ^b	21.3 ^a
LPHE _b	12.1 ^b	38.9 ^{de}	8.3 ^a	43.7 ^c	1.8 ^a	1.5 ^{ab}	8.8 ^a	0.3 ^a	6.6 ^{abc}	0.6 ^b	7.5 ^{bc}	47.1 ^c	31.0 ^b	16.1 ^a
LPLE _a	13.4 ^c	31.8 ^a	9.3 ^b	43.6 ^c	2.5 ^b	2.6 ^{ab}	11.2 ^b	0.4 ^a	6.4 ^{abc}	1.2 ^b	6.3 ^{ab}	34.0 ^a	17.7 ^a	16.2 ^a
LPLE _b	12.8 ^{ab}	37.6 ^{cde}	8.7 ^c	26.0 ^a	3.1 ^d	2.5 ^{ab}	14.1 ^c	0.2 ^{ab}	7.4 ^{cd}	1.2 ^b	5.9 ^a	43.1 ^b	26.5 ^{ab}	16.6 ^a
Ref. ⁵	8.7	-	-	59.0	6.9	-	-	-	8.9	-	8.2	49.6	27.2	22.2

^{a-h}Means within a column followed by different superscripts differ ($p < 0.05$).

¹The diets are described in more detail in Table 4.1.

²DM=Dry matter content (105 °C); C/N=Total Carbon to total N Nitrogen ratio.

³TAN=Total Ammoniacal Nitrogen content ($\text{N-NH}_{3,\text{aq}} + \text{N-NH}_4^+$).

⁴N_{org}=Organic Nitrogen content; calculated as the difference between Total N and TAN content.

⁵Average composition of wastewater free dairy cattle slurry in the Netherlands (Blgg, 2005)

Total Mg content of the slurries varied from 5.9 (g kg DM⁻¹) for the HPLE_b-treatment to 9.4 (g kg DM⁻¹) for the HPHE_b-treatment. For all feeding regimes the mean daily Mg amount excreted was 31 ± 7 g (cow day)⁻¹. All slurries, except HPHE_b, had a Mg content comparable to the mean Mg content found in dairy slurries (Table 4.2), whereas total Mg content of the HPHE_b-slurry was ca. 34% lower. The Mg content of the slurries were positively correlated with the total K content of the slurries (Pearson= 0.744; $p < 0.001$). A higher K content in manure slurries is the result of an increase in the dietary K content. A high dietary K content has a negative effect on the digestibility of Mg (Schonewille *et al.*, 1999; Weiss, 2004), leading to an increase in the amount of Mg excreted by the animal.

The total N content of slurries, produced by dry cows fed feeding regimes containing a high protein and a high energy content (regimes HPHE_a and HPHE_b), were ca. 54% higher than the mean N content found in dairy slurries (Table 4.2). All other treatments had total N content comparable with the mean N content found in dairy slurries, except for the LPLE_a-slurry, which was ca. 32% lower than the mean N content. The total N content of slurries, produced by non-lactating cows fed feeding regimes containing a high protein content or a high energy content, were on average 56% and 27% higher (CP: $p < 0.001$; NEm: $p < 0.01$) than the N content of slurries produced by cows fed diets with a low protein or a low energy content. In contrast, Broderick (2003) observed a decrease in total N excreted in manure slurry of lactating dairy cows, when the energy content of the feed increased. These contradicting observations are likely to be related to the lower N-use efficiency of non-lactating cows (this experiment) compared to lactating cows (Broderick, 2003). In the case of non-lactating cows (this experiment) no N is converted into milk N protein and N requirements for maintenance are lower. As a consequence, feeding of extra CP or energy to the cow will result in higher amounts of excreted N than in the case of lactating cows.

Only the total P content of the slurry based on the highly digestible grass silage treatment (HPHE_b; 14 g kg DM⁻¹) was higher ($p < 0.05$) than the mean total P content found in dairy slurry (8.2 g kg DM⁻¹). The P content of the other treatments varied from 5.9 (LPLE_b) to 10.3 g kg DM⁻¹ (HPHE_a). Similar to the total N content, the total P content of the slurries increased with higher protein (48%) or energy content (39%) of the feeding regimes, or both ($p < 0.01$). The N/P-ratios of the slurries varied from 5.4 to 9.0 and were on average 10% higher than the mean N/P ratio found in manure slurries (6.1; Table 4.2). The N/P ratio required

by crops is about 8 (Lefcourt and Meisinger, 2001). Because manure application is primarily based on N-requirements of plants, a higher N/P ratio of slurry manure will lower the amount P applied (in excess) to the soil, which will reduce the risk of P-loading of soils. Total P present in manure slurry was positively related with the Mg and the TAN content of manure slurry, which might relate to the potential presence of minerals (e.g. struvite) in manure slurry (Van der Stelt *et al.*, 2005).

'Free' nutrient content

Almost all Na present in manure slurry exists as 'free' ions (73-102%), whereas only small amounts of the divalent cations Ca and Mg were present in manure slurry as 'free' ions (1-10% and 0-19%, respectively). The relative 'free' Na content in the slurries correlates (Pearson= 0.714, $p < 0.05$) with the Na content of the feed (Table 4.1), which indicates that Na supply was sufficient during the feeding trial. The low 'free' Ca and Mg content are most likely caused by precipitation of minerals (e.g. CaCO_3 (calcite) and $\text{MgNH}_4\text{PO}_4 \cdot \text{H}_2\text{O}$ (struvite)) and/or adsorption to organic matter (Sommer and Husted, 1995).

Changes in the protein content or in the energy content/digestibility of the feed did not significantly affect the relative 'free' nutrient content of Na, Ca and Mg.

The TAN amounted to 52-77% of the total N content present in manure slurry. Both the TAN content and the organic N content of the slurries were higher ($p < 0.01$) at higher CP content of the feed. However the increase in TAN was 3 times higher than the increase in organic N, indicating that at higher CP content of the feed more N is excreted via the urinary tract. Similar observations were reported by Broderick (2003). In contrast to the observations of Broderick (2003) was the TAN content in our experiment higher for the high energy diets (ca. 32%; $p < 0.001$). This discrepancy is related to the higher N intake of the cows receiving the high energy diets, in combination with the physiological status of the cows. Unlike lactating cows, non-lactating cows are not able to use this extra N taken up efficiently through the production of milk. Therefore the extra N uptake has been excreted in urine, causing a higher TAN content in the slurry.

Both, the 'free' Ca^{2+} and the 'free' Mg^{2+} content were negatively correlated to the TAN content present in manure slurry (Ca^{2+} : Pearson= -0.462 ($p < 0.05$) and Mg^{2+} : Pearson= -0.514, ($p < 0.05$)). This is probably related to the exchange

of Ca^{2+} and Mg^{2+} from the liquid phase with NH_4^+ adsorbed to organic matter, which was previously described by Sommer *et al.* (2003).

Ammonia volatilization

The chemical composition of the (diluted) manure slurries used in the ammonia volatilization experiment is given in Table 4.3. The ionic strengths of the slurries were higher when either the protein content or the energy content of the feeding regimes was higher, ranging from 0.17 M in case of the straw based regime with a low protein content (LPLE_b), to 0.36 M for the regimes high in energy and high in protein content (treatments HPHE_a and HPHE_b). The ionic strengths of the slurries are within the range commonly found for cattle slurries (e.g. 0.1-0.4 M; Sommer *et al.*, 2003).

The pHs of the manure slurries at the start of the experiment ranged from 7.2 to 7.6 for the feeding regimes low in protein content and from 7.6 to 8.4 for the regimes high in protein content. Similar, Paul *et al.* (1998) and Külling *et al.* (2001) measured higher slurry pHs when dairy cattle were fed more CP. Slurry pHs were lower when slurries were obtained from feeding regimes containing a high energy content. At higher dietary energy content, more feed is taken in by the animal per unit of time (Table 4.1) and hence the passage rate of the feeds through the animal is higher. Feeding energy rich diets will stimulate volatile fatty acids formation in the hindgut, which will decrease slurry pH, as was shown to occur for energy rich diets in feeding trials with growing pigs (Canh *et al.*, 1998) and with lactating cows (Reynolds *et al.*, 2001).

The $\text{NH}_{3,\text{aq}}$ content of manure slurries produced from feeding regimes with a high CP content were considerably higher than the $\text{NH}_{3,\text{aq}}$ content of slurries produced from regimes with a low CP content (on average 10 times higher). Moreover, the range in $\text{NH}_{3,\text{aq}}$ content was quite variable for the slurries based on feeds containing a high CP content, e.g. varying from 0.8 to 4.2 g N kg DM⁻¹. Comparable differences in slurry $\text{NH}_{3,\text{aq}}$ content were calculated for slurries studied in feeding trials by Paul *et al.* (1998), e.g. $\text{NH}_{3,\text{aq}}$ content were more than 4 times higher when the feed contained 15.3% CP instead of 12.3%. The $\text{NH}_{3,\text{aq}}$ content of the slurries was hardly affected by change in energy content of the feed. The $\text{NH}_{3,\text{aq}}$ content of both straw based treatments (HPLE_b and LPLE_b) were 2 times higher than the other treatments containing a similar protein content of the feed. The higher $\text{NH}_{3,\text{aq}}$ content for the straw treatments are most likely caused by the high carbon content of the slurries (Table 4.2), which stimulated

the ammonification process (Külling *et al.*, 2001) and because of the higher pH, which shifted the $\text{NH}_4^+/\text{NH}_{3,\text{aq}}$ -equilibrium in favor of $\text{NH}_{3,\text{aq}}$.

Table 4.3. Chemical properties, dilution characteristics and the distribution of TAN¹ over NH_4^+ and $\text{NH}_{3,\text{aq}}$ of the manure slurries used in the ammonia volatilization experiment.

Code ²	DM ³ [%]	Dilution [-]	pH-H ₂ O [-]	I ³ [M]	N-components ⁴ [g N kg DM ⁻¹]			
					TAN ⁴	NH ₄ ⁺	NH _{3,aq}	% NH _{3,aq} TAN ⁻¹
HPHE _a	9.33 ^a	0.00	7.64 ^c	0.36 ^e	51.0 (66%)	50.2 (65%)	0.8	1.6
HPHE _b	10.18 ^{ab}	0.00	8.03 ^d	0.36 ^e	53.6 (71%)	51.5 (68%)	2.0	3.8
HPLE _a	13.49 ^c	0.00	8.24 ^{de}	0.28 ^d	32.3 (56%)	30.3 (53%)	2.0	6.1
HPLE _b	9.73 ^a	0.11	8.39 ^e	0.27 ^{cd}	49.5 (77%)	45.3 (70%)	4.2	8.4
LPHE _a	11.03 ^b	0.09	7.23 ^a	0.26 ^c	31.1 (59%)	30.9 (59%)	0.2	0.6
LPHE _b	11.19 ^b	0.07	7.17 ^a	0.20 ^b	31.0 (66%)	30.8 (65%)	0.2	0.6
LPLE _a	12.89 ^c	0.04	7.37 ^{ab}	0.18 ^{ab}	17.7 (52%)	17.5 (52%)	0.2	0.9
LPLE _b	10.39 ^{ab}	0.19	7.59 ^{bc}	0.17 ^b	26.5 (62%)	26.1 (61%)	0.4	1.5

^{a-e}Means within a column followed by different superscripts differ ($p < 0.05$).

¹TAN= Total Ammoniacal Nitrogen content ($\text{NH}_{3,\text{aq}} + \text{NH}_4^+$)

²The diets are described in more detail in Table 4.1.

³DM= Dry matter content (105 °C); I= Ionic strength.

⁴The percentages of TAN and NH_4^+ compared to the total N content of the manure slurries are given between brackets.

Figure 4.1a shows the mean cumulative NH_3 amounts volatilized per day from the different manure slurries incubated at 12 °C for 32 days. The absolute NH_3 amounts volatilized are expressed per cow per day to correct for differences in amounts of manure slurry produced per treatment. The amounts of NH_3 volatilized per cow per day varied for the low CP-treatments between 0.13 and 0.21 g NH_3 and for the high CP-content between 0.93 and 1.41 g NH_3 . Compared to previous studies the amounts of NH_3 volatilized in 32 days were low, e.g. Misselbrook *et al.* (2005) reported that similar amounts of NH_3 were volatilized from cattle slurry surfaces in 50 hours of incubation. Under normal conditions, NH_3 emissions from manure slurries are the combined results of diffusion and convectional processes occurring at the slurry surface (Sommer and Hutchings, 2001). In most laboratory studies NH_3 volatilization is simulated by transporting air over the slurry surface (as is the case for the study by Misselbrook *et al.* (2005)). In our experiment active transport of air was minimized, and thus NH_3 emissions due to convectional processes should be minimized. Hence, lower amounts of NH_3 will volatilize per unit of time. Extrapolating the measured data to conditions in which both, diffusional and convectional processes occur, is

problematic since diffusion is determined mainly by the concentration gradient (e.g. solubility of NH_3), whereas convection is mainly determined by air velocity and temperature. However, our method is suited to relatively compare NH_3 volatilization from different treatments and to assess the effects of dietary changes on NH_3 volatilization.

Between the low CP-treatments only a difference in volatilized NH_3 amounts was observed between the LPLE_a and LPHE_a slurries (ca. 38%; $p < 0.05$). At high protein content, more NH_3 volatilized (28% on average) from slurries based on feeding regimes with a low energy content than from regimes with a high energy content ($p < 0.05$), which could be correlated with the pHs of the slurries (Pearson = 0.953, $p < 0.01$). As was previously explained, at increasing energy content of the feed more volatile fatty acids will be produced in slurry, which will lower pH, thereby decreasing the $\text{NH}_3/\text{NH}_4^+$ ratio and thus decreasing the tendency for NH_3 volatilization. Model calculations by Kebreab *et al.* (2002) showed that total N excreted by lactating cows, as well as the urinary N fraction and NH_3 emissions are reduced when lactating cows are fed regimes with higher energy concentrations. Although in this trial more N was excreted by the non-lactating cows fed high energy containing feeding regimes, relatively more N was excreted via the feces (data not shown), which reduced ammonia emissions.

The effects of changing dietary CP or energy content on NH_3 emissions from the manure slurries become clearer when the (cumulative) NH_3 amounts volatilized were expressed in proportion to the amount of slurry produced (Figure 4.1b), or in proportion to the TAN content of the slurries (Figure 4.1c). Expressed per kg wet manure slurry, the NH_3 amounts volatilized from slurries based on low CP (108 g kg⁻¹ DM) feeding regimes were on average 78% lower than the NH_3 amounts volatilized from slurries based on high CP regimes (190 g kg⁻¹ DM). Külling *et al.* (2001) reported a 69% decrease in the rate of NH_3 emissions when cows were fed 125 g CP per kg DM instead of 175 g kg⁻¹. At high CP content, on average 66% more NH_3 volatilized from manure slurries based on low energy feeds. The differences in amounts of slurry excreted led to similar NH_3 emissions at low energy content when NH_3 is expressed as mg N- NH_3 per kg manure (e.g. HPLE_a and HPLE_b; 32 and 41 L day⁻¹), whereas the difference became more evident for the slurries at high energy content (e.g. HPHE_a and HPHE_b; 54 and 43 L day⁻¹). At low CP content, no effects of energy content of the feed on the amount of NH_3 emitted per kg of manure slurry were observed and amounts of NH_3 volatilized increased in the order: LPLE_a ≤ LPHE_b < LPHE_a ≤ LPLE_b.

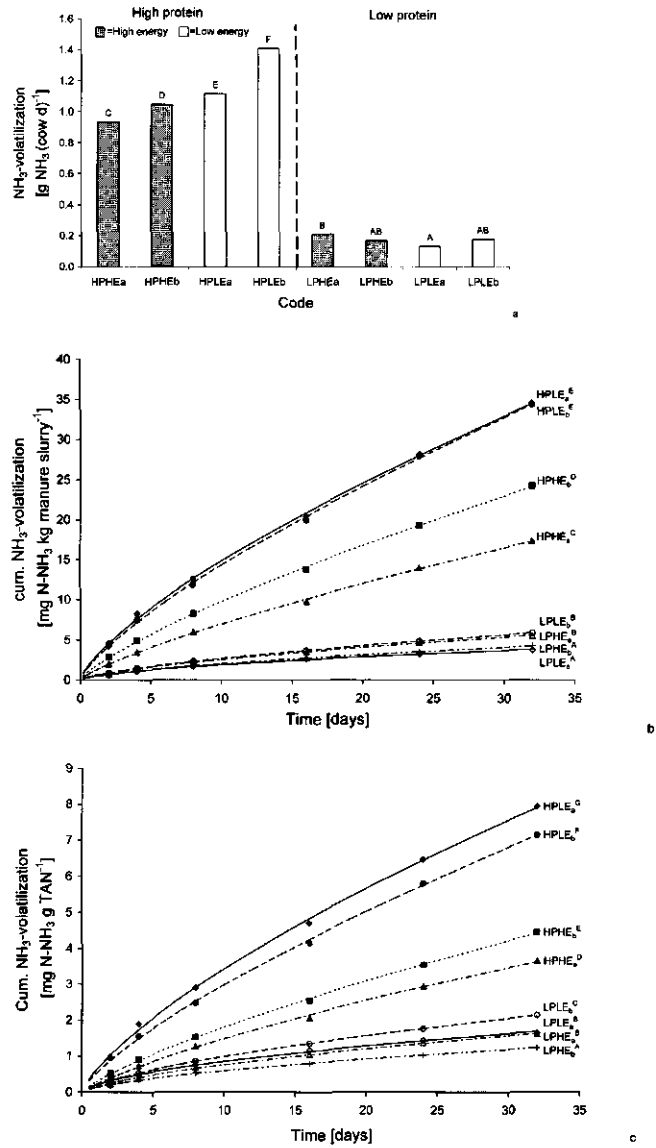


Figure 4.1. The cumulative NH₃ emissions of the different manure slurries incubated at 12 °C for 32 days: a) expressed as g N-NH₃ per cow per day; b) as mg N-NH₃ per kg manure slurry; and c) as mg N-NH₃ per g TAN. Points denote measured data, lines represent modelled NH₃-emissions. The dietary compositions on which the slurries are based are described in more detail in Table 4.1. Different indices in the graph denote differences ($p < 0.05$) between the treatments.

When NH_3 emissions are expressed as a proportion of the TAN content of the slurries (Figure 4.1c), the order of the NH_3 emissions reflects the four dietary groups. However at low CP content, no statistical differences ($p < 0.05$) in amounts of NH_3 volatilized were observed between slurries LPHE_a and LPLE_a. Again, NH_3 emissions are much lower when the CP content of the feedings regimes were lower (71%) and increasing the energy content of the regimes resulted in lower NH_3 emissions, both at high (86%) and low (33%) CP content of the feeding regimes.

Dietary changes to improve manure quality and hence reduce environmental losses are primarily based on reducing CP content and increasing the fibre content (lowering the net energy content of diets). Our data confirm that lowering dietary CP content can be an effective way to reduce N emissions. In contrast, the effects of increasing the fibre content of the feeds on NH_3 volatilization from manure slurry are not as well defined. Increasing the fibre content of the diet will in general lower the OM content which is fermented in the rumen. Dry matter intake and the passage rate of OM will be lower, which will reduce the efficiency of microbial N synthesis in the rumen (Oba and Allen, 2003). Hence, more N in the rumen will be transformed into NH_3 , which will subsequently be transformed into urea and is (partly) excreted via the urinary tract.

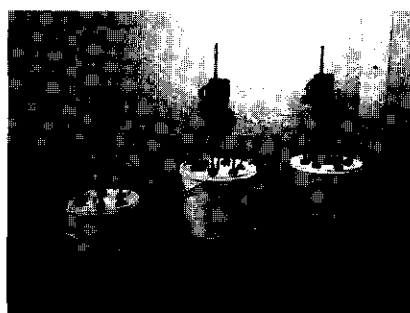
Conclusions

Adjusting dietary composition is considered to be an important way to improve the sustainability of farming. Changes in the protein content or the energy content of the feed, did not significantly affect the 'free'-to-total ratios of Na, Ca and Mg content of the slurries.

Lowering the dietary CP content markedly decreased the total N and total P content of slurries and reduced the potential risk of NH_3 volatilization. Compared to the CP content, the effects of reducing the energy content on manure composition and ammonia volatilization were not as straightforward. Total N, total P and TAN content in manure slurry decreased, but the amount NH_3 volatilized increased.

5

Measurement of anions using the Donnan Membrane Technique and its application to manure slurry



B. van der Stelt, E.C.H.M. van Boekel, E.J.M. Temminghoff, L. Weng,
and W.H. van Riemsdijk
Department of Soil Quality, Wageningen University, The Netherlands

To be submitted

Abstract

The Donnan membrane technique (DMT) was tested for the application to measure anion speciation, using an anion exchange membrane instead of a cation exchange membrane. Besides testing the technique in synthetic solutions, the technique was also tested in three manure slurries. Insight in the chemical speciation of manure nutrients can help to improve nutrient management and to reduce nutrient losses to the environment. The DMT tests in the synthetic solutions showed that Donnan equilibrium time was, as expected, affected by the charge of the of the P species: for H_2PO_4^- it took about five days to reach 95% of the Donnan equilibrium concentration, whereas for HPO_4^{2-} it took about sixteen days. Moreover, a study with a multi-component system (NH_4NO_3 , K_2HPO_4 , K_2SO_4) showed that Donnan equilibrium time increased, besides charge, with the effective diffusion coefficients of the different species. Humic acid (representing complicated anionic molecules) was not transported over the membrane, but citrate (representing a simple small organic molecule) was. Next, the cell was tested in three manure slurries. After seven days of incubating dissolved 'free' P concentrations were rather constant, indicating that Donnan equilibrium for P was reached. For each slurry, the 'free' dissolved P concentration (largely HPO_4^{2-} at pH 8) was less than 1% of the total P concentration. The percentage 'free' S concentration (largely HS^- at pH 8) of one slurry was less than 2%, while the 'free' S concentration of the other two slurries were 9 and 13%, respectively.

Introduction

The Donnan Membrane Technique (DMT) can be used to measure charged ion concentrations that can move freely through the environment (e.g. 'free' ions). Recently the DMT technique has been further developed (Temminghoff *et al.*, 2000) and applied to measure the concentration of free cations in soil solutions (Weng *et al.*, 2001; Cancès *et al.*, 2003), in surface waters (Temminghoff *et al.*, 2000; Kalis *et al.*, 2006b; Kalis *et al.*, 2007), and in manure slurries (Van der Stelt *et al.*, 2005). The progress has been due to a better theoretical understanding of the technique in combination with a lowering of the detection limit (Weng *et al.*, 2005; Kalis *et al.*, 2006b) and by the development of new cell designs (Van der Stelt *et al.*, 2005). The objective of the present study was to determine if DMT could be used to measure 'free' anion concentrations, by using an anion-exchange membrane instead of a cation-exchange membrane. In theory, replacing the cation exchange membrane with an anionic exchange membrane would result in the opposite effect, hence transport of 'free' anions over the membrane. The suitability of the cells for anion measurement was first tested in synthetic solutions. Next, the DMT technique was used to measure 'free' anion concentrations in manure slurries. Finally, the availability of P and S in the manure slurries was determined by sequential fractionation.

In the past few decades the intensity and productivity of livestock farming systems in the Netherlands increased considerably. This increase was mainly realized by large inputs of imported feed and fertilizers (Van Keulen *et al.*, 2000; Oenema, 2004). Since nutrient use efficiencies of cows are low, varying between 15 and 30% for nitrogen (N), phosphorus (Aarts *et al.*, 1992) (P) and sulphur (S; McNeill *et al.*, 2005), most nutrients fed will be excreted by the animal in the feces or via the urinary tract. In dairy farming systems, feces and urine are commonly stored together as slurry, which additionally may contain some waste water and bedding material. Excessive land application of manure slurry has had a negative impact on the environment, leading to environmental problems like soil acidification, eutrophication (Tilman *et al.*, 2001) and metal contamination (Nicholson *et al.*, 1999; Brock *et al.*, 2006). To reduce the environmental impact of agricultural activities, the government imposed restraints on the use of nutrients and on the quantities of manure produced (Neeteson, 2000), which led to a rediscovery of manure as a valuable source of nutrients.

Besides biologically controlling processes, nutrient bioavailability and transport in soils is also determined by the chemical speciation of nutrients in

both, manure slurry and soil. Hence, insight (qualitatively as well as quantitatively) in the chemical species of the various nutrients in animal manure can contribute to an effective nutrient management. Most research has been focused on the composition of N in manure, as it is the most abundant nutrient in manure and N losses can occur to ground- and surface waters, as well as to the atmosphere (Rotz, 2004).

Phosphorus speciation in manure is often studied by sequential fractionation, sometimes combined with spectrometry. Dou *et al.* (2002) observed an increase in total P content of dairy feces, as well as an increase in the proportion of the water soluble P fraction, when dietary P levels were raised by feeding P containing minerals. The water-soluble P fraction has been shown to be linearly related with the amount of P leached from manure and compost (Sharpley and Moyer, 2000). Most of the P present in animal slurry is inorganic, *e.g.* making up for 75 to 95% of the total slurry P content (Dou *et al.*, 2000; Sharpley and Moyer, 2000; Dou *et al.*, 2002; He *et al.*, 2004). Nevertheless, organic P has frequently been found to be the main P fraction in soil leachates (Chardon *et al.*, 1997; McDowell and Koopmans, 2006).

Excretion of S from domestic animals is estimated to correspond to about 13% of the world S production (Eriksen, 2002). Sulphate (SO_4^{2-}) is very mobile and can easily be lost from fertilized soils by leaching (McNeill *et al.*, 2005). During storage of manure under anaerobic conditions, SO_4^{2-} can be transformed by microbes into organic S and gaseous compounds, which are susceptible for volatilization (Eriksen, 2002). Emissions of hydrogen sulphide from animal slurries during storage, mixing and treatment can cause a human health hazard (Stevens *et al.*, 1993).

Speciation of nutrients in manure slurry can be affected by the pre-treatment of the slurry, *i.e.* drying of manure has been shown to affect the speciation of P (Chapuis-Lardy *et al.*, 2004). In contrast, the DMT technique is a speciation technique, which can measure 'free' ionic species, without (hardly) affecting the substrate.

Experimental

Donnan Membrane Technique

In the DMT technique 'free' ion concentrations are determined by separating the solution of interest (donor) from an acceptor solution using a charged ion-exchange membrane. When 'free' anionic species are to be determined, a positively charged anion-exchange membrane has to be used, which allows the transport of negative charged ions over the membrane, while transport of positive charged ions and neutral complexes is restricted. In time, 'free' anion concentrations in the acceptor solution become in (Donnan) equilibrium with 'free' anion concentrations in the donor solution. To determine the 'free' anion concentrations in the donor solution, the measured 'free' anion concentrations in the acceptor solution have to be corrected for the difference in ionic strength between both solutions (Helleferich, 1962):

$$[c_{i,d}] = [c_{i,a}] \cdot \left[\frac{c_{Cl,d}}{c_{Cl,a}} \right]^{z_i} \quad (5.1)$$

where c_i represents the concentration of anion i in the donor (d) or in the acceptor (a) solution, c_{Cl} represents the concentration of chloride (Cl) and z_i is the charge of anion i . Chloride is used to correct for differences in ionic strength between donor and acceptor solution, because it hardly forms any complexes, but is almost entirely present as 'free' ions.

The anion concentration in the acceptor solution at a certain moment can be calculated by (Temminghoff *et al.*, 2000):

$$[c_{i,a}]_t = [c_{i,a}]_{t_0} + ([c_{i,a}]_{t_\infty} - [c_{i,a}]_{t_0}) \cdot (1 - e^{-bt}) \quad (5.2)$$

where c_i represents the 'free' anion concentration of anion i in the acceptor (a) solution, while b is a constant related to ion transport kinetics. The parameters t , t_0 , and t_∞ stands for the starting time (t_0), the time of interest (t), and the time when Donnan equilibrium is reached (t_∞), respectively.

Recently the kinetics of the DMT for measurement of 'free' trace cation concentrations in aqueous solutions were studied in more detail by Weng *et al.* (2005). According to Weng *et al.* (2005) Donnan equilibrium time is either controlled by diffusion in solution, by diffusion in the membrane, or by both diffusion processes. When diffusion in solution controls Donnan Equilibrium time and i) all the species of the ion of interest diffuse with the same diffusion coefficient; ii) the total-to-'free' ratio of the ion of interest is the same for the donor and ac-

ceptor solution; and iii) mass of the ion of interest in the membrane is neglected; the Donnan equilibrium time can be determined by:

$$t_{95\%} = -Ln(0.05) \frac{V_{acc} \delta}{A_e D_i} \quad (5.3)$$

where $t_{95\%}$ is the time [s] after which the concentration in the acceptor solution is at 95% of the Donnan Equilibrium concentration, V_{acc} is the volume [m³] of the acceptor solution, δ is the thickness [m] of the diffusion layer in solution, A_e is the effective surface area [m²] of the membrane; and D_i represents the diffusion coefficient of ion i [m² s⁻¹] in water.

When diffusion in the membrane limits transport and i) the 'free' ion is the (only) dominating species; ii) the total-to-'free' ratio of the ion of interest remains constant; and iii) mass of the ion of interest in the membrane is neglected; Donnan equilibrium time can be calculated by:

$$t_{95\%} = -Ln(0.05) \frac{V_{acc} \delta_m P_{i,acc}}{A_e D_{i,m} B^{|z|}} \quad (5.4)$$

where $t_{95\%}$ is the time (s) after which the concentration in the acceptor solution is at 95% of the Donnan Equilibrium concentration; V_{acc} is the volume [m³] of the acceptor solution; δ_m is the thickness [m] of the membrane solution; $P_{i,acc}$ is the total to 'free' ratio of the ion of interest; A_e is the effective surface area [m²] of the membrane; $D_{i,m}$ represents the apparent diffusion coefficient of ion i [m² s⁻¹] in the membrane; B is the Boltzmann factor [-] for the Donnan phase in the membrane; and z is the charge [-] of the ion.

When membrane diffusion is limiting the transport and Donnan membrane equilibrium has not been reached, the 'free' ion concentration in the donor solution can be calculated by:

$$C_{i,don} = \frac{C_{i,acc}}{P_{i,acc} \left(1 - e^{-\frac{A_e D_{i,m} B^{|z|}}{P_{i,acc} \delta_m} t}\right)} \quad (5.5)$$

in which $C_{i,don}$ represents the 'free' ion concentration [mol m⁻³] in the donor solution.

In the situation that the measured 'free' ion concentration is far from the Donnan equilibrium concentration, hence the 'free' ion concentration in the acceptor solution is much lower than in the donor solution, and transport of the ion is limited by membrane diffusion, the Donnan equilibrium concentration can be calculated by:

$$C_{i,don} = \frac{V_{acc}}{A_e} \frac{\delta_m}{D_{i,m} B^{|z_i|}} \frac{C_{i,tot,acc}}{t} \quad (5.6)$$

DMT-manure cell

The cell used to characterize 'free' anionic species in manure slurries is identical to the one used by Van der Stelt *et al.* (2005) to measure 'free' cations in manure slurry, except that in this case positively charged anion-exchange membranes (BDH 55164 2S) have been used. The membranes consist of a matrix of polystyrene, cross-linked with divinylbenzene, to which quaternary ammonium ($-NR_3^+$) groups are attached (BDH, 1981). The membranes are 0.11-0.14 mm thick. Before each experiment all materials were cleaned, by successively washing with 0.1 M HCl, Ultra Pure Water (UPW), 10 times concentrated acceptor solution, UPW, and 4 times the acceptor solution. All other materials were washed with 0.1 M HCl, UPW, and the acceptor solution.

Synthetic solutions

Speciation of P

In this experiment the time needed to reach Donnan equilibrium for solutions that are dominated by different species of P was investigated. Therefore two synthetic solutions (5 L each) consisting of 0.3 M KCl were prepared. The ionic strength of the synthetic solutions was chosen to be similar to that found in cattle slurries (Sommer *et al.*, 2003). Each solution was divided into two parts, 0.5 L was used as acceptor solution and 4.5 L was used as donor solution. Both donor solutions were transferred to 5 L buckets, and to each KH_2PO_4 was added to a final concentration of 15 mM P. The amount of P added was based on the average amount of P that can be found in manure slurry (Mooij, 1996). The pHs of the donor solutions were set at pH 4.5 and 9.8, respectively, by using NaOH. At pH 4.5, more than 97% of the added P is present as $H_2PO_4^-$, while at pH 9.8 almost all P ($\approx 99\%$) is present as HPO_4^{2-} , as was calculated using the speciation program Ecosat (Keizer and Van Riemsdijk, 2002). To maintain the pH of the donor solution at pH 9.8 nitrogen gas flowed continuously through the system. Per bucket, three cells filled with acceptor solution were immersed in the synthetic solution. At 0, 2.5, 5, 24, 48, 72, 168, and 336 hours after the start of the experiment, both, the acceptor and the donor solutions were sampled. Phosphorus concentrations were measured by inductively coupled plasma atomic

emission spectrometry (ICP-AES; Varian, Vista-pro), while Cl was measured with a chlorocounter (Marius Instrumenten, Nieuwegein, the Netherlands). At each sampling moment, pHs of the acceptor, as well as pHs of the donor solutions were measured using a combined glass-calomel electrode.

Multi-component system

Next, the time needed to reach Donnan equilibrium for some anions in a multi-component system was determined. The set-up of the experiment was identical to the previously described experiment (see Speciation of P). However, in addition to KH_2PO_4 , also K_2SO_4 and NH_4NO_3 were added to the synthetic (donor) solution, to final concentrations of 15 mM P, 25 mM S, and 93 mM N- NO_3^- , respectively. In this experiment the pH of the solution was set, using NaOH, on 5.4.

Simultaneously, it was tested if the presence of organic molecules would affect Donnan equilibrium time in a multi-component system. Purified humic acid (HA) from forest floor material of the Tongbersven forest (The Netherlands; Temminghoff *et al.*, 1997) was used to test if complicated organic anionic molecules would be transported over the membrane. Therefore, purified HA was added to one bucket of synthetic solution, to a final concentration of 100 mg L^{-1} .

The transport of small negatively charged organic acids over the membrane was investigated using citrate. A synthetic background solution consisting of 0.3 M KCl was prepared. Additionally, citrate was added to the donor solution to a final concentration of 22 mM citrate. To prevent degradation of citrate by microorganisms during the incubation, a bactericide (NaN_3) was added to both, the donor and the acceptor solution, to a final concentration of 1 mM. Besides citrate, NaCl was added to the donor solution to a final concentration of 108 mM to test if Na^+ would be transported over the membrane. The pH of the synthetic solution containing citrate was set at 5.5. Speciation calculations (Keizer and Van Riemsdijk, 2002) showed that at this pH about 37% of the citrate is trivalent, 59% is divalent, either because of protonation (ca. 49.9%) or because of formation of complexes with Na (ca. 9%) and about 4% of the citrate is monovalent due to protonation.

The synthetic and acceptor solutions were sampled, 0, 2.5, 5, 24, 48, 72, 168, 216, and 336 hours after the start of the experiment. Phosphorus, S, and Na were measured by ICP-AES, whereas, NO_3^- , NH_4^+ , and (total) C were measured by SFA.

Manure slurry

Manure slurry analysis

Electric conductivities (EC), dry matter contents (DM 105 °C), organic matter contents (OM), redox-potentials and pHs of the manure slurries were determined at the beginning of the experiment. The EC was measured using a conductivity meter (Eijkelkamp 18.21 multi-parameter analyzer) and was subsequently converted into the ionic strength (I) of the slurries by using the equation described by Griffin and Jurinak (1973):

$$I \cong 0.013 \cdot EC \quad (5.7)$$

in which I is expressed as mol L⁻¹ and EC as millimhos cm⁻¹ (at 25 °C). The organic matter content of the slurries was determined by loss-on-ignition. Redox-potential was measured using a platinum-electrode (Radiometer analytical, MC305 1Pt-9) and pH was measured using a combined glass-calomel electrode.

To determine total nutrient concentrations in the slurries, the slurry samples were first dried at 70 °C for three days and subsequently ground (<1 mm). Prior to the drying, the slurries were acidified below pH 4, in order to prevent N volatilization during drying (Velthof *et al.*, 2005; McDowell and Koopmans, 2006). Total N and P were analyzed by SFA, after the dried slurry material was digested with H₂SO₄, salicyclic acid, H₂O₂ and selenium (Novozamsky *et al.*, 1983). To determine the total S content of the slurries, the dried material was digested (in a microwave) with HNO₃, H₂O₂ and HF (Novozamsky *et al.*, 1996) and analyzed by ICP-AES. The Cl concentrations of the slurries were measured on a chlorocounter in the supernatant of manure samples. Therefore, the slurries were centrifuged at 15000 rpm and 4 °C for 30 minutes, after which the samples were filtered (<0.45 µm). Also the total ammoniacal nitrogen content (TAN= NH₄⁺ + NH_{3,aq}) and the dissolved organic carbon content (DOC) were measured by SFA in the supernatant of the slurries.

Experimental set-up

Three slurries were obtained from a feeding trial in which different diets (Table 5.1), varying in energy and protein content, were fed to non-lactating cows (Reijs *et al.*, 2007). Slurry I was produced from a diet rich in protein content and rich in energy content and the main ingredients were maize (60%) and young grass (40%). Slurry II came from cows that were fed low digestible grass

silage, which had a high protein content and a low energy content. Finally, slurry III originated from a diet consisting of low digestible grass silage, with a low protein content and a low energy content.

After collection, the manure slurries were stored at 4 °C until the start of the experiment. It is assumed that at this temperature microbial degradation of the slurries is minimized. Before the slurries were used, the slurries were homogenized by mixing and subsequently 11 kg of each slurry was transferred to a 15 L bucket and placed in a conditioned room, at 20 °C. Three DMT-manure cells were placed in each bucket. During the experiment the slurries were continuously mixed to prevent settling of solids. To minimize volatilization of gasses, the buckets were closed by lids, which contained small openings to allow mixing. The acceptor solution consisted of 0.3 M KCl. From each DMT-manure cell 3 ml of the acceptor solution was sampled at day 0, 7, 11, and 14. At the same time about 100 ml of manure slurry was collected from each slurry (in triplicate). The pH of all samples was determined. The acceptor solutions were directly analyzed for P, S, and Ca by ICP-AES, while the slurry samples had to be pretreated first (see Manure slurry analysis).

Table 5.1. Main dietary characteristics (including organic matter digestibility) of the manures tested in the experiment.

Diet	Forages ^a	Intake [kg DM d ⁻¹]	Concentrates ^b	Intake [kg DM d ⁻¹]	C/N ^c [-]	Dietary characteristics ^d [kg ⁻¹ DM]				
						NEm	CP	NDF	ADF	Dig. OM
	Type		Type			[kJ]	[g]	[g]	[g]	[%]
I	HDGS	9.3	40% S + 60% M	3.4	13.0	6800	199	405	247	80
II	LDGS	7.4	100% S	2.8	14.3	5300	188	483	324	68
III	LDGS	7.2	19% S + 81% B	2.6	22.5	5170	110	539	350	64

^aForages: HDGS= High Digestible Grass Silage; LDGS= Low Digestible Grass silage.

^bConcentrates: S= Soybean meal; M= Maize; B= Beet pulp.

^cTotal Carbon-to-Nitrogen ratio of the diets.

^dDietary characteristics: NEm= Net Energy for maintenance; CP= Crude Protein;

Dig. OM= Digested Organic Matter; NDF= Neutral Detergent Fiber; ADF= Acid Detergent Fiber.

P and S fractionation

Manure slurries I, II, and III were fractionated following the procedure described by He *et al.* (2004). Therefore, part of the slurries sampled at day 14 was dried (70 °C) and ground without acidification, so P and S speciation would not be affected. From each slurry 0.3 g dried material was first extracted at 22 °C for 2 h with 30 ml of UPW, using an orbital shaker (250 rpm). Samples were then

centrifuged at 15000 rpm for 1 h at 4 °C. The supernatant was decanted and filtered through an 0.45 µm filter. Subsequently, the remaining fraction was extracted for 16 h with 30 ml 0.5 M NaHCO₃. After the extraction, the samples were again centrifuged for 1 h at 15000 rpm, decanted and filtered (0.45 µm). Finally, the remaining fraction was extracted for 16 h with 0.5 M NaOH, centrifuged, after which the supernatant was decanted and filtered again. Total P and S-concentrations were measured by ICP-AES. The experiment was performed in triplicate.

Statistical analysis

The data have statistically been analyzed using the computer program "Statistical Package for the Social Sciences" (SPSS®; SPSS Inc., 2003). If necessary, data were transformed to obtain equality of variances.

Results and discussion

Synthetic solutions

Speciation of P

During the first hours of the experiment the pHs of both acceptor solutions increased rapidly, reaching in both cases a similar pH as the synthetic solutions approximately one day after the start of the experiment (data not shown). In contrast, the increase in P concentration in the acceptor compartment of the DMT-manure cell was much slower and depended on the charge of the P species (Figure 5.1). From Figure 5.1 and equation 5.2 it could be calculated that 95% of the Donnan equilibrium concentration was reached in about five days for H₂PO₄⁻ and in about sixteen days for HPO₄²⁻. Under the assumption that the charge density of the anion exchange membrane is the same as the absolute charge density of the cation exchange membrane used to determine 'free' cation concentrations in surface waters (Weng *et al.*, 2005), the difference in Donnan equilibrium time between the two species of P could be explained to be the result of both, a slower diffusion rate of HPO₄²⁻ ($0.439 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$) compared to H₂PO₄⁻ ($0.879 \cdot 10^{-9} \text{ m}^2 \text{ s}^{-1}$; Marcus, 1997) and more accumulation in the membrane. The pH of manure slurry is normally in the range 7 to 8 (Sommer *et al.*, 2003). At pH 7, about 37% of the total 'free' inorganic P concentration exists as H₂PO₄⁻ and about

63% as HPO_4^{2-} , whereas at pH 8 about 5% is present as H_2PO_4^- and the rest is present as HPO_4^{2-} . In solution, H_2PO_4^- and HPO_4^{2-} are rapidly transformed into each other. Therefore, it is assumed that Donnan equilibrium time for P in manure slurry will be between five and sixteen days.

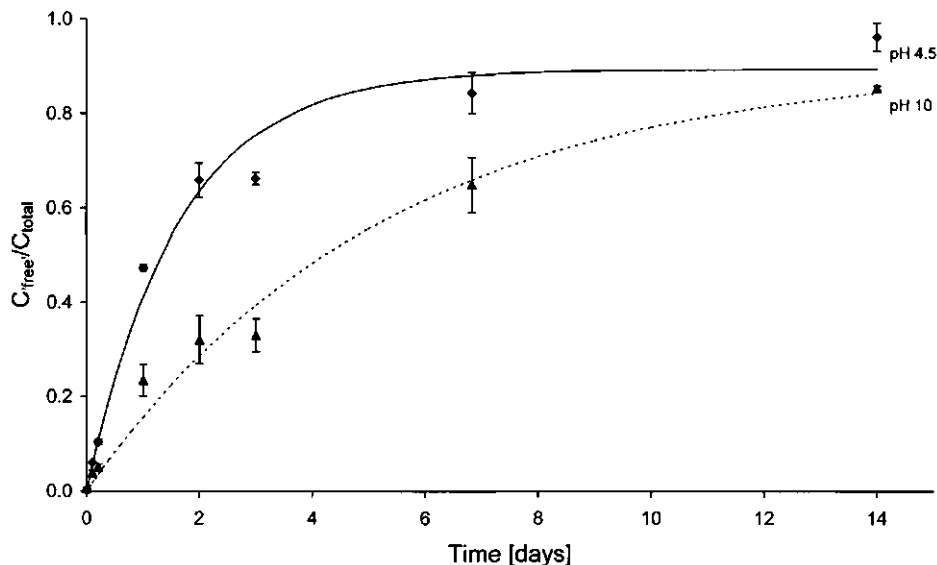


Figure 5.1. Ratios of the 'free'-to-total P concentrations measured by DMT as a function of time at pH 4.5 (species H_2PO_4^-) and pH 9.8 (species HPO_4^{2-}). Lines were calculated using equation 5.2 and vertical bars represent standard deviations.

Multi-component system

There were large differences in Donnan equilibrium time for the different species studied in the multi-component system, as can be seen in Figure 5.2. Within a half day, the NO_3^- concentration was already at 95% of the Donnan equilibrium concentration. In contrast, about 3 days were needed for S to obtain 95% of its Donnan equilibrium concentration and about six days for P. The pH of the synthetic solution was set at 5.4, at which P is mainly present ($\approx 94\%$) as H_2PO_4^- , while S is present as SO_4^{2-} (62.5%) and as KSO_4^- (37.5%). Compared to the first experiment, Donnan equilibrium time for HPO_4^- was about one day longer, which is likely caused by the extra sampling session at day 9 of the experiment, because the ratios 'free'-to-total P concentration measured at the sampling sessions before day 9 were comparable.

The ratio 'free'-to-total concentration at which Donnan equilibrium was reached was not equal to one (as was expected) and differed between the species. At each sampling round, about half of the acceptor volume was removed for analysis and replaced with fresh acceptor solution. Consequently, the concentration in the remaining acceptor solution was reduced by approximately 50%. As can be derived from equation 5.2, Donnan equilibrium time depends on the concentration difference between the donor and acceptor solution. Compared to the initial situation, this concentration difference becomes smaller and thus Donnan equilibrium time increases. It is likely, that the frequency of sampling has resulted in a kind of pseudo-Donnan equilibrium. However, this is somewhat in disagreement with the fast Donnan equilibrium time found for NO_3^- .

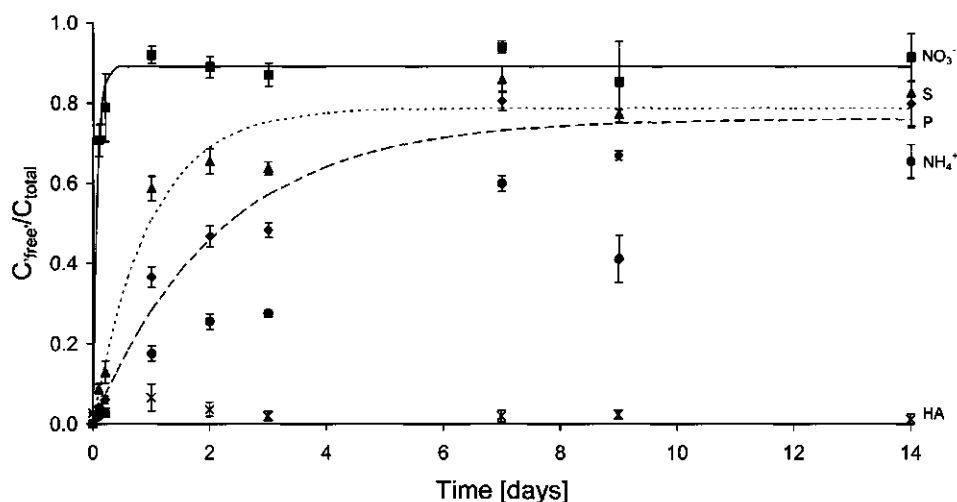


Figure 5.2. Ratios of the 'free'-to-total concentrations of the compounds studied in the multi-component system as a function of time. Lines were calculated using equation 5.2 and vertical bars represent standard deviations.

Despite the positive charge of the membrane, Na^+ and NH_4^+ were transported over the membrane into the acceptor solution, but both species did not reach Donnan equilibrium within 14 days of the experiment (Figure 5.2 and 5.3). Due to the positive electrostatic potential of the membrane, the concentration of cations in the membrane is in principle lower than in the solution. In combination with an apparent diffusion coefficient that is very likely lower than in the solution, the cation transport is controlled by diffusion in the membrane, which is

slower than in the solution. The Na^+ measured at the acceptor side may also be due to transport over the membrane of Na complexed as negatively charged citric species.

Addition of HA to the multi-component system had no influence on Donnan equilibrium time and speciation of the components in the system (Figure 5.2). The amount of HA transported over the membrane was negligible, which is likely due to the relatively large size of the molecules.

In contrast, citrate was easily transported over the membrane (Figure 5.3), reaching 95% of its Donnan equilibrium concentration within four days. The transport of citrate over the membrane suggests that other small organic acids, like volatile fatty acids (VFAs), can also pass the membrane.

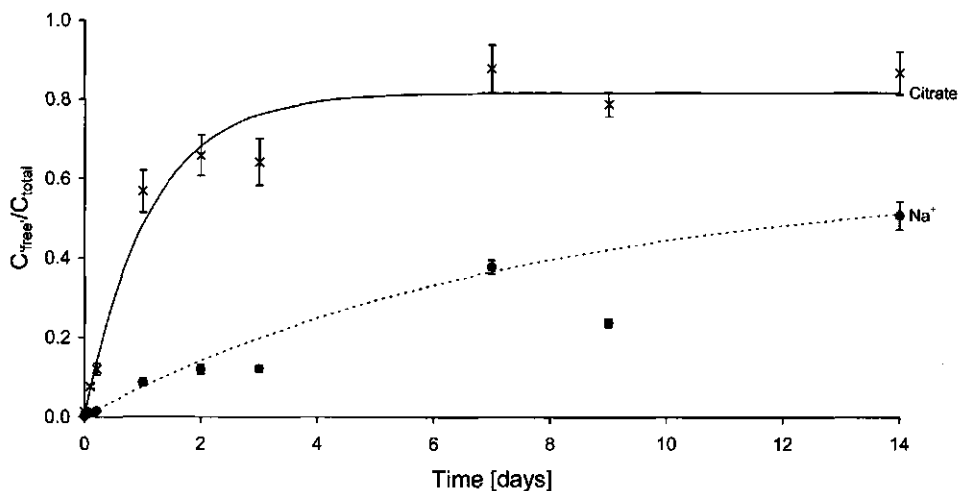


Figure 5.3. Ratios of the 'free'-to-total concentrations of citrate and Na measured by DMT as a function of time. Measured data are represented by symbols (including error bars), whereas lines represent the calculated ratios (using equation 5.2).

Manure slurry

Slurry characteristics

The main initial slurry characteristics are presented in Table 5.2. The DM (dry matter) content of slurries I and II are in line with DM content normally found in dairy slurries produced in the Netherlands ($DM = 8.7 \pm 4.0\% \text{ ww}^{-1}$; Blgg, 2005). In contrast, the DM content of slurry II was significantly higher ($p < 0.05$), but corresponded with DM content reported by Holter and Urban (1992) for slurries produced by (pregnant) dry cows (13.4% and 14.9%, respectively), when it was assumed that urine contained 3.5% DM (Oenema *et al.*, 2000). The OM content of slurries II and III were significantly higher ($p < 0.05$) than the mean OM content found in Dutch dairy slurries ($OM = 6.5 \pm 2.2\% \text{ ww}^{-1}$; (Blgg, 2005)). Due to chemical and biological processes (e.g. CO_2 release), occurring continuously during the experiment, the pHs of the slurries increased in 14 days with 0.6 pH-unit for slurries I and II and with 1.5 pH-unit for slurry III. The ionic strengths of the manure slurries were within the range commonly found for animal slurry ($0.1\text{--}0.5 \text{ mol L}^{-1}$), as reported by Sommer *et al.* (2003). Despite of the mixing of the slurries, the redox potentials measured in the slurries, three days after the start of the experiment, were between -384 and -285 Eh , which indicates that the effect of mixing on the anaerobic slurry environment was minimal.

Table 5.2. Some characteristics^a of the (initial) manure slurries used in the experiment.

Slurry	DM [%]	OM [%]	pH [-]	I [Mol L ⁻¹]	Redox [Eh]
I	10.4 ^a 0.2	8.0 ^a 0.0	7.65 ^b 0.09	0.41 ^y 0.02	-384 ^a 14
II	14.2 ^y 0.2	10.8 ^y 0.0	7.69 ^b 0.07	0.37 ^b 0.01	-285 ^b 21
III	12.9 ^b 0.0	9.6 ^b 0.0	7.03 ^a 0.01	0.24 ^a 0.00	-287 ^b 19

^{a-y}Means within a column followed by different superscripts differ ($p < 0.05$).

^aSlurry characteristics: DM= dry matter content (105 °C); OM= organic matter content; I= Ionic strength.

DMT experiment

Total P, total N, and the Tan content of slurries I and II were higher ($p < 0.05$) and those of slurry III similar to the mean total P, N, and TAN content found in (waste water free) dairy slurries produced in the Netherlands (Table 5.3). Taking the limited number of slurries investigated in this study into account, the higher amounts of P, N, and TAN found in slurries produced from diets with a high protein content (e.g. slurries I and II), were likely caused by the fact that extra nutrients consumed by the (non-lactating) cows, were not used for milk production or for growth, but were rather excreted by the animal.

In contrast, the S content of slurries I, II, and III were in the range of 4.4 to 21.9 mM, which is within the range commonly found for manure S (Eriksen *et al.*, 1995). About 40% of the N present in slurry III was of mineral origin, whereas in slurries I and II the TAN fraction was about 55%. In agreement with our observations, Paul *et al.* (1998) reported an increase in NH_4^+ concentrations with increasing protein content of the diet.

'Free' dissolved P concentrations did not systematically increase with sampling time, but were rather 'constant' after 7 days of incubation, indicating that Donnan equilibrium for P was reached well within the 14 days of measurement. Less than 1% of the P content present in manure slurries I and II was present as 'free' dissolved P. Since slurry pHs were above 8, HPO_4^{2-} would be the dominating inorganic P species. Unfortunately, due to the dilution of the acceptor samples, which was required to meet the conditions of the ICP-AES, 'free' dissolved P concentrations could not be determined for slurry III. The 'free' dissolved S fraction in slurry III was less than 2% of total S, but the 'free' dissolved S fractions of slurry I and II were considerably higher, 13% and 9%, respectively. Since all slurries were anaerobic (Table 5.2), 'free' dissolved S was likely to exist in all slurries as sulphide (HS^-). Stevens *et al.* (1993) observed an increase in sulphide concentrations with an increase in protein content of the feed, which is in agreement with our observations; although in our case it could not be tested for significance.

The DOC concentrations varied between 1191 and 1506 mM and were significantly higher ($p < 0.05$) for the slurries produced from diets with a high protein content. 'Free' ionized C concentrations measured by DMT in the slurries were similar, varying from 151 to 210 mM (Table 5.3). When it is assumed that all C originated from monovalent VFAs, the total VFA concentration present in the slurries could be estimated. Since the final pH for each slurry was above 8,

nearly all VFA present in the slurry was present in an ionized form. VFA concentrations were within the VFA concentration range found for cattle slurries (2-206 mM) by Sommer and Husted. (1995).

As mentioned above (section Multi-component system), NH_4^+ and Na^+ are to some degree transported over the positively charged membrane. Since the low binding affinities of these species, their presence on equilibrium concentrations of anionic species is small. To check if divalent cations could also pass the positive charged membranes, Ca concentrations in the acceptor solutions were determined at the start and at the end of the experiment. The Ca concentrations did not increase during the experiment, suggesting that the double layer overlap in the pores of the membranes is sufficient to prevent free diffusion of Ca. Due to its higher positive charge, Ca^{2+} is repelled stronger by the positive electrostatic potential of the membrane, leading to much smaller Ca^{2+} transport over the membrane than for Na^+ .

Table 5.3. Total and 'free' nutrient concentrations of P, S, C, and N present in the manure slurries studied.

Slurry	Nutrients [mM]							
	P		S		C		N	
	Total	'free'	Total	'free'	DOC	'free' ^a	Total	TAN ^b
I	47.1 ^y	0.5 ^β	17.9 ^β	2.9 ^y	1462 ^β	210 ^α	540 ^β	295 ^β
	<i>3.7</i>	<i>0.1</i>	<i>0.7</i>	<i>0.5</i>	<i>120</i>	<i>27</i>	<i>34</i>	<i>5</i>
II	41.1 ^β	0.3 ^α	17.0 ^β	1.9 ^β	1506 ^β	172 ^α	586 ^β	319 ^β
	<i>4.2</i>	<i>0.1</i>	<i>0.5</i>	<i>0.5</i>	<i>116</i>	<i>71</i>	<i>35</i>	<i>7</i>
III	24.5 ^α	^c	12.1 ^α	0.2 ^α	1191 ^α	151 ^α	312 ^α	125 ^α
	<i>2.6</i>		<i>0.6</i>	<i>0.0</i>	<i>32</i>	<i>38</i>	<i>20</i>	<i>7</i>
Ref. ^d	23.0	-	-	-	-	-	308	169
	<i>14.6</i>	-	-	-	-	-	<i>164</i>	<i>104</i>

Standard deviations are given in italics.

^{α,γ}Means within a column followed by different superscripts differ ($p < 0.05$).

^aIonized C 'species' measured by DMT.

^bTotal ammoniacal nitrogen (TAN = $\text{NH}_4^+ + \text{NH}_3, \text{aq}$).

^cLess than the detection limit of the ICP-AES.

^dMean composition of wastewater free dairy cattle slurry in the Netherlands (Bggg, 2005).

P and S fractionation

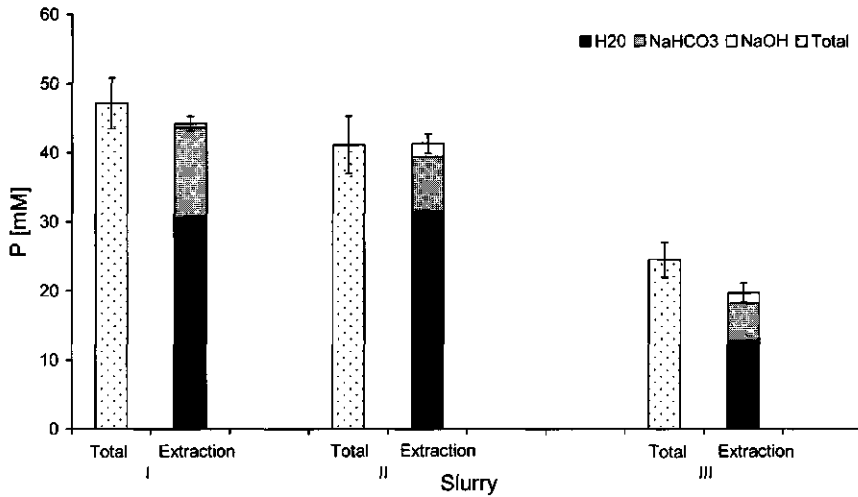
Sequentially extracting the manure slurries with H_2O , NaHCO_3 and NaOH resulted in P fractions of 52-77%, 19-29% and 1-6% (Figure 5.4a), respectively. Similar P distributions in dairy slurries were reported by Dou *et al.* (2000) and Sharpley and Moyer (2000).

The low 'free' P concentrations measured with DMT in this experiment suggests that in manure most water-extractable P is either present in a mineral form as precipitates, or as a constituent in (dissolved) organic matter (*e.g.* phospholids, simple phosphate monoesters) that do not pass the membrane. Phosphate containing minerals found in cattle slurry are struvite ($\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$), trimagnesium phosphate ($\text{Mg}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$), octacalcium phosphate ($\text{Ca}_8\text{H}(\text{PO}_4)_3 \cdot 3\text{H}_2\text{O}$), dicalcium phosphate ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) (Sharpley and Moyer, 2000). The dissolved 'free' P concentrations measured in slurries I and II are several times higher than the minimum 'free' P concentration (0.02 mM) required for precipitation of struvite, which was calculated for a similar cattle slurry by Van der Stelt *et al.* (2005). Phosphorus can also be present in ammonium calcium organic precipitates ($\text{NH}_4\text{CaPO}_4 \cdot \text{H}_2\text{O}$; Sharpley and Moyer, 2000).

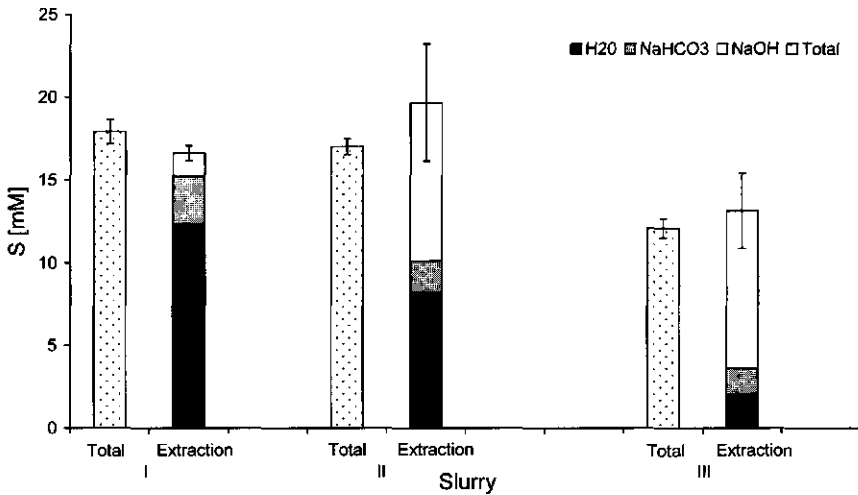
The added amounts of P sequentially extracted in slurries I and II agreed with the total P content of those slurries. In contrast, a substantial part of P (ca. 20%) remained in the solid phase of slurry III. The P extracted with H_2O and NaHCO_3 is commonly considered to represent the readily soluble pool (Turner and Leytem, 2004), although it is questioned if the complete organic P fraction in both, the water extracts (Hayes *et al.*, 2000), as well as in the NaHCO_3 extracts (Hayes *et al.*, 2000; He and Honeycutt, 2001) should be included. In this experiment more than 90% of P was readily soluble in case of slurries I and II, and about 75% in case of slurry III.

Compared to P, the distribution of S over the different extracts was more distinct (Fig. 5.4b). The amount of S extracted by H_2O was 74% of the total S content for slurry I, 42% for slurry II and 16% for slurry III. Extraction with NaHCO_3 resulted in similar amounts of S which were extracted, ca. 17%, 9% and 12% for slurries I, II and III, respectively. For all slurries, the remaining S was extracted with NaOH , hence 9% for slurry I, 49% for slurry II and 72% for slurry III. The range in readily soluble S, assuming that, analogous to P, the readily soluble S fraction consists of the H_2O and NaHCO_3 extractable S fractions, is also much wider, ca. 85% for slurry I, ca. 59% for slurry II, and ca. 30% for slurry III and

seems to correspond with the nutrient richness of the diet (Table 5.1). However, this effect could not be tested for significance, because of the limited number of slurries studied.



a



b

Figure 5.4. Total content and fractions of P (a) and S (b) extracted by sequential fractionation, for the manure slurries collected at the end of the DMT-experiment. Vertical bars represent standard deviations.

Conclusions

The Donnan Membrane Technique is a relatively easy method to determine speciation of nutrients in synthetic solutions and manure slurries, because soluble 'free' anions are determined in a simple salt solution, instead of in the complex slurry mixture. The design of the cell is such that its usefulness is not restricted to manure slurries alone, but can be used in other aqueous solutions, for instance surface waters.

In this study it was shown that soluble 'free' anion concentrations could be determined within a reasonable time frame. Donnan equilibrium time varied from shorter than half a day for NO_3^- to about sixteen days for HPO_4^{2-} . Also, small organic acids were transported over the membrane and as they might contain P it can result in some over estimation of the 'free' soluble P content.

The three manure slurries investigated in this study were obtained from a feeding trial in which non-lactating cows were fed diets mainly varying in either protein, or energy content. Only a small part of the readily soluble amounts of P (<2%) and S (<16%) are present in a 'free' dissolved ionic form as determined by DMT. In contrast, the readily soluble amounts of P and S, as determined by sequential fractionation, were much higher, ranging from 74% to more than 90% for P and from 30% to 85% for S. In general 'free' concentrations of P, S, and C increased with increasing protein content of the diet, but this could not be statistically tested, because of the limited number of slurries used.

6

Effects of organic matter content on nitrogen dynamics in grassland soils



B. van der Stelt, P.C.J. van Vliet, E.J.M. Temminghoff,
and W.H. van Riemsdijk
Department of Soil Quality, Wageningen University, the Netherlands

Submitted

Abstract

In a field trial, combined with incubation experiments, was investigated how nitrogen (N) dynamics in grasslands were affected by differences in soil organic matter content (SOM). It was expected that N mineralization increased with increasing SOM content and that N-mineralization at higher SOM content would occur more gradually. In total, 18 grasslands situated on sandy soils varying in SOM content were selected on 9 dairy farms. The fields were managed according to the farmers own farming style. Potential N mineralization rates of the soils (e.g. incubated at 20 °C and 60% water holding capacity) could be predicted well using a model based on the initial dissolved organic nitrogen (DON) content of the soil. Potential N mineralization rates of the soils were also related to the dissolved organic C (DOC) content of the soils. Nitrogen mineralization rates of soils incubated under field conditions were weakly related to the DOC (June samples), or to the DON content (September samples), or to none of the measured soil characteristics (April samples). Total soluble N (N_{ts}) was present during the whole growing season. During the growing season two peaks in N_{ts} were observed for all grassland soils, which were preceded (in both cases) by a period of drought. No relationships were observed between the peaks in N_{ts} and the time of manure application. Maximum N_{ts} and NO_3^- content were higher in soils with a lower SOM content. This is probably caused by faster water depletion of soils with a lower SOM content during drought periods, which hampers N uptake by plants. No effect of organic manure management on soil N mineralization was observed. Despite of the large fertilizer quantities supplied to the grasslands, N leaching was minimal and was for all grasslands below the legal standard of 50 mg L⁻¹.

Introduction

Agriculture is an important branch in the Netherlands, more than 10% of all economic activities are related to agriculture and it covers about 60% of the total surface area of the Netherlands (CBS, 2006b). The main sector in Dutch agriculture is dairy farming, which combines plant and animal production. During the sixties and seventies of the last century milk production at dairy farms intensified considerably by increasing inputs of fertilizers and concentrates. Concurrently, the nutrient use efficiency by the animals decreased, leading to high excretions of nutrients. As a consequence, large nutrient surpluses were applied to farmland, and nutrient losses to the environment increased (Neeteson, 2000).

Nitrogen (N) is one of the most important nutrients related to environmental problems. Soils which receive surpluses of N are susceptible for ammonia (NH_3) volatilization, nitrate (NO_3^-) leaching to groundwater, N runoff to surface waters, and nitrous oxide (N_2O) emissions during denitrification (Tilman *et al.*, 2001).

To minimize the environmental impact of dairy farming, several European policies and measures have been implemented, of which the most important are the Agenda 2000, the Water Framework Directive (including the Nitrate Directive) and the EU Air Quality Directive (NH_3 and N-oxides; Oenema *et al.*, 2004). The main objectives of these policies are to ensure that NO_3^- concentration in upper groundwater do not exceed 50 mg L^{-1} , NH_3 emissions from manure to the atmosphere are not higher than $30 \text{ kg N (ha year)}^{-1}$ and N_2O emissions are below $3 \text{ kg N (ha year)}^{-1}$.

To meet environmental legislation demands dairy farmers are forced to change their management style and to implement N conserving technologies. Nutrient losses reduced by adjustments based on a whole-farm approach are preferred over compartment based adjustments, because an integrated approach will reduce the risks of transferring nutrient losses from one compartment to another compartment of the farming system (Zhu, 2000).

The initial results obtained at the experimental farm 'De Marke' showed that strict environmental demands can be obtained within short time, using a whole farm approach, while productivity is maintained (Aarts *et al.*, 2000; Van Keulen *et al.*, 2000).

Farmers within the VEL-VANLA project also try to reduce nutrient losses (especially N) to the environment using an integrated approach (Verhoeven *et al.*, 2003). The main characteristics of this approach are reducing the crude

protein content and increasing the fiber content of the feeds, which will result in manure slurry with a lower N content, of which a higher fraction is present as organic N. The lower inorganic N content of the manure slurry will lower the amounts of N volatilized to the atmosphere during housing, storing and application of the slurry. To fertilize optimal, to restrict environmental losses, insight in the N-supplying capacity of the soil is required. Besides soil characteristics, the N-supplying capacity of the soil depends on climatic conditions and on field management. The aim of this study is to investigate how nitrogen availability during the growing season is affected by differences in soil organic matter (SOM) content and manure management within the VEL-VANLA project.

We hypothesize that N-mineralization not only increases with increasing SOM content, but that a higher SOM content will also lead to a more constant N-mineralization. To test the hypothesis 18 grasslands within the VEL-VANLA project, located on 9 farms (2 grasslands per farm), were selected for a field trial. The 18 grassland soils were of similar texture, but varied in SOM content. Grasslands located on the same farm were applied with the same manure. The fields were managed by the farmers according to their own practice, although all farming practices were conform the VEL-VANLA approach.

During the growing season field measurements were carried out at regular time-intervals, in which samples were taken from both, soil and grass, to study N availability. Additionally, at four times soil N mineralization rates were determined by incubation studies (including respiration measurements and counting of bacteria), once under optimized conditions and three times under field conditions. Furthermore, it was tested if the empirical relationship found by Bregliani *et al.* (2006) between N mineralization and the dissolved organic N (DON) content in arable soils could be used to predict N mineralization in grassland soils. Finally, the obtained data were used to derive mineral N balances for the different fields.

Materials and Methods

Site description and field selection

The field experiment was conducted at 9 dairy farms located in the Vel-Vanla area (Figure 6.1), the Netherlands, during the 2004 growing season. At each farm 2 sandy soils were selected, which belonged to the cHn23 soil series (StiBoKa, 1981), which can be classified as Plagganthreptic Alorthod soils (Soil Survey Staff, 1998). The soils had similar groundwater tables (Figure 6.1) and had been in use as grasslands for at least 10 years.

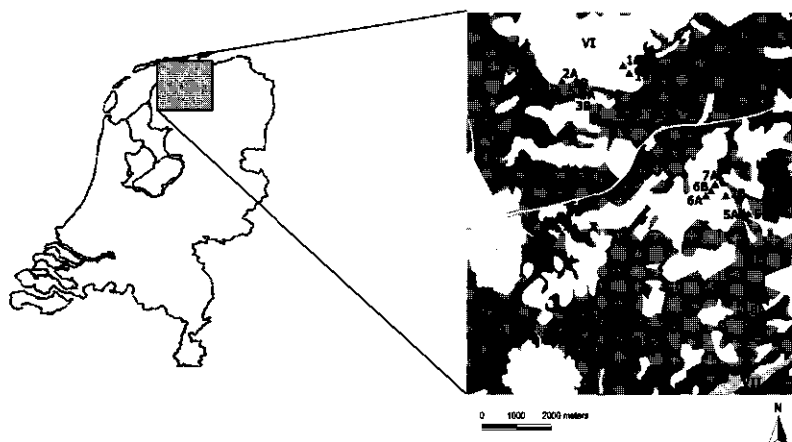


Figure 6.1. Location of the grasslands in the Netherlands (56:16°N; 6:05°E). The numbers represent different farms. Letters A and B correspond (per farm) to grassland plots with a low (A) or a high (B) organic matter content. Roman numerals represent different groundwater table classes.

Some soil characteristics of the different fields investigated are given in Table 6.1. The silt fraction ranged from 11 to 19.5% and the clay content was for all soils less than 7%. The organic matter (OM) content ranged from 5.7 to 11.2% ww^{-1} , which is quite a broad range considering that all fields had the same soil type.

The pHs of most soils studied were within the range 4.8-5.5. In agricultural soils, pH is usually measured in a 1 M KCl extraction solution. In contrast, in this study soil pH was measured in a 0.01M CaCl₂-extraction solution. However, no difference in soil pH was measured when both extraction methods were applied to the same soil (data not shown). Soils within the 4.8-5.5 pH-range are considered to be optimal soils for managing grasslands (LTO, 2005). The carbon and N content varied between 3.25-5.98% and 0.27-0.48%, respectively, and the C-to-N ratio varied between 11.3-15.4.

Manure slurries produced at farms 6-9 were enriched with the additive Euro Mest-mix®. This additive is claimed, by the producers, to improve manure characteristics and to reduce NH₃ volatilization. No additives were applied to manure slurries from farms 1-5.

Table 6.1. Soil properties and total nitrogen and carbon content of the soils studied in the field trial.

Nr.	Silt (2-50 μ m) [%]	Clay (<2 μ m) [%]	O.M. [% ww ⁻¹]	pH _{CaCl2} [-]	%C	%N	C/N [-]
Depth (cm)	0-20	0-20	0-10	0-10	0-10	0-10	0-10
1a	11.1	4.2	5.68	4.98	4.21	0.33	12.53
1b	16.2	4.3	7.43	5.22	3.25	0.27	12.06
2a	19.5	5.7	7.89	4.69	4.38	0.34	12.90
2b	17.1	6.8	9.81	4.62	5.67	0.48	11.95
3a	17.1	6.8	8.32	4.84	3.82	0.30	12.91
3b	16.8	5.7	10.45	4.95	5.72	0.47	12.10
4a	15.2	3.3	6.77	5.45	3.44	0.27	12.72
4b	17.3	5.4	8.44	5.40	4.53	0.40	11.32
5a	12.0	4.1	9.06	5.11	4.26	0.36	11.81
5b	17.8	4.8	11.12	4.72	5.92	0.45	13.30
6a	15.8	5.1	8.63	4.53	4.44	0.36	12.49
6b	16.9	2.5	9.60	4.78	4.94	0.39	12.63
7a	13.1	4.4	7.82	5.41	3.51	0.28	12.62
7b	13.1	5.6	10.14	5.05	5.09	0.44	11.55
8a	15.7	4.9	10.38	5.00	5.98	0.50	12.12
8b	13.0	3.4	11.05	5.06	5.07	0.41	12.36
9a	11.0	4.7	7.83	5.26	4.31	0.28	15.39
9b	13.8	5.7	11.22	5.32	5.94	0.48	12.39

Manure slurry analysis

Manure slurry was collected at each farm during spring, except at farm 9, because at the time of collection no slurry was in storage at the latter farm. Prior to manure analysis, all slurries were homogenized, acidified to $\text{pH} < 4$ (to avoid N volatilization (Velthof *et al.*, 2000)) and dried at $105\text{ }^{\circ}\text{C}$ for at least 3 days. After drying, the slurries were ground ($< 1\text{ mm}$). Next, the slurry samples were digested by H_2SO_4 , salicyclic acid and H_2O_2 (Novozamsky *et al.*, 1983) to measure total N content. Total N content was measured by segmented-flow-analysis (SFA; Skalar). Ammonium (NH_4^+) and NO_3^- content were measured by SFA after (dried and ground) slurry samples were extracted (1:10) with distilled water. Total organic carbon content of the manure slurries were measured by Kurmies (Heanes, 1984). The composition of manure slurry produced at farm 9 has been estimated by calculating the average nutrient content of the manure slurry produced at this farm from 1999-2004. The amount of N applied to the grasslands by means of artificial fertilizers was calculated using the 'composition descriptions' given by the manufacturers.

Soil sampling and preparation

Within each grassland soil, a plot covering a surface area of $240\text{--}250\text{ m}^2$ was selected. At regular intervals during February-October 2004 bulk soil samples of the 0-10 cm soil layer were collected, each consisting of 50 replicate soil cores. A steel nematode-auger was used for soil sampling. The bulk soil samples collected in February were dried by air, homogenized and sieved ($< 2\text{ mm}$) to remove root material. All other soil samples taken were homogenized and sieved ($< 5\text{ mm}$) while they still were moist and were stored at $4\text{ }^{\circ}\text{C}$ until they could be analyzed, or they could be used in the incubation experiments.

Incubation studies

Next to the field measurements, N dynamics in the 18 soils were studied in more detail by means of two sets of incubation experiments: i) an incubation study at the beginning of the growing season under optimal mineralization conditions to determine the potential N mineralization and ii) Incubation studies at 3 sampling times (April, June and September), in which soils were incubated under field conditions, measured at the time of sampling.

To determine potential N-mineralization rates, dried soil samples from the February sampling session were rewetted with demineralized water to field capacity (e.g. 60% of the water holding capacity). Per soil, 12 bottles were filled with an amount of soil comparable to 50 g of dry weight soil, closed with a wad of cotton and preincubated at 20 °C for 5 days.

At 0, 4, 6 and 12 weeks after start of the experiment 2 bottles per soil were taken and the amount of CO₂ respired, N and C mineralized, and the amount of bacteria present in the samples were measured (see next sections). Each week the soil samples were flushed with compressed air to prevent accumulation of CO₂. After flushing, the soil samples were rewetted with distilled water to maintain a constant moisture level. The net potential N mineralization rates were calculated using linear regression of the mineral N content with time from weeks 2, 6 and 12.

For the incubations under field conditions 50 g of moist soil was transferred to a bottle (4 bottles for each soil), which was (again) closed with a wad of cotton. The moisture content of the soils, sampled during the April, June and September sampling rounds, were on average 37%, 19% and 33%, respectively. Incubation temperatures, representing the soil temperature at the time of sampling, were calculated using the equation:

$$X_b = 0.7X_a + 3.5 \quad (6.1)$$

in which X_b corresponds to the soil temperature in the 10 cm soil layer below the surface (Verschoor, 2001). The air temperature (X_a) and precipitation data were obtained from a nearby weather station (KNMI, 2004). The calculated soil temperatures at the sampling rounds of April, June and September, were 8 , 14, and 15 °C, respectively.

The results from the potential N mineralization studies indicated that pre-incubating the soils for 5 days was too short to overcome the disturbance of the soil. Therefore pre-incubation time for the incubation studies under field conditions were extended to 1 week. In contrast to the potential N mineralization incubation, soils were sampled only 2 times, at the beginning (week 0) and 6 weeks after the start of the experiment. Again the soils were analyzed for respired C, mineralized C and N. However, the amount of bacteria present in the soil samples were only determined for the incubations studies performed on soils from the April sampling session. Net mineralization rates were calculated by subtracting the mineral N content at week 0 from the mineral N content at week 6, divided over time (6 weeks).

Soil analysis

Dry matter content of the soils were determined by drying the soil samples at 105 °C for 3 days. Organic matter content of the soils were determined by loss-on-ignition (550 °C). A part of the dried and ground soil samples, collected during the February and October sampling sessions, were milled (<250 µm) and analyzed for total C and total N content, using a CHN-analyzer (Fisons Instruments EA 1108). Silt and clay data were obtained by the sieve-pipette method, which was carried out by the Agricultural laboratory Northern Netherlands. Dissolved nitrogen concentrations (N_{ts} , NO_3^- and NH_4^+) were measured by SFA, after 4 g of moist soil had been extracted with 30 ml 0.01 M $CaCl_2$ (Houba *et al.*, 2000). DON was calculated by subtracting NO_3^- and NH_4^+ concentrations from N_{ts} concentrations. Dissolved organic carbon (DOC) was measured on a TOC/DOC analyzer (Skalar). Soil pH was measured in the extracts by a combined glass electrode. All soil analyses were performed in duplicate and for each soil sample the moisture content was determined.

CO₂-respiration

The amount of CO₂ respired in the soils was determined on a photo-acoustic multi-gas monitor (Innova Air Tech Instruments). One day before each measurement the soil samples were flushed with compressed air, after which the bottles were closed with a septum. 24 hours after closing the bottles, CO₂ respiration was measured.

Microbial analyses

The bacterial biomass present in the soil samples was determined by confocal laser scanning microscopy and image analysis (Bloem and Vos, 2004). In contrast to this method, soil samples were dried during the night after 10 µL soil suspension was smeared out evenly on a glass slide. After drying, procedures were followed according to the method described by Bloem and Vos (2004).

The Microbial Biomass Quotient (MBQ), which is a measure for the activity of micro-organisms in soil, was calculated by dividing the amount CO₂ respired by the micro-organisms in time over the amount of biomass present in soil.

Grass yield and nutrient concentrations

The amount of grass produced during the growing season was determined for each field by cutting 1 m² of grass. The grass samples were taken from within a cage, which prevented grazing by the cows. After sampling, the remaining grass inside the cage was cut and removed from the cage. The collected grass samples were dried at 70 °C, after which they were ground. Total N content of the grass samples were determined in a similar way as the total N content of the manure slurries were. Furthermore, farmers were asked to administrate all field activities on a 'grassland calendar'.

N mineral balances

For each field a mineral N balance was constructed, which can be described as a 'soil system' type of balance. This type of balance is considered suitable to identify the fate of nutrient surpluses (Oenema *et al.*, 2003).

N applied to soil by means of artificial fertilizer or manure slurry, or removed by harvesting, were determined by field measurements (see above sections). The amount of N supplied to the soil during grazing was estimated by multiplying grazing time with the number of animals allowed grazing and with the average N excretion per animal (LNV, 2006), corrected for the size of the grazed fields.

From the national monitoring network it was derived that atmospheric deposition of N in the 'VEL-VANLA area' was $\approx 23 \pm 2$ kg N (ha year)⁻¹ (CBS, 2005). Nitrogen input via legumes (e.g. clover) was negligible for all fields. It was assumed that mineralization of roots in the beginning of the growing season was similar to root mineralization at the end of the growing season, so no net root mineralization occurred.

Ammonia volatilization was considered dependent on the manure application technique and was estimated to be $6 \pm 2.9\%$ of the mineral N content of the applied manure slurry when slurry was injected into the soil (farms 1 and 5) and $20 \pm 26.9\%$ when slurry was applied to the soil by narrow-band application (Huijsmans *et al.*, 2001). Furthermore, 1.6% of the applied amount of fertilizer was estimated to volatilize (Harrison and Webb, 2001). In accordance with Velthof and Oenema (2001), the mineral N content of the soils sampled in autumn (e.g. October soil samples) were taken as an indicator for the amount of N which is lost from the soil by leaching and denitrification. Soil mineralization during the growing season, including mineralization of organic N in manure, was

calculated as the weighted mean of the actual mineralization rates measured during the growing season.

Modelling N mineralization

A good correlation ($R^2 = 0.87$) was found by Bregliani *et al.* (2006) between the organic N amount mineralized during a 60 days incubation of 18 arable Dutch soils at field capacity and the initial DON content of the incubated soils, as determined by CaCl_2 -extractions. The data could be described with the equation (Bregliani *et al.*, 2006):

$$[N_{\text{org min}}] = 7.769 \cdot [\text{DON}] - 0.646 \quad (6.2)$$

in which the amount organic N mineralized ($N_{\text{org min}}$) and DON are expressed in mg N kg^{-1} dry soil.

In this study, equation 6.2 was used to predict soil N mineralization rates for the soils incubated under optimized conditions (e.g. 20 °C and field capacity). N mineralization was estimated using the DON content measured in the air dried soil samples of the February sampling session. Subsequently, the calculated N mineralization rates were compared with the measured N mineralization rates.

Statistical analysis

Statistical analysis were conducted with the software package SPSS (SPSS Inc., 2003). Stepwise linear regression was used to study the relationships between N mineralization rates and soil characteristics. Mean values were compared by using t-tests at the 0.05 probability level.

Results

N-availability during the growing season

The grasslands were fertilized regularly during the growing season. From the first of February till around the 24th of March grasslands were mainly fertilized by manure slurry. A second round of manure slurry was applied after the first grass harvest (Figure 6.2; period III) and a third round of manure was applied during the second half of August till the first half of September. On average $204 \text{ kg N (ha year)}^{-1}$ was applied to the grasslands by manure slurry application

(Table 6.2), of which most was applied during the spring season. The total N content of manure slurries, which were enriched with Euro Mest-mix®, were lower ($p < 0.05$) than the total N content of slurries to which no additive was added.

Artificial fertilizer was especially applied from the second half of March till the first half of August. The total N amount applied to grassland by artificial fertilizer ranged between 56 and 192 kg N (ha year)⁻¹, and was more or less equally distributed over the growing season.

There was a big difference in grazing time between the fields, varying from no or only a few days of grazing till a grazing time of more than 70 days. Also grazing intensity varied considerably between the fields, ranging from 0 till 125 cows ha⁻¹. As a consequence the amount of N supplied to the soil by animal excretion during grazing varied between 0 and 277 kg N ha⁻¹. Removal of N by grass harvesting and grazing of animals were in the range of 315 and 536 kg N (ha year)⁻¹. No difference in N removal by grass relative to the total N input was observed between soils to which manure, enriched or not enriched with Euro Mest-mix®, was applied. Root biomass increased with increasing OM content of the soils ($p < 0.05$). However, a significant increase in grass yield with increasing OM content was only observed during the sampling session of June.

The pH of the soils remained constant during the growing season. Bulk density decreased and moisture content of the soils increased with increasing OM content of the soils ($p < 0.05$). The availabilities of soil N components and DOC throughout the growing season were for all studied grasslands similar, although the amounts of components varied. Figure 6.2 shows the mean content of N components and DOC measured at the different sampling dates. Measurements from the February sampling session were excluded because those samples were air dried before analysis and N_{ts}, NH₄⁺, DON, and DOC content were 2 to 8 times higher compared to the values measured in the (field moist) soil samples taken at the other sampling dates.

Table 6.2. Management characteristics of the different grasslands for the 2004 growing season.

Characteristic	Avg	Min-Max
Manure application [kg N (ha year) ⁻¹]	204	69-340
Artificial fertilizer application [kg N (ha year) ⁻¹]	133	56-192
Manure deposit during grazing [kg N (ha year) ⁻¹]	106	0-277
Harvest [kg N (ha year) ⁻¹]	452	381-536

In general, NH_4^+ content was low during the growing season, except when measurements were carried out within a few days after manure slurry application, which primarily occurred during the first sampling session of June. Total soluble N and NO_3^- content varied during the season with a high peak in June and a smaller peak in September. Total soluble N peaks in June were higher ($p=0.08$) for soils with a lower SOM content. The trend for the N_{ts} peaks in September were also higher for soils with a lower SOM content. In line with the incubation studies, DON and DOC content remained rather constant from April till October, but DOC content were higher when soils contained more organic matter ($p<0.05$).

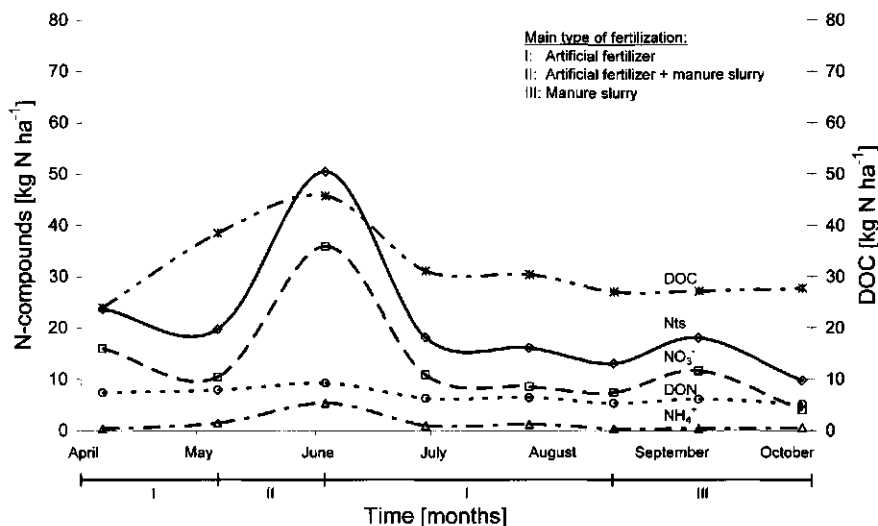


Figure 6.2. The mean availabilities of total soluble N (N_{ts}), nitrate (NO_3^-), ammonium (NH_4^+), dissolved organic nitrogen (DON), and the dissolved organic carbon (DOC) content of the moist soils studied in the field experiment. Main type of fertilizer applied to the grasslands during the growing season are represented by roman numbers (I-III).

N mineralization

Potential mineralization rates predicted by the model of Bregliani (eq. 6.2) correlated very well ($R^2 = 0.86$; see Figure 6.3a) with the potential mineralization rates measured in the incubation experiment under optimized conditions, although the calculated mineralization rates were slightly higher. Soils 6A, 6B, 8A,

and 9B were excluded because manure slurry was already applied before soil sampling occurred.

Figure 6.3b represents the change in N compounds, DOC and pH in soil 1a during the 12 weeks of incubating under optimized conditions. Similar changes were observed for all other soils. The pH's of the soils decreased on average with more than 0.4 pH-unit during incubation. Total soluble N and NO_3^- content increased strongly in the first 2 weeks of incubation, after which N_{ts} and NO_3^- content increased linearly in time. Ammonium content were only measured in the soil solution at the start of the incubations, whereas DON and DOC content remained rather constant during the experiment. It was concluded that pre-incubating for 5 days was too short to overcome the disturbance of the soil environment due to handling of the soil. Therefore potential N mineralization rates have been determined by linear regression of mineral N content measured at weeks 2, 6 and 12. The potential mineralization rates varied between 0.61 and 1.36 mg N (kg dw soil day) $^{-1}$ and were reasonably related ($R^2 = 0.64$) to the DOC-content of the soils, as is shown in Figure 6.4. The potential mineralization rates could also be related to the OM content ($R^2 = 0.24$), total C ($R^2 = 0.25$); total N ($R^2 = 0.27$) and the DON content ($R^2 = 0.37$) of the soils, although the correlations were rather poor.

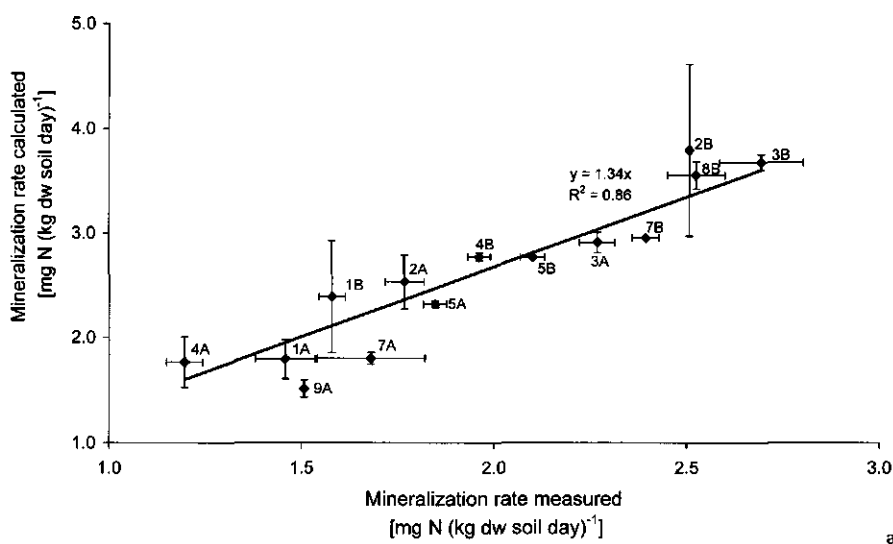
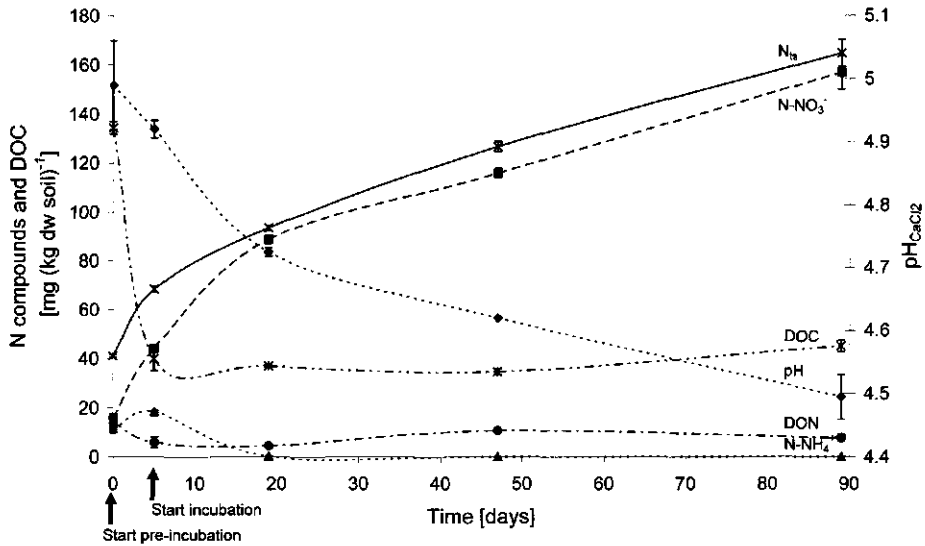


Figure 6.3a. Comparing N mineralization rates calculated according to the empirical relationship described by Bregliani *et al.* (2006) with measured N mineralization rates in incubated soils at 20 °C and field capacity. Standard deviations are represented by error bars



b

Figure 6.3b. Change in content of N species (N_{ts} , NO_3^- , NH_4^+ , and DON), DOC, and the pH during the incubation of soil 1a. Standard deviations are represented by error bars.

Nitrogen mineralization rates measured in soils incubated under field conditions were near the mineralization rates determined under optimal conditions (e.g. 20 °C and field capacity). On average, N mineralization rates were ca. 78% in April, 90% in June and 66% in September compared to the N mineralization rates under optimized conditions. Nitrogen mineralization rates of soils incubated under field conditions were weakly related to either the DOC, or the DON content, except for the mineralization rates of soils sampled in April, which could not be related to any of the soil characteristics measured. In June, N mineralization rates were related to the DOC content of the soil solutions ($R^2 = 0.35$), but not to the DON-content of the soil solutions. Including an additional parameter into the regression analysis did not increase the relationship. In September, N mineralization rates could only be related to the DON-content of the soil solutions ($R^2 = 0.20$).

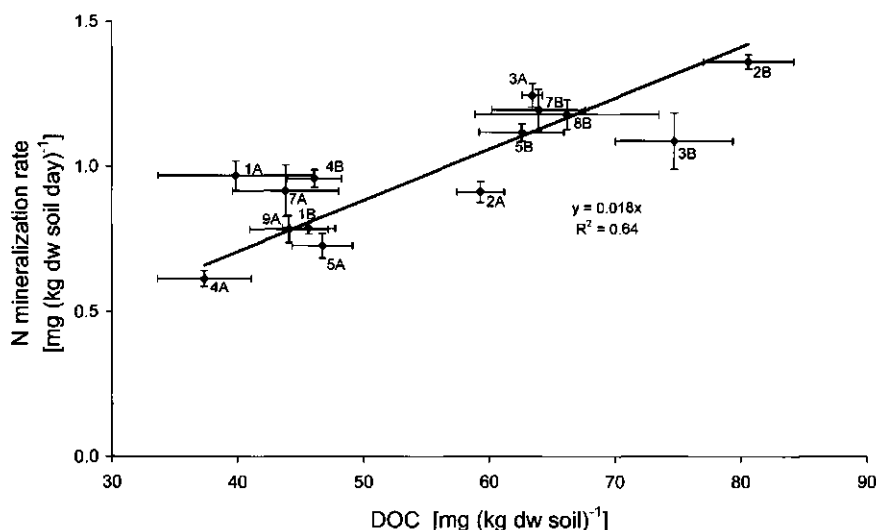


Figure 6.4. Relationship between the N mineralization rate and the dissolved organic carbon (DOC) content of the soils used in the field trial (after drying and rewetting) and incubated under optimal conditions ($T = 20\text{ }^{\circ}\text{C}$ and field capacity).

CO₂ respiration, bacterial biomass and the microbial biomass quotient (MBQ)

The amounts of CO₂ respired in the incubation experiments under optimized conditions were well related to the DOC content of the soils at each time interval. For example, Figure 6.5 shows the relationship between the amounts CO₂ respired and the DOC content of the soils incubated for two weeks. The correlation coefficients for the respiration measurements at 0, 2, 6, and 12 weeks after the start of the incubation were 0.70; 0.86; 0.85; 0.66, respectively. In contrast, no relationships were found between CO₂ respired and DOC measured in the soil incubations under field conditions, except for the 6 weeks incubated soils from the June sampling ($R^2 = 0.61$). During the incubation under optimized conditions bacterial biomass decreased on average with 66% during the first six weeks of incubation, after which bacterial biomass returned to their starting amount at week 12 (Table 6.3).

The bacterial biomass in the incubated soils from the April soil sampling more than doubled during the 6 weeks of incubation (Table 6.3). There was a wide range in calculated MBQs between the incubated soils, varying between 0.013 and 0.265.

Table 6.3. The mean, median, minimum, and maximum values found for the respiration data, bacterial biomasses, and the microbial biomass quotients (MBQ) measured in the soils incubated under optimized conditions ($T = 20\text{ }^{\circ}\text{C}$ and field capacity) at different times, or incubated under the field conditions of the April sampling session ($T = 8\text{ }^{\circ}\text{C}$ and field moisture content) at two times.

Time (weeks)	Respiration [$\mu\text{g C dw soil}^{-1}$]				Bact. Biomass [$\mu\text{g C dw soil}^{-1}$]				MBQ			
	Avg.	Med.	Min.	Max.	Avg.	Med.	Min.	Max.	Avg.	Med.	Min.	Max.
<i>Potential</i>												
0	41	41	27	54	319	306	104	541	0.14	0.14	0.07	0.22
2	17	18	13	22	211	215	74	360	0.10	0.09	0.05	0.16
6	12	13	10	15	108	104	34	202	0.13	0.14	0.07	0.26
12	10	10	7	13	301	276	167	576	0.04	0.04	0.02	0.05
<i>Actual</i>												
0	21	22	17	26	201	203	89	337	0.11	0.11	0.06	0.27
6	10	10	8	14	489	474	299	710	0.02	0.02	0.01	0.03

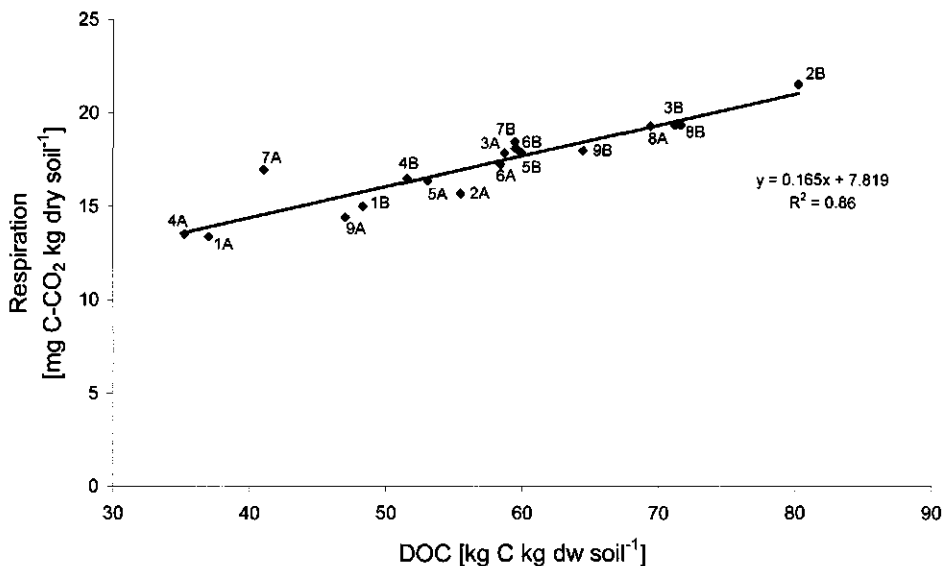


Figure 6.5. The relation between the CO₂ respiration rate and the dissolved organic carbon (DOC) content of the soils used in the field trial and incubated (after drying and rewetting) for two weeks under optimal conditions ($T = 20\text{ }^{\circ}\text{C}$ and field capacity).

N mineral balance

Total N inputs into the soils varied from 375 to 637 kg N (ha year)⁻¹ and total N outputs varied from 420 to 582 kg N (ha year)⁻¹. The mineral N amount present in the soil during the growing season was estimated to vary between 323 and 580 kg N (ha year)⁻¹. The mineral N gift to the soils by applying artificial fertilizer represented on average 30% of the total mineral N amount. The mineral part of slurry N applied to the soil by manure application or by grazing accounted on average for 29% of the total mineral N amount. At most, 7% of the total mineral N input was caused by atmospheric N-deposition in this area. Mineralization of organic N in the soil during the growing season accounted on average for about one third of the total mineral N present in the soil.

About 95% of N withdrawn from the soil was incorporated into grass. Nitrogen volatilization accounted on average for 3% of the total mineral N output and N loss by leaching or denitrification accounted for 1%. The mineral N surpluses for all grasslands are given in Figure 6.6. The high standard deviations are primarily caused by the large differences in grass yields. At $p < 0.1$, mineral N inputs and outputs were in balance. There were no differences in grass and soil N mineralization efficiencies observed for the fields under different organic manure management.

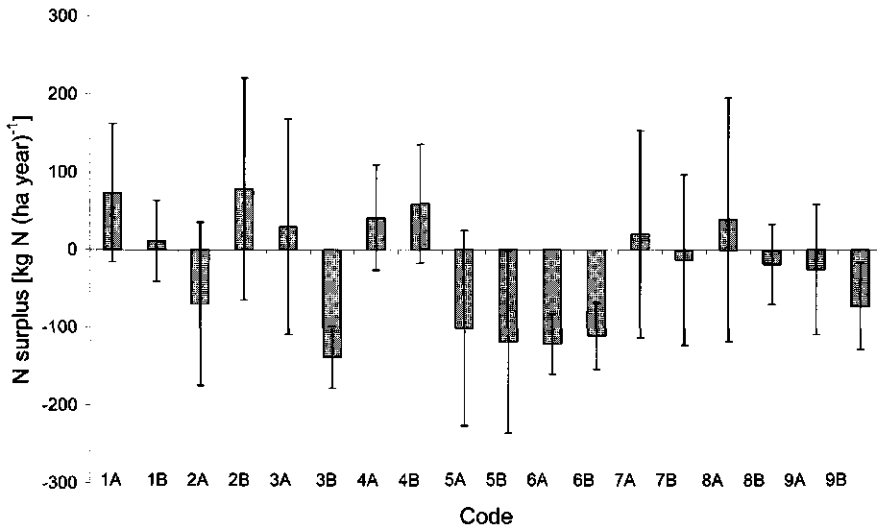


Figure 6.6. Mineral N surpluses for the grasslands studied in the field experiment.

Discussion

N-availability during the growing season

The amounts of grass harvested were in line with estimated grass yields by the farmers (plus the removal of N during grazing), which suggests that the short growing periods of the grass between sampling dates, did not stimulate exponential plant growth within the grass cages.

Statistical analysis of the manure slurries produced at the farms in previous years did only show difference in total N content between the two types of organic manure management in the years 2002 and 2004. Euro Mest-mix® has been used at farms 6-9 at least since 1998 and it is therefore unlikely that the lower total N content in manure slurries produced at those farms are related to the use of the additive. It is more likely that the lower N content in the slurries is related to the type of feed given to the cows. The feed fed to cows at farms 6-9 during the winter of 2001-2002 contained significantly less N ($p < 0.05$). Unfortunately, the N content of the feed fed during winter 2003-2004 was not measured.

No differences between type of farm management could be found for the amounts of N applied to the grasslands via slurry application or by application of artificial fertilizers, nor was there a difference in the organic matter content of the soils. In general, grazing intensity was more than twice as high at farms 6-9 compared to farms 1-5, which resulted in higher N inputs into the soils by animal excretions ($p = 0.02$).

The two peaks for both N_{ts} , as well as for NO_3^- , occurring in the beginning of June and in the beginning of September were, in both cases, preceded by a period of drought (Figure 6.7). Under dry conditions and mild temperatures, organic N is mineralized in higher quantities than mineral N is taken up by plants, and as a consequence mineral N will accumulate in the soil. The second peak was observed in all soils. However, not all soils were fertilized between the August and September sampling sessions. This suggests that manure application was not the cause of the second peak, but rather the preceding period of drought. Total soluble N peaks were in June higher ($p = 0.08$) for soils lower in organic matter content. A similar trend was observed for the N_{ts} availability for the September sampling session. The water holding capacity of soils increases with increase in SOM content (Sumner, 1999), and therefore plant uptake of N during

periods of drought will be higher in soils with a higher OM content, which will lead to a lower N_{ts} concentration in the soil solution.

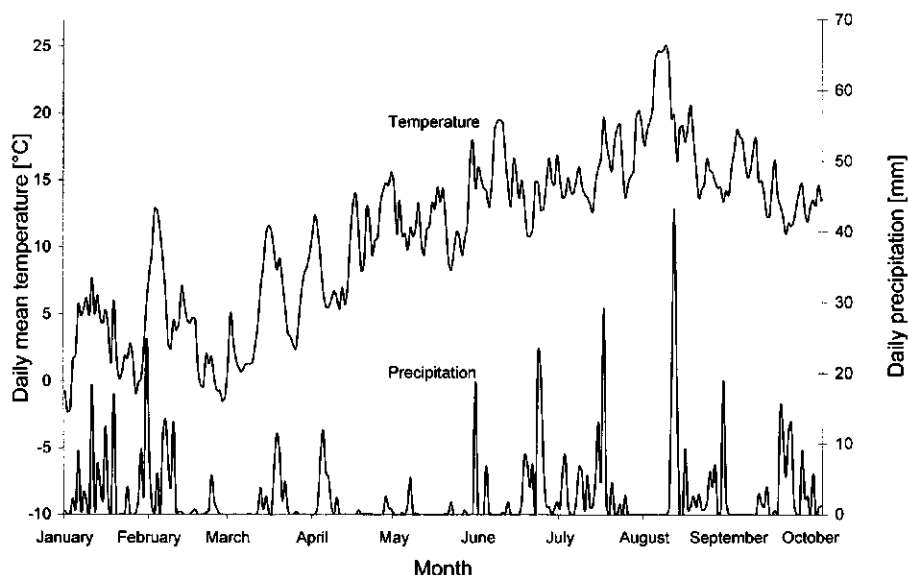


Figure 6.7. Daily mean temperature [°C] and daily precipitation [mm] during the 2004 growing season. Meteorological data were derived from the Eelde weather station (KNMI, 2004).

N mineralization

The initial DON content of the air dried soils is a reasonable predictor of potential N mineralization rates, when soil mineralization is determined without taking into account the initial flush of N, which occurs in the first days of the incubation.

The slightly higher N mineralization rates predicted by the Bregliani model compared to the N mineralization rates measured might be caused by the difference in experimental set-up between the two studies. The relationship between the initial DON content and the N mineralization rates found by Bregliani *et al.* (2006) is based on an incubation experiment in which agricultural soils were incubated at field capacity, in oxygen permeable plastic bags, in a greenhouse. The minimal temperature in the greenhouse was set at 20 °C, but during daytime higher temperatures could occur. In contrast, in this experiment temperature was maintained at 20 °C during the entire incubation period. Since N mineraliza-

tion is enhanced by temperature increase (Benbi and Richter, 2002), the higher temperatures during daytime in the greenhouse probably caused additional N mineralization.

The higher DOC and DON content measured in the February soil samples compared to the DOC and DON content measured in the other field samples are most likely related to the drying (and sieving) of the soils, prior to the CaCl_2 -extractions, in case of the former soils. Drying of soil reduces microbial activity and causes lyses of microbial biomass. In addition, drying disrupts the stability of soil aggregates (West *et al.*, 1992; Van Gestel *et al.*, 1993). The higher N_{ts} and NH_4^+ content measured in the air dried soils compared to the N_{ts} and NH_4^+ content measured in field moist soils were probably also the result of drying. The NO_3^- content of the air dried soils were similar to the NO_3^- content measured in field moist soils, which suggests that nitrification stopped upon drying and thus that microbial activity is indeed reduced upon drying.

Mineralization of organic N and conversion of NH_4^+ into NO_3^- during the incubation resulted in H^+ release into the soil solution. The H^+ increase in the soil solution exceeded the buffer capacity of the (incubated) soils, causing a decrease of soil pH. In contrast, the pH in the CaCl_2 extractions of the field soils remained constant during the growing season, which suggests that N mineralization was lower in the field than in the incubated soils, or that NO_3^- uptake by plants released OH^- into the soil solution, or that H^+ leached from the 0-10 cm soil layer.

Potential N mineralization rates measured were in line with N mineralization rates reported by Hassink (1994) and by Velthof (2003). Besides temperature, soil N mineralization is affected by soil moisture content (Benbi and Richter, 2002). Temperature and moisture content of the soils incubated under field conditions were lower than under optimized conditions. However, the supply of easily decomposable organic matter via slurry application to the soil resulted in similar N mineralization rates in both type of soils.

Our results showed that the DOC and, or the DON content of the soil were the best indicators for N mineralization. Similar findings were reported by Velthof *et al.* (2000) for N-mineralization in arable soils.

CO₂ respiration, bacterial biomass and the microbial biomass quotient

The good correlations between the DOC content and the amounts CO₂ evolved from the incubated soils under optimized conditions, as opposed to the soils incubated under field conditions, suggest that the 'extra' carbon which became available due to drying could easily be utilized by micro-organisms after rewetting of the soils.

This pool of easily decomposable carbon diminished during incubation and as a consequence respiration and bacterial biomass decreased. The increase in bacterial biomass at week 12 is most likely caused by the release of easily decomposable carbon, due to degradation of deceased micro-organisms.

Dissolved organic carbon present in soil solutions of field moist soils can not readily be utilized by micro-organisms. This suggests that the fraction of microbial derived carbohydrates to the total DOC content is low, and most DOC consists of humic and fulvic acids. The measured MBQs of the studied grasslands were similar to MBQs found for grasslands on sandy soils located in the research area (Schouten *et al.*, 2002).

N mineral balance

Although large amounts of N are applied to the grasslands, the surpluses of N did not exceed the 'farm-based' legislation surplus of 250 kg N (ha year)⁻¹ (European Commission, 2005). Mineralization of organic N is a significant contributor to the amount of mineral N available in the soil during the growing season. Efforts to reduce the uncertainty in the N mineral balance should be primarily aimed at reducing the uncertainty in N removal by grass harvest. Input and output of the mineral N balance seems to be in balance, indicating that most important factors determining the mineral N content in the soil are accounted for. The data further suggests that N losses through leaching or denitrification were limited. Even if no denitrification would occur in the soils, still NO₃⁻ concentrations in the groundwater would not exceed the legal limit of 50 mg L⁻¹. Moreover, as can be seen from Figure 6.2 the NO₃⁻ content in the soil solution tended to decline in the fall. Since most NO₃⁻ is leached during wintertime, this reduces the risks of NO₃⁻ leaching even more.

The data did not provide evidence of any effects of organic manure management on grass yields, nor to the nutrient use efficiency of grasslands. However, the amount of data was scarce and the range in soil types was small.

References



-
- Aarts, H.F.M., Biewinga, E.E., and Van Keulen, H., 1992. Dairy farming systems based on efficient nutrient management. *Neth. J. Agr. Sci.* **40**:285-299.
- Aarts, H.F.M., Habbekotté, B., and Van Keulen, H. 2000. Nitrogen (N) management in the 'De Marke' dairy farming system. *Nutr. Cycl. Agroecosys.* **56**:231-240.
- Amlinger, F., Götz, B., Dreher, P., Geszti, J., and Weissteiner, C. 2003. Nitrogen in biowaste and yard waste compost: dynamics of mobilisation and availability- a review. *Eur. J. Soil Biol.* **39**:107-116.
- Amon, B., Kryvoruchko, V., Amon, T., and Moitzi, G., 2005. Can the additive "Effective Micro-organisms (EM)" reduce ammonia and greenhouse gas emissions from slurry stores? In: Bernal, M.P., Moral, R., Clemente, R., and Paredes, C. (Eds.), *Sustainable Organic Waste Management for Environmental Protection and Food Safety*. FAO and CSIC, Murcia, pp. 233-236.
- ASAE, 2005. *Manure production and characteristics*, American Society of Agricultural Engineers, St. Joseph, pp. 19.
- BDH, 1981. *Ion exchange resins*, BDH Chemicals Ltd, Poole, pp. 110.
- Benbi, D.K., and Richter, J. 2002. A critical review of some approaches to modelling nitrogen mineralization. *Biol Fert. Soils* **35**: 168-183.
- Blgg, 2005. *Dutch manure dataset 2002 - 2005*. Blgg Oosterbeek, the Netherlands.
- Bloem, J., and Vos A., 2004. Fluorescent staining of microbes for total direct counts, p. 861-874, In G. A. Kowalchuk, et al., eds. *Molecular Microbial Ecology Manual, 2nd edition*. Kluwer Academic Publishers, Dordrecht, the Netherlands.
- Børsting, C.F., Kristensen, T., Misciattelli, L., Hvelplund, T., and Weisbjerg, M.R., 2003. Reducing nitrogen surplus from dairy farms. Effects of feeding and management. *Livest. Prod. Sci.* **83**:165-178.
- Borucki Castro, S.I., Phillip, L.E., Girard, V., and Tremblay, A., 2004. Altering dietary cation-anion difference in lactating dairy cows to reduce phosphorus excretion to the environment. *J. Dairy Sci.* **87**:1751-1757.
- Bregliani, M.M., Temminghoff, E.J.M., Van Riemsdijk, W.H., and Haggi, E.S., 2006. Nitrogen fractions in arable soils in relation to nitrogen mineralization and plant uptake. *Commun. Soil Sci. Plant Anal.* **37**:1571-1586.
- Bril, J., Salomons, W., 1990. Chemical composition of animal manure: a modelling approach. *Neth. J. Agr. Sci.* **38**:333-351.

- Brock, E.H., Ketterings, Q.M., and McBride, M., 2006. Copper and zinc accumulation in poultry and dairy manure-amended fields. *Soil Sci.* **171**:388-399.
- Broderick, G.A., 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. *J. Dairy Sci.* **86**:1370-1381.
- Bussink, D.W., and Oenema, O., 1998. Ammonia volatilization from dairy farming systems in temperate areas: a review. *Nutr. Cycl. Agroecosys.* **51**: 19-33.
- Cancès, B., Ponthieu, M., Castrec-Rouelle, M., Aubry, E., and Benedetti, M.F., 2003. Metal ions speciation in a soil and its solution: experimental data and model results. *Geoderma* **113**:341-355.
- Canh, T.T., Sutton, A.L., Aarnink, A.J.A., Verstegen, M.W.A., Schrama, J.W., and Bakker, G.C.M., 1998. Dietary carbohydrates alter the fecal composition and pH and the ammonia emission from slurry of growing pigs. *J. Anim. Sci.* **76**:1887-1895.
- Castillo, A.R., Kebreab, E., Beever, D.E., Barbi, J.H., Sutton, J.D., Kirby, H.C., and France, J., 2001. The effect of energy supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. *J. Anim. Sci.* **79**:240-246.
- CBS, 2005. *Milieucompendium (in Dutch)*. Statistics Netherlands/ National institute for public health and the environment (<http://www.mnp.nl>).
- CBS, 2006a. *CBS Statline*. Central Bureau for Statistics (<http://statline.cbs.nl>).
- CBS, 2006b. *Productie van dierlijke mest en mineralen naar diercategorie (in Dutch)*, CBS. Statistics Netherlands.
- Chapuis-Lardy, L., Fiorini, J., Toth, J., and Dou, Z., 2004. Phosphorus concentration and solubility in dairy feces: variability and affecting factors. *J. Dairy Sci.* **87**:4334-4341.
- Chardon, W.J., Oenema, O., Del Castilho, P., Vriesema, R., Japenga, J., and Blaauw, D., 1997. Organic phosphorus in solutions and leachates from soils treated with animal slurries. *J. Environ. Qual.* **26**:372-378.
- Chung, J.B., and Zasoski, R.J., 1994. Ammonium-Potassium and Ammonium-Calcium Exchange Equilibria in Bulk and Rhizosphere Soil. *Soil Sci. Soc. Am. J.* **58**:1368-1375.
- CVB, 2003. *Animal feed table 2003 (in Dutch)*. Centraal Veevoederbureau, Lelystad, the Netherlands.

-
- Dach, J., and Starmans D., 2005. Heavy metals balance in Polish and Dutch agronomy: Actual state and previsions for the future. *Agr. Ecosyst. Environ.* **107**:309-316.
- De Clercq, P., Gersis, A.C., Hofman, G., Jarvis, S.C., Neeteson, J.J., and Sinabell, F., 2001., *Nutrient management legislation in European countries*. Wageningen Pers, Wageningen, the Netherlands, pp. 347.
- De Hoop, D.W., Hubeek, F.B., and Van der Schans, J.W., 2004. *Evaluatie van mestafzetovereenkomsten en dierrechten: Studie in het kader van evaluatie Meststoffenwet 2004 (in Dutch)*, LEI, Den Haag, pp. 109.
- De Vries, J., 1974. *The Dutch rural economy in the golden age, 1500-1700*, Yale Series in Economic History. Yale University Press, Ltd., London, pp. 316.
- Delaune, P.B., Moore, P.A., Jr., Daniel, T.C., and Lemunyon, J.L., 2004. Effect of chemical and microbial amendments on ammonia volatilization from composting poultry litter. *J. Environ. Qual.* **33**:728-734.
- Dewes, T., 1996. Effect of pH, temperature, amount of litter and storage density on ammonia emissions from stable manure. *J. Agric. Sci.* **127**:501-509.
- Dewes, T., 1999. Ammonia emissions during the initial phase of microbial degradation of solid and liquid cattle manure. *Bioresource Technol.* **70**:245-248.
- Dou, Z., Toth, J.D., Galligan, D.T., Ramberg Jr., C.F., and J.D., F., 2000. Laboratory procedures for characterizing manure phosphorus. *J. Environ. Qual.* **29**:508-514.
- Dou, Z., Knowlton, K.F., Kohn, R.A., Wu, Z., Satter, L.D., Zhang, G., Toth, J.D., and J.D., F., 2002. Phosphorus characteristics of dairy feces affected by diets. *J. Environ. Qual.* **31**:2058-2065.
- Edmeades, D.C., 2003. The long-term effects of manures and fertilisers on soil productivity and quality: a review. *Nutr. Cycl. Agroecosys.* **66**:165-180.
- Eriksen, J., Mortensen, J.V., Kjellerup, V.K., and Kristjansen, O., 1995. Forms and plant-availability of sulfur in cattle and pig slurry. *Z. Pflanz. Bodenkunde* **158**:113-116.
- Eriksen, J., 2002. *Organic manures as sources of fertiliser sulphur*. The International Fertiliser Society, Cambridge, pp. 1-19.
- European Commission, 2005. Commission Decision of 8 December 2005 granting a derogation requested by the Netherlands pursuant to Council Directive 91/676/EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources (C(2005) 4778). *Official Journal of the European Union*, pp. 89-93.

- Griffin, R.A., and Jurinak, J.J., 1973. Estimation of activity coefficients from the electrical conductivity of natural aquatic systems and soil extracts. *Soil Sci.* **116**:26-30.
- Griffin, T.S., He, Z., Honeycutt, C.W., Jones, D.L., Healey, J.R., Willett, V.B., Farrar, J.F., and Hodge, A., 2005. Manure composition affects net transformation of nitrogen from dairy manures. *Plant Soil* **273**:29-38.
- Groot, J.C.J., Cone, J.W., Williams, B.A., Debersaques, F.M.A., and Lantinga, E.A., 1996. Multiphasic analysis of gas production kinetics for in vitro fermentation of ruminant feeds. *Anim. Feed Sci. Tech.* **64**:77-89.
- Harrison, R., and Webb, J., 2001. A review of the effect of N fertilizer type on gaseous emissions. *Adv. Agron.* **73**:65-108.
- Hassink, J., 1994. Effects of soil texture and grassland management on soil organic C and N and rates of C and N mineralization. *Soil Biol. Biochem.* **26**:1221-1231.
- Hayes, J.E., Richarson, A.E., and Simpson, R.J., 2000. Components of organic phosphorus in soil extracts that are hydrolysed by phytase and acid phosphatase. *Biol. Fertil. Soils* **32**:279-286.
- He, Z., and Honeycutt, C.W., 2001. Enzymatic characterization of organic phosphorus in animal manure. *J. Environ. Qual.* **30**:1685-1692.
- He, Z., Griffin, T.S., and Honeycutt, C.W., 2004. Phosphorus distribution in dairy manures. *J. Environ. Qual.* **33**:1528-1534.
- Heanes, D.L., 1984. Determination of total organic c in soils by an improved chromic acid digestion and spectrophotometric procedure. *Commun. Soil Sci. Plant Anal.* **15**:1191-1213.
- Helleferich, F., 1962. *Ion exchange*. McGraw-Hill, New York.
- Henkens, P.L.C.M., and Van Keulen, H., 2001. Mineral policy in the Netherlands and nitrate policy within the European Community. *Neth. J. Agr. Sci.* **49**:117-134.
- Holter, J.B., and Urban, J., W.E., 1992. Water partitioning and intake prediction in dry and lactating Holstein cows. *J. Dairy Sci.* **75**:1472-1479.
- Houba, V.J.G., Temminghoff, E.J.M., Gaikhorst, G.A., and Van Vark, W., 2000. Soil analysis procedures using 0.01 M calcium chloride as extraction reagent. *Commun. Soil Sci. Plant Anal.* **31**:1299-1396.
- Huijsmans, J.F.M., Hol, J.M.G., and Hendriks, M.M.W.B., 2001. Effect of application technique, manure characteristics, weather and field conditions on ammonia volatilization from manure applied to grassland. *Neth. J. Agr. Sci.* **49**:323-342.

-
- Husted, S., Jensen, L.S., and Jorgensen, S.S., 1991. Reducing ammonia loss from cattle slurry by the use of acidifying additives: the role of the buffer system. *J. Sci. Food Agric* **57**:335-349.
- Husted, S., 1994. Seasonal variation in methane emission from stored slurry and solid manures. *J. Environ. Qual.* **23**:585-592.
- ISO5983, 1998. *Animal feeding stuffs - Determination of nitrogen content and calculation of crude protein content*. International Organization for Standardization, Geneva, Switzerland.
- James, T., Meyer, D., Esparza, E., Depeters, E.J., and Perez-Honti, H., 1999. Effects of dietary nitrogen manipulation on ammonia volatilization from manure from Holstein Heifers. *J. Dairy Sci.* **82**:2430-2439.
- Japenga, J., Harmsen, K., 1990. Determination of mass balances and ionic balances in animal manure. *Neth. J. Agr. Sci.* **38**:353-367.
- Jarvis, S.C., and Pain, B.F., 1990. Ammonia volatilisation from agricultural land. *Proc. Fert. Soc.* **298**:3-33.
- Jones, D.L., Healey, J.R., Willett, V.B., Farrar, J.F., and Hodge, A., 2005. Dissolved organic nitrogen uptake by plants -an important N uptake pathway. *Soil Biol. Biochem.* **37**:413-423.
- Kalis, E.J.J., Temminghoff, E.J.M., Weng, L.P., and Van Riemsdijk, W.H., 2006a. Effects of humic acid and competing cations on metal uptake by *Lolium Perenne*. *Environ. Chem. Toxicol.* **25**:702-711.
- Kalis, E.J.J., Weng, L., Dousma, F., Temminghoff, E.J.M., and Van Riemsdijk, W.H., 2006b. Measuring free metal ion concentrations in situ in natural water using the Donnan Membrane Technique. *Environ. Sci. Technol.* **40**:955-961.
- Kalis, E.J.J., Temminghoff, E.J.M., Weng, L., and Van Riemsdijk, W.H., 2007. Measuring free metal ion concentrations in multicomponent solutions using Donnan Membrane Technique. *Anal. Chem.*, **79**: 1555-1563.
- Kebreab, E., France, J., Mills, J.A.N., Allison, R., and Dijkstra, J., 2002. A dynamic model of N metabolism in the lactating dairy cow and an assessment of impact of N excretion on the environment. *J. Anim. Sci.* **80**:248-259.
- Keizer, M.G., and Van Riemsdijk, W.H., 2002. *A computer program for the equilibrium calculation of speciation and transport in soil-water systems (ECOSAT 4.7)*. Wageningen University, Wageningen, pp. 76.
- Kinniburgh, D.G., Van Riemsdijk, W.H., Koopal, L.K., Borkovec, M., Benedetti, M.F., and Avena, M.J., 1999. Ion binding to natural organic matter:

- competition, heterogeneity, stoichiometry and thermodynamic consistency. *Colloid. Surface. A.* **151**:147-166.
- KNMI, 2004. *Climatic data*, Royal Netherlands Meteorological Institute, De Bilt.
- Külling, D.R., Menzi, H., Kröber, T.F., Neftel, A., Sutter, F., Lischer, P., and Kreuzer, M., 2001. Emissions of ammonia, nitrous oxide and methane from different types of dairy manure during storage as affected by dietary protein content. *J. Agr. Sci.* **137**:235-250.
- Lefcourt, A.M., and Meisinger, J.J., 2001. Effect of adding alum or zeolite to dairy slurry on ammonia volatilization and chemical composition. *J. Dairy Sci.* **84**:1814-1821.
- Lindsay, W.L., 1979. *Chemical Equilibria in soils*. John Wiley & sons, New York, pp. 162-205.
- LVN, 2006. *Mestbeleid 2006 (in Dutch), Tabellenbrochure Mestbeleid*. Ministry of Agriculture, Nature and Food Quality, The Hague.
- LTO, 2005. *Adviesbasis bemesting grasland en voedergewassen (in Dutch)*, LTO-Nederland, Lelystad, pp.170.
- Marcus, Y., 1997. *Ion properties*. Marcel Dekker Inc., New York, pp. 272.
- McCrary, D.F., and Hobbs, P.J., 2001. Additives to reduce ammonia and odor emissions from livestock wastes: a review. *J. Environ. Qual.* **30**:345-355.
- McDowell, R.W., and Koopmans, G.F., 2006. Assessing the bioavailability of dissolved organic phosphorus in pasture and cultivated soils treated with different rates of nitrogen fertiliser. *Soil Biol. Biochem.* **38**:61-70.
- McNeill, A.M., Eriksen, J., Bergström, L., Smith, K.A., Marstorp, H., Kirchmann, H., and Nilsson, I., 2005. Nitrogen and sulphur management: challenges for organic sources in temperate agricultural systems. *Soil Use Manage* **21**:82-93.
- McQuarrie, D.A., and Simon, J.D., 1997. Chemical kinetics I: Rate laws. In: McGuire, A. (Ed.), *Physical chemistry, a molecular approach*. University Science Books, Sausalito, pp. 1046-1089.
- Misselbrook, T.H., Powell, J.M., Broderick, G.A., and Grabber, J.H., 2005. Dietary manipulation in dairy cattle: laboratory experiments to assess the influence on ammonia emissions. *J. Dairy Sci.* **88**:1765-1777.
- Monteny, G.J., Smits, M.C.J., Van Duinkerken, G., Mollenhorst, H., and De Boer, I.J.M., 2002. Prediction of ammonia emission from dairy barns using feed characteristics part II: Relation between urinary urea concentration and ammonia emission. *J. Dairy Sci.* **85**:3389-3394.
- Mooij, M., 1996. Composition of animal manures. *Meststoffen* 38-40.

-
- Mosier, A., Kroeze, C., Nevison, C., Oenema, O., Seitzinger, S., and Van Cleemput, O., 1998. Closing the global N₂O budget: Nitrous oxide emissions through the agricultural nitrogen cycle. *Nutr. Cycl. Agroecosys.* **52**:225-248.
- Myrold, D.D., 1998. *Transformations of Nitrogen, Principles and applications of microbiology*. Prentice-Hall, New-Jersey, pp. 259-294.
- Neeteson, J.J., 2000. Nitrogen and phosphorus management on Dutch dairy farms: legislation and strategies employed to meet the regulation. *Biol. Fertil. Soils.* **30**:566-572.
- Nennich, T.D., Harrison, J.H., Van Wieringen, L.M., Meyer, D., Heinrichs, A.J., Weiss, W.P., St Pierre, N.R., Kincaid, R.L., Davidson, D.L., and Block, E., 2005. Prediction of Manure and Nutrient Excretion from Dairy Cattle. *J. Dairy Sci.* **88**:3721-3733.
- Ni, J., 1999. Mechanistic models of ammonia release from liquid manure: a review. *J. Agric. Eng. Res.* **72**: 1-17.
- Ni, J.-Q., Hendriks, J., Vinckier, C., and Coenegrachts, J., 2000. A new concept of carbon dioxide accelerated ammonia release from liquid manure in pig house. *Environ. Int.* **26**:97-104.
- Nicholson, F.A., Chambers, B.J., Williams, J.R., Unwin, R.J., 1999. Heavy metal contents of livestock feeds and animal manures in England and Wales. *Bioresource Technol.* **70**:23-31.
- Novozamsky, I., Houba, V.J.G., Van Eck, R., and Van Vark, W., 1983. A novel digestion technique for multi-element plant analysis. *Commun. Soil Sci. Plant Anal.* **14**:239-249.
- Novozamsky, I., Van Eck, R., Houba, V.J.G., and Van der Lee, J.J., 1996. Solubilization of plant tissue with nitric acid - hydrofluoric acid - hydrogen peroxide in a closed system microwave digester. *Commun. Soil Sci. Plant Anal.* **27**:867-875.
- Oba, M., and Allen, M.S., 2003. Effects of diet fermentability on efficiency of microbial nitrogen production in lactating dairy cows. *J. Dairy Sci.* **86**:195-207.
- Oenema, O., Velthof, G.L., Groot Koerkamp, P.W.G., Monteny, G.J., Bannink, A., Van der Meer, H.G., and Van der Hoek, K.W., 2000. *Forfaitaire waarden voor gasvormige stikstofverliezen uit stallen en mestopslagen (in Dutch)*, Alterra, Wageningen, pp. 185.

- Oenema, O., Kros, H., and De Vries, W., 2003. Approaches and uncertainties in nutrient budgets: implications for nutrient management and environmental policies. *Eur. J. Agron.* **20**:3-16.
- Oenema, O., 2004. Governmental policies and measures regulating nitrogen and phosphorus from animal manure in European agriculture. *J. Anim. Sci.* **82**:E196-E206.
- Oenema, O., Van Lieere, L., Plette, S., Prins, T., Van Zeijts, H., Schoumans, O., and Jarvis, S.C., 2004. Environmental effects of manure policy options in the Netherlands. *Water Sci. Technol.* **49**:101-108.
- Oenema, O., Wrage, N., Velthof, G.L., Van Groenigen, J.W., Dolfing, J., and Kuikman, P.J., 2005. Trends in global nitrous oxide emissions from animal production systems. *Nutr. Cycl. Agroecosys.* **72**:51-65.
- Osté, L.A., Temminghoff, E.J.M., Lexmond, T.M., and Van Riemsdijk, W.H., 2002. Measuring and modeling zinc and cadmium binding by humic acid. *Anal Chem* **74**:856-862.
- Paul, J.W., Dinn, N.E., Kannangara, T., and Fisher, L.J., 1998. Protein content in dairy cattle diets affects ammonia losses and fertilizer nitrogen value. *J. Environ. Qual.* **27**:258-534.
- Phillips, V.R., Cowell, D.A., Sneath, R.W., Cumby, T.R., Williams, G., Demmers, T.G.M., and Sandars, D.L., 1999. An assessment of ways to abate ammonia emissions from UK livestock buildings and waste stores. Part 1: ranking exercise. *Bioresource Technol.* **70**:143-155.
- Reijs, J., Sonneveld, M.P.W., Sørensen, P., Schils, R.L.M., Groot, J.C.J., and Lantinga, E.A., 2007. Effects of different diets on utilization of nitrogen from cattle slurry applied to grassland on a sandy soil in the Netherlands. *Agr. Ecosyst. Environ.* **118**:65-79.
- Reynolds, C.K., Cammell, S.B., Humphries, D.J., Beever, D.E., Sutton, J.D., and Newbold, J.R., 2001. Effects of postrumen starch infusion on milk production and energy metabolism in dairy cows. *J. Dairy Sci.* **84**:2250-2259.
- RIVM, 2002. *Minas en Milieu Balans en Verkenning (in Dutch)*, National institute for public health and the environment, Bilthoven, rep. nr. 718201005, pp. 206.
- Rotz, C.A., 2004. Management to reduce nitrogen losses in animal production. *J. Anim. Sci.* **82**:E119-E137.

-
- Rotz, C.A., Taube, F., Russelle, M.P., Oenema, J., Sanderson, M.A., and Wachendorf, M., 2005. Whole-farm perspectives of nutrient flows in grassland agriculture. *Crop Sci.* **45**:2139-2159.
- Schonewille, J.T., Van 't Klooster, A.T., Wouterse, H., and Beynen, A.C., 1999. Effects of intrinsic potassium in artificially dried grass and supplemental potassium bicarbonate on apparent magnesium absorption in dry cows. *J. Dairy Sci.* **82**:1824-1830.
- Schouten, A.J., Bloem, J., Didden, W., Jagers op Akkerhuis, G., Keidel, H., and Rutgers, M., 2002. *Bodembioologische Indicator 1999. Ecologische kwaliteit van graslanden op zand bij drie categorieën melkveehouderij-bedrijven (in Dutch)*. National Institute for Public Health and the Environment, Bilthoven, rep. nr 607604003, pp. 107.
- Sharpley, A., and Moyer, B., 2000. Phosphorus forms in manure and compost and their release during stimulated rainfall. *J. Environ. Qual.* **29**:1462-1469.
- Smits, J.P., 2006. *Technological change, institutional development and economic growth in Dutch Agriculture, 1870-1939*, IEHC 2006. Groningen Growth and Development Centre, Helsinki, pp. 23.
- Smits, M.C.J., Monteny, G.J., and Van Duinkerken, G., 2003. Effect of nutrition and management factors on ammonia emission from dairy cow herds: models and field observations. *Livest. Prod. Sci.* **84**:113-123.
- Soil Survey Staff, 1998. *Keys to soil taxonomy*, U.S. Gov. Print. Office, Washington, D.C.
- Sommer, S.G., and Olesen, J.E., 1991. Effects of Dry Matter Content and Temperature on Ammonia Loss from Surface-Applied Cattle Slurry. *J. Environ. Qual.* **20**:679-683.
- Sommer, S.G., Olesen, J.E., and Christensen, B.T., 1991. Effects of temperature, wind speed and air humidity on ammonia volatilization from surface applied cattle slurry. *J. Agric. Sci.* **117**:91-100.
- Sommer, S.G., Christensen, B.T., Nielsen, N.E., and Schjorring, J.K., 1993. Ammonia volatilization during storage of cattle and pig slurry: effect of surface cover. *J. Agr. Sci.* **121**:63-71.
- Sommer, S.G., and Husted, S., 1995. The chemical, buffer system in raw and digested animal slurry. *J. Agric. Sci.* **124**:45-53.
- Sommer, S.G., and Hutchings, N., 1995. Techniques and strategies for the reduction of ammonia emission from agriculture. *Water Air Soil Poll.* **85**:237-248.

- Sommer, S.G., Petersen, S.O., and Sørensen, H.T., 2000. Greenhouse gas emission from stored livestock slurry. *J. Environ. Qual.* **29**:744-751.
- Sommer, S.G., and Hutchings, N.J., 2001. Ammonia emission from field applied manure and its reduction. *Eur. J. Agron.* **15**:1-15.
- Sommer, S.G., Générmont, S., Cellier, P., Hutchings, N.J., Olesen, J.E., and Morvan, T., 2003. Processes controlling ammonia emission from livestock slurry in the field. *Eur. J. Agron.* **19**:465-486.
- Sørensen, P., Weisbjerg, M.R., and Lund, P., 2003. Dietary effects on the composition and plant utilization of nitrogen in dairy cattle manure. *J. Agr. Sci.* **141**:79-91.
- SPSS Inc., 2003. *SPSS 12.0 Brief Guide*. SPSS Inc., Chicago, pp. 218.
- Stevens, R.J., and Laughlin, R.J., 1997. The impact of cattle slurries and their management on ammonia and nitrous oxide emissions from grassland. In: S.C. Jarvis, and Pain, B.F. (Eds.), *Gaseous nitrogen emissions from grasslands*. CAB International, Oxon, UK, pp. 233-256.
- Stevens, R.J., Laughlin, R.J., and Frost, J.P., 1993. Effects of diet and storage time on the concentration of sulphide in dairy-cow slurry. *Bioresource Technol.* **45**:13-16.
- StiBoKa, 1981. *Bodemkaart van Nederland 1:50.000: toelichting bij de kaartbladen 6 West Leeuwarden 6 Oost Leeuwarden en het vaste land van de kaartbladen 2 West Schiermonnikoog en 2 Oost Schiermonnikoog*. Stiboka, Wageningen.
- Stuiver, M., Van Der Ploeg, J.D., and Leeuwis, C., 2003. The VEL and VANLA environmental co-operatives as field laboratories. *Neth. J. Agr. Sci.* **51**:27-39.
- Sumner, M.E., 1999. *Handbook of soil science*. CRC Press, Washington D.C., pp. 2112.
- Temminghoff, E.J.M., Plette, A.C.C., Van der Zee, S.E.A.T.M., Van Riemsdijk, W.H., 1997. Speciation of heavy metals in soil in relation to availability and mobility. In: S.G. Pandalai (Ed), *Recent Research Developments in Soil Science*: pp. 55-65.
- Temminghoff, E.J.M., Plette, A.C.C., Van Eck, R., and Van Riemsdijk, W.H., 2000. Determination of the chemical speciation of trace metals in aqueous systems by the Wageningen Donnan Membrane Technique. *Anal. Chim. Acta.* **412**:149-157.
- Thompson, R.B., Pain, B.F., and Rees, Y.J., 1990. Ammonia Volatilization from Cattle Slurry Following Surface Application to Grassland II. Influence of

-
- Application Rate Wind Speed and Applying Slurry in Narrow Bands. *Plant Soil* **125**:119-128.
- Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W.H., Simberloff, D., and Swackhamer, D., 2001. Forecasting Agriculturally driven global environmental change. *Science* **292**:281-284.
- Turner, B.L., and Leytem, A.B., 2004. Phosphorus compounds in sequential extracts of animal manures: chemical speciation and a novel fractionation procedure. *Environ. Sci. Technol.* **38**:6101-6108.
- Valadares, R.F.D., Broderick, G.A., Valadares Filho, S.C., and Clayton, M.K., 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. *J. Dairy Sci.* **82**:2686-2696.
- Van der Stelt, B., Temminghoff, E.J.M., and Van Riemsdijk, W.H., 2005. Measurement of ion speciation in animal slurries using the Donnan Membrane Technique. *Anal. Chim. Acta.* **552**:135-140.
- Van Gestel, M., Merckx, R., and Vlassak, K., 1993. Microbial biomass responses to soil drying and rewetting: The fate of fast- and slow-growing microorganisms in soils from different climates. *Soil Biol. Biochem.* **25**:109-123.
- Van Horne, P., and Prins, H., 2002. *Development of dairy farming in the Netherlands in the period 1960-2000*. Agricultural Economics Research Institute, The Hague, rep. nr. 2.02.07, pp. 26.
- Van Keulen, H., Aarts, H.F.M., Habbekotté, B., Van der Meer, H.G., and Spiertz, J.H.J., 2000. Soil-plant-animal relations in nutrient cycling: the case of dairy farming system 'De Marke'. *Eur. J. Agron.* **13**:245-261.
- Van Vliet, P.C.J., Bloem, J., and de Goede, R.G.M., 2005. Microbial diversity, nitrogen loss and grass production after addition of effective micro-organisms (EM) to slurry manure. *Appl. Soil Ecol.* **32**:188-198.
- Van Zanden, J.L., 1991. The first green revolution: The growth of production and productivity in European Agriculture, 1870-1914. *Econ. Hist. Rev.* **44**:215-239.
- Velthof, G.L., Oenema, J., and Nelemans, J.A., 2000. Comparison of nitrogen mineralization indices for arable land (in Dutch). *Meststoffen* 45-52.
- Velthof, G.L., and Oenema, O., 2001. *Effects of ageing and cultivation of grassland on soil nitrogen*, Alterra, Wageningen, pp. 53.

- Velthof, G.L., 2003. *Relaties tussen mineralisatie, denitrificatie en indicatoren voor bodemkwaliteit in landbouwgronden*, Alterra, Wageningen, pp. 38.
- Velthof, G.L., Kuikman, P.J., and Oenema, O., 2003. Nitrous oxide emission from animal manures applied to soil under controlled conditions. *Biol. Fertil. Soils* **37**:221-230.
- Velthof, G.L., Nelemans, J.A., Oenema, O., and Kuikman, P.J., 2005. Gaseous nitrogen and carbon losses from pig manure derived from different diets. *J. Environ. Qual.* **34**:698-706.
- Verhoeven, F.P.M., Reijers, J.W., and Van Der Ploeg, J.D., 2003. Re-balancing soil-plant-animal interactions: towards reduction of nitrogen losses. *Neth. J. Agr. Sci.* **51**:147-164.
- Vérité, R., Delaby, L., 2000. Relation between nutrition, performances and nitrogen excretion in dairy cows. *Ann. Zootech.* **49**:217-239.
- Verschoor, B.C., 2001. *Nematode-plant interactions in grasslands under restoration management*, PhD-thesis, Wageningen University, pp.197.
- Webb, J., Menzi, H., Pain, B.F., Misselbrook, T.H., Dämmgen, U., Hendriks, H., and Döhler, H., 2005. Managing ammonia emissions from livestock production in Europe. *Environ. Pollut.* **135**:399-406.
- Weiss, W.P., 2004. Macromineral digestion by lactating dairy cows: factors affecting digestibility of magnesium. *J. Dairy Sci.* **87**:2167-2171.
- Weng, L., Temminghoff, E.J.M., Van Riemsdijk, W.H., 2001a. Contribution of individual sorbents to the control of heavy metal activity in sandy soil. *Environ. Sci. Technol.* **35**:4436-4443.
- Weng, L., Temminghoff, E.J.M., Van Riemsdijk, W.H., 2001b. Determination of the free ion concentration of trace metals in soil solution using a soil column Donnan membrane technique. *Eur. J. Soil Sci.* **52**:629-637.
- Weng, L., Van Riemsdijk, W.H., and Temminghoff, E.J.M., 2005. Kinetic aspects of Donnan Membrane Technique for measuring free trace cation concentration. *Anal. Chem.* **77**:2852-2861.
- West, A.W., Sparling, G.P., Feltham, G.W., and Reynolds, J., 1992. Microbial activity and survival in soils dried at different rates. *Aust. J. Soil. Res.* **30**:209-222.
- Wiskerke, J.S.C., Verhoeven, F.P.M., Brussaard, L., Struik, P.C., and Wienk, J.F., 2003. Rethinking environmental management in Dutch dairy farming: a multidisciplinary farmer-driven approach (Preface). *Neth. J. Agr. Sci.* **51**:3-7.

-
- Witehead, D.C., 1995. *Grassland Nitrogen*. CAB International, Wallingford, pp. 397.
- Wrage, N., Velthof, G.L., Laanbroek, H.J., and Oenema, O., 2004. Nitrous oxide production in grassland soils: Assessing the contribution of nitrifier denitrification. *Soil Biol. Biochem.* **36**:229-236.
- Zhu, J., 2000. A review of microbiology in swine manure odor control. *Agr. Ecosyst. Environ.* **78**:93-106.

Summary/Samenvatting



Summary

During the last century agricultural intensity and productivity increased remarkably in Europe, especially in the Netherlands. Dairy farming in which plant and livestock production are combined, became the main sector in Dutch agriculture. The increase in agricultural intensity and productivity was mainly realized by an increased use of imported feed and high inputs of artificial fertilizer, which led to a decoupling of the internal farm chain crop-cow-manure-soil. For instance, the nutrient use efficiency of dairy cows is low (ca. 15-30% for N) and hence most nutrients fed are excreted. Also, large amounts of nutrients were applied as manure to a relatively small area of agricultural soils.

Application of manure at higher rates than needed for crop production or out of sync with the uptake of plants can result in a nutrient build up in the soil. When the nutrient binding capacity of the soil is exceeded, nutrients are susceptible for losses to the environment by leaching and/or runoff. Losses from manure to the environment can also occur by volatilization during accumulation or storage of manure, or during and after application of manure. Environmental problems related to agricultural activities are soil acidification, contamination of groundwater, and eutrophication of nutrient 'poor' ecosystems.

To reduce the impact of agricultural activities on the environment the Dutch government introduced several policies and measures, thereby setting conditions for the use of manure. Since January 2006 the manure policy of the Netherlands is, in accordance with European legislation, based on application standards, which allows a certain amount of livestock manure to be applied to agricultural soils. In order to comply with the manure application standards, while maintaining or even increasing agricultural productivity, the nutrient use efficiency of manure needs to be improved. Manure composition is one of the factors determining the nutrient use efficiency of manure as fertilizer. The main objectives of the thesis were to gain more insight in manure composition and ammonia (NH_3) volatilization and to gain more insight into the relationships between soil organic matter content and nitrogen (N) flows in grasslands.

One of the possibilities to reduce NH_3 volatilization is to apply additives to manure. The effect of three manure additives (Euro Mest-mix[®], Effective Micro-organisms[®], and Agri-mest[®]) on NH_3 volatilization from manure was tested in an incubation experiment (Chapter 2). Furthermore, the effects of mixing and temperature (4, 20, and 35 °C) on NH_3 volatilization were included in the experi-

ment. The incubation study showed that NH_3 volatilization increased with temperature increase and mixing of the manures, except for the incubation at 35 °C. The reduction in NH_3 volatilization when manures were mixed at 35 °C were related to a reduced crust resistance to gaseous transport at higher temperatures and a higher overpressure of NH_3 when slurries were not mixed. Moreover, mixing introduced oxygen into the anaerobic manure environment, which slowed down anaerobic microbial activity.

The use of additives did not change manure characteristics (pH, dry matter, N_{total} , N_{mineral} , C/N, and C/ N_{organic}) and did in general not result in a significant ($p < 0.05$) decrease in NH_3 emissions. Only at 4 °C and no mixing of the manures, a significant decrease of 34% in NH_3 volatilization was observed, when a combination of the additives Agri-mest® and Effective Micro-organisms® was applied to manure.

To study manure composition in detail the Donnan Membrane Technique (DMT) was adapted to measure 'free' cation concentrations in manure (Chapter 3). The developed DMT-manure cell consists of an acceptor compartment, which is separated from manure by two negatively charged cation-exchange membranes, which only allow exchange of 'free' cations between the manure and the acceptor solution. Equilibrium tests with a synthetic solution showed that Donnan equilibrium between 'free' cation concentrations in the acceptor solution and 'free' cation concentrations in the synthetic solution was obtained after approximately four days.

The DMT-manure cell was used to study the effect of dilution of animal manure (with distilled water) on 'free' dissolved ionic species (K^+ , Mg^{2+} , Na^+ , Ca^{2+} , and NH_4^+). Total nutrient concentrations, as well as the monovalent 'free' cation concentrations (K^+ , Na^+ , and NH_4^+) studied decreased proportionately with increasing dilution. In contrast, the 'free' dissolved concentrations of divalent cations (Ca^{2+} and Mg^{2+}) remained more or less constant with dilution, which is most probably the result of the release of these ions from organic matter. Also, dissolution of phosphate minerals (struvite and whitlockite), which were likely present in the initial manure, may have contributed to the buffering of the 'free' Ca^{2+} and Mg^{2+} concentrations.

Effects of various dietary protein and energy levels on manure composition (Ca, Mg, K, Na, N, P, and pH) were studied by determining total and direct available ('free') nutrient concentrations in eight manures obtained from a feeding trial with non-lactating cows (Chapter 4). Furthermore, the effects of dietary changes on NH_3 volatilization from manures were studied. Both, raising the crude

protein content of the feed, or raising the energy content of the feed resulted in an increase in total N and total P content of the manures. A low protein content, or a low energy content of the diets reduced total ammoniacal nitrogen (TAN) concentrations in the manures. Changes in the protein content or the energy content of the feed did not significantly affect the 'free'-to-total ratios of Ca and Mg content of the manures.

The NH_3 volatilization study showed that NH_3 volatilization from manure was higher for manures produced from diets with high protein content. In contrast, NH_3 volatilization from manures produced from diets with a high energy content were lower than from manures produced from diets with a low energy content, although the TAN content of the former manures were higher, but manure pHs were lower, which shifted the $\text{NH}_{3,\text{aq}}\text{-NH}_4^+$ equilibrium in favor of NH_4^+ .

Also insight in the chemical speciation of anionic species (e.g. HPO_4^{2-} , PO_4^{3-} , HS^- , and SO_4^{2-}) in manure is needed to improve nutrient management and to reduce nutrient losses to the environment. Therefore, it was tested if the DMT-manure cell, by using a hydrophilic anion-exchange membrane, could be used to measure 'free' anionic species in manure (Chapter 5). The suitability of the cells for anion measurement was tested in synthetic solutions. Donnan equilibrium time was affected by the charge of the P species. Within five days 95% of the Donnan equilibrium concentration for H_2PO_4^- was reached, whereas for HPO_4^{2-} it took about sixteen days. A study with a multi-component system showed that Donnan equilibrium time increased, besides charge, with the effective diffusion coefficients of the species. In contrast to complex anionic molecules like humic acid, simple organic molecules (e.g. citrate) were transported over the membrane as may be expected.

Next, the cell was tested in three manures. Only a small part of total P (<1%) and total S (<13%) was present in a 'free' dissolved ionic form. In contrast, the readily soluble amounts of P and S, as determined by sequential extraction, were much higher, ranging from 74% to more than 90% for P and from 30 to 85% for S.

In a field trial (Chapter 6), combined with incubation experiments, it was investigated how N dynamics in grasslands were affected by differences in soil organic matter content (SOM). It was expected that N mineralization would increase with an increasing SOM content and that mineralization during the growing season would occur more gradually at higher SOM content. Eighteen grasslands situated on sandy soils varying in SOM content were selected on nine

dairy farms. The grasslands were managed by the farmers according to their farming style. Potential N mineralization rates (measured at 20 °C and 60% water holding capacity) could be predicted well using a model that was based on the initial dissolved organic nitrogen (DON) content of the soil as measured in 0.01 M CaCl₂ extract. Furthermore, potential N mineralization rates were also related to the dissolved organic carbon (DOC) content of the soils. However, only weak relationships at best were found between N mineralization rates in soils incubated under field conditions and measured soil characteristics. During the whole growing season total soluble N was found in all grassland soils. Two peaks in total soluble N were observed, which were both preceded by a period of drought. The peaks could not be related to the time of manure application. Maximum total soluble N and nitrate (NO₃⁻) content were higher in soils with a lower SOM content. This is probably caused by faster water depletion of soils with a lower SOM content during drought periods, which will hamper N uptake by plants. No effect of organic manure management on soil N mineralization was observed. Despite of the large fertilizer quantities supplied to the grasslands, N leaching was minimal and was for all grasslands below the legal standard of 50 mg L⁻¹ NO₃⁻.

Overall, the Donnan Membrane Technique is a relatively easy method to determine speciation of nutrients in manure. The design of the cell was such that its usefulness is not restricted to manure alone, but can be used in other aqueous solutions (e.g. surface waters) as well.

For farmers it is difficult to quantify the effects additives have on manure quality, because farming management changes continuously, making it hard for farmers to have a representative control sample. The results presented in this thesis indicate that it is unlikely that Euro Mest-mix®, Effective Micro-organisms®, and Agri-mest® will reduce NH₃ emissions from manure in daily practices.

Adjusting the dietary protein (and/or energy content) proved to be a much more effective way to reduce NH₃ volatilization from manure. Considerable lower amounts of NH₃ were emitted from manures produced by non-lactating cows which were fed diets with a lower dietary protein content. Moreover, adjusting the diet will reduce NH₃ volatilization at any time of manure handling, e.g. during housing, storage, and during and after application of manure, which is not always the case for NH₃ volatilization reduction techniques.

Although promising, more study on the relationships between the initial DON content and N mineralization rates is needed before the initial DON content can be used to estimate the amount of N which is mineralized in the soil during the growing season.

In today's farming practices the organic matter content of soils is sometimes depleted. However, as is presented in this thesis, depleting the organic matter content of soils can lead to higher fluctuations of total soluble N, which may lead to more leaching and thereby polluting the environment with N. Applying manure with a higher DM content to soils will lead to an increase in SOM content, thereby increasing the nutrient binding capacity and the water holding capacity of the soil and will improve soil structure.

Samenvatting

De intensiteit en de productiviteit van de Europese landbouw is gedurende de vorige eeuw sterk toegenomen, met name in Nederland. Binnen de Nederlandse landbouw vormt de melkveehouderij, waarin het houden van een melkveestapel wordt gecombineerd met gewasproductie, momenteel de belangrijkste sector. De intensivering en de toename in de productiviteit van de landbouw waren vooral mogelijk door een stijging van de geïmporteerde hoeveelheid veevoer en door het toenemend gebruik van kunstmest. Dit heeft echter wel geleid tot een verstoring van de keten gewas-koe-mest-grond. De mate van benutting van voedingsstoffen door melkkoeien is laag (ca. 15-30% voor stikstof) en de meeste voedingsstoffen verlaten de koe dan ook via de feces of via de urine. De mest (combinatie van feces, urine, spoelwater en strooisel) werd toegediend op een relatief klein areaal van landbouwgronden.

Wanneer bemesting plaatsvindt in grotere hoeveelheden dan nodig is voor gewasproductie, of op het verkeerde moment, kan dit leiden tot een verrijking van de bodem met nutriënten. Als de bindingscapaciteit van de bodem wordt overschreden, kan dit leiden tot uitspoeling of afspoeling van nutriënten, waardoor deze in het milieu terecht kunnen komen. Nutriëntverliezen vanuit de mest naar het milieu kunnen ook optreden door middel van vervluchtiging, tijdens het verzamelen en de opslag van mest, of tijdens en na toepassing van mest. Milieu-problemen gerelateerd aan de agrarische sector zijn bodemverzuring, verontreiniging van grondwater en eutrofiëring van nutriënt-arme ecosystemen.

Om de milieubelasting van de agrarische sector te verminderen heeft de Nederlandse overheid verschillende beleidsmaatregelen genomen, daarmee voorwaarden stellend aan het gebruik van mest. Sinds januari 2006 is het Nederlandse mestbeleid, conform Europese regelgeving, gebaseerd op gebruiksnormen. De gebruiksnormen stellen een limiet aan de hoeveelheid mest die op een landbouwgrond mag worden aangebracht. Om aan de gebruiksnormen te kunnen voldoen en tegelijkertijd de agrarische productiviteit te kunnen handhaven, of zelfs verhogen, dient de benuttingefficiëntie van mest te worden verhoogd. De samenstelling van mest is één van de factoren die bepalend is voor de mate waarin mest als voedingsbron door planten kan worden benut. Dit onderzoek is uitgevoerd om meer inzicht te krijgen in de samenstelling van mest en de daarmee samenhangende ammoniak (NH_3) vervluchtiging. Bovendien is getracht om meer inzicht te krijgen in de relaties tussen het bodem organische stofgehalte en stikstof (N) stromen in graslanden.

Een manier om NH_3 vervluchtiging uit mest te verminderen is door middel van het gebruik van additieven. In een incubatie-experiment (Hoofdstuk 2) zijn drie mestadditieven (Euro Mest-mix[®], Effectieve Micro-organismen[®] en Agri-Mest[®]) getest voor de mate waarin ze NH_3 vervluchtiging uit mest beïnvloeden. Tevens is onderzocht wat de invloeden zijn van mixen en incubatietemperatuur (4, 20 en 35 °C) op NH_3 vervluchtiging. Ammoniak vervluchtiging nam toe bij toenemende incubatietemperatuur en bij mixen van de mest, m.u.v. de incubatie bij 35 °C. De verlaging in NH_3 vervluchtiging, wanneer de mesten bij 35 °C werden gemixed, is het gevolg van een afname van de korstweerstand tegen gas-transport bij stijging van de incubatietemperatuur. Bovendien is de NH_3 overdruk hoger wanneer er niet gemixed wordt, waardoor de korstweerstand afneemt. Daarnaast zorgt het mixen ervoor dat zuurstof wordt geïntroduceerd in het anaërobe mestmilieu, waardoor de activiteit van anaerobe micro-organismen wordt afgeremd.

De toevoeging van additieven aan mest had geen effect op de onderzochte mestkenmerken (pH, droge stof, N_{totaal} , N_{mineraal} , C/N, en C/N_{organisch}) en in het algemeen werd er geen significante ($p < 0.05$) daling in NH_3 vervluchtiging waargenomen. Uitsluitend in de situatie dat Agri-mest[®] en Effectieve Micro-organismen[®] beiden aan de mest werden toegevoegd en de mest geïncubeerd werd bij 4 °C zonder mixen, werd er een significante daling van 34% in NH_3 vervluchtiging waargenomen.

Om de mestsamenstelling nader te kunnen bestuderen is de Donnan Membraan Techniek (DMT) zodanig aangepast dat 'vrije' kation concentraties in mest bepaald kunnen worden (Hoofdstuk 3). De ontwikkelde DMT-mestcel bestaat uit een acceptorcompartiment, dat van de mest is afgescheiden door middel van twee negatief geladen kation uitwisselingsmembranen. Enkel 'vrije' kationen kunnen door de membranen vrij worden uitgewisseld tussen de mest en de oplossing in het acceptorcompartiment. Laboratoriumtests toonden aan dat de evenwichtstijd benodigd voor de instelling van het Donnan evenwicht tussen 'vrije' kation concentraties in de acceptoroplossing en 'vrije' kation concentraties in de gebruikte synthetische oplossing ongeveer vier dagen bedraagt.

Vervolgens is de DMT-mestcel gebruikt om het effect van verdunning van dierlijke mest (met gedestilleerd water) op vrije kation concentraties (K^+ , Mg^{2+} , Na^+ , Ca^{2+} en NH_4^+) te bestuderen. De totaal concentraties, als mede de 'vrije' kation concentraties of de bestudeerde monovalente kationen (K^+ , Na^+ en NH_4^+) daalden evenredig met de toenemende verdunning. Dit in tegenstelling tot de

gemeten 'vrije' divalente ion concentraties (Ca^{2+} en Mg^{2+}), welke nauwelijks veranderden wanneer de mest verdund werd. Dit is waarschijnlijk het gevolg van desorptie van Ca^{2+} en Mg^{2+} van het organisch materiaal in de mest. Ook kan het oplossen van fosfaat mineralen (m.n. struviet en whitlockiet) hebben bijgedragen aan de buffering van de 'vrije' Ca^{2+} en Mg^{2+} concentraties.

De effecten van veranderingen in eiwit en energieniveau van het rantsoen op de mestsamenstelling zijn bestudeerd door totaal en direct beschikbare ('vrije') nutriënt concentraties (Ca, Mg, K, Na, N, P en pH) te bepalen voor acht mesten die waren verkregen uit een dieetproef met niet-melkgevende (droogstaande) koeien (Hoofdstuk 4). Tevens is onderzocht wat het gevolg was van veranderingen in de dieetsamenstelling op de NH_3 vervluchtiging. Zowel een stijging in het ruwe eiwit gehalte van het voedsel, als een verhoging van de energie inhoud van het rantsoen, resulteerden in hogere N en P totaal gehalten in de mest. Een laag eiwitgehalte, of een lage energie inhoud van de rantsoenen hadden een verlaging van de ammoniakale stikstof (TAN) tot gevolg. Veranderingen in het eiwitgehalte of in de energie inhoud hadden geen effect op de concentratie ratio's 'vrij':totaal van Ca of Mg in de mesten.

De NH_3 vervluchtigingstudie toonde aan dat NH_3 vervluchtiging van mest hoger was, wanneer deze afkomstig was van een rantsoen met een hoog eiwitgehalte. Ammoniak vervluchtiging van mesten afkomstig van 'rantsoenen met een hoge energie inhoud' waren lager dan die van mesten die afkomstig waren van 'laag energierantsoenen', ondanks dat de TAN gehalten van de eerst genoemde mesten hoger waren. Echter, de pH van de mesten afkomstig van 'hoog energierantsoenen' waren lager dan die van mesten afkomstig van 'laag energierantsoenen', waardoor het $\text{NH}_{3,\text{aq}}\text{-NH}_4^+$ evenwicht meer richting NH_4^+ ligt.

Voor een betere benutting van meststoffen, waardoor verliezen naar het milieu kunnen worden beperkt, is ook inzicht nodig in de chemische speciatie van anionen (bv. HPO_4^{2-} , PO_4^{3-} , HS^- , en SO_4^{2-}). Daarom is onderzocht of dat de DMT-mestcel, door middel van het gebruik van een hydrofiel anion uitwisselingsmembraan, toepasbaar gemaakt kon worden voor het meten van 'vrije' anionen in mest (Hoofdstuk 5). De geschiktheid van de mestcel voor anionmeting werd getest met behulp van synthetische oplossingen. De tijd benodigd voor het verkrijgen van Donnan evenwicht is afhankelijk van de speciatie van P; binnen vijf dagen werd voor H_2PO_4^- 95% van de Donnan evenwichtconcentratie bereikt, terwijl dit voor HPO_4^{2-} ongeveer zestien dagen duurde. In een multi-component systeem werd aangetoond dat Donnan evenwichtstijd toenam met, naast lading, de (effectieve) diffusie coëfficiënten van de species. In tegenstelling tot complexe

negatief geladen moleculen zoals humuszuren, werden eenvoudige organische moleculen (bv. citraat) zoals verwacht wel over het membraan getransporteerd.

Hierna is de cel toegepast in drie mesten. Slechts een kleine fractie van de totale hoeveelheid P (<1%) en van de totale hoeveelheid S (<13%) was aanwezig in de mest als 'vrij' P of 'vrij' S. Dit in tegenstelling tot de makkelijk oplosbare hoeveelheden P en S, welke respectievelijk 74-90% (voor P) en 30-85% (voor S) bedroegen, als werd aangetoond met behulp van sequentiële extractie.

Middels een combinatie van een veldexperiment met incubatieproeven (Hoofdstuk 6), werd onderzocht hoe N stromen in graslanden werden beïnvloed door verschillen in bodem organische stof (SOM). De verwachting was dat N mineralisatie zou toenemen met een toename in het SOM gehaltes en dat mineralisatie bij een hoger SOM gehalte van het grasland geleidelijker gedurende het groeiseizoen zou verlopen. De graslanden werden door de boeren beheerd naar eigen inzicht. Potentiële N mineralisatiesnelheden, gemeten bij 20 °C en bij 60% van het waterhoudend vermogen (veldcapaciteit), konden modelmatig goed voorspeld worden op basis van het oplosbaar organisch N gehalte (DON), wat gemeten werd in 0.01M CaCl₂ extracten aan het begin van de incubatieproeven. Daarnaast waren de potentiële N mineralisatiesnelheden ook gerelateerd aan het opgelost organisch koolstof (DOC) gehalte van de gronden. Echter, N mineralisatiesnelheden gemeten in gronden welke geïncubeerd waren onder veldomstandigheden toonden in het gunstigste geval maar een zwakke relatie met de gemeten bodemkarakteristieken. Gedurende het gehele groeiseizoen werd oplosbaar totaal N gemeten in alle graslanden. Twee pieken in oplosbaar totaal N zijn waargenomen, die allebei werden voorafgegaan door een periode van droogte. Geen van de pieken kon worden gerelateerd aan het tijdstip van bemesting. De maximale gehalten aan oplosbaar totaal N en nitraat (NO₃⁻) waren hoger voor gronden met een lager SOM gehalte. Dit wordt waarschijnlijk veroorzaakt door een snellere uitdroging van deze gronden (gedurende droogteperiodes), waardoor de N opname door planten geremd wordt. Er is geen effect waargenomen van het organisch mestbeheer op de N mineralisatie in de bodem. Ondanks dat grote hoeveelheden meststoffen op de graslanden zijn aangebracht, was de uitspoeling van N minimaal en bleef in alle gevallen beneden de wettelijke norm van 50 mg NO₃⁻ L⁻¹.

In het algemeen kan de Donnan Membraan Techniek beschouwd worden als een relatief eenvoudige methode om de speciatie van nutriënten in mest nader te onderzoeken. Het ontwerp van de DMT-mestcel was dusdanig dat het ge-

bruik ervan niet beperkt is tot mest alleen, maar dat de cel ook gebruikt kan worden in andere waterige oplossingen (bv. oppervlaktewater).

Voor boeren is het vaak moeilijk om effecten van additieven op mestkwaliteit goed te kunnen beoordelen, omdat hun 'bedrijfsmanagement' continu verandert en er dus nauwelijks sprake kan zijn van een representatief controlemonster. De resultaten beschreven in dit onderzoek duiden erop dat het in de dagelijkse praktijk onwaarschijnlijk is dat het gebruik van Euro Mest-mix[®], Effectieve Micro-organismen[®], of Agri-mest[®] zal leiden tot een verlaging van NH₃ emissies.

Aanpassingen in het eiwitgehalte van het rantsoen (en/of de energie-inhoud) bleek een veel effectievere methoden te zijn om NH₃ vervluchtiging van mest te verminderen. Aanzienlijk lagere hoeveelheden NH₃ vervluchtigden van mesten die geproduceerd waren door droogstaande koeien welke gevoerd werden met laag eiwitrantsoenen. Bovendien, zal een aanpassing in de rantsoensamenstelling effect hebben op de NH₃ vervluchtiging in de gehele mestketen (tijdens huisvesting, opslag en tijdens mestaanwending), wat niet vanzelfsprekend is voor alle beschikbare NH₃ vervluchtigingsreductietechnieken.

Hoewel veelbelovend, dient er nader onderzoek verricht te worden naar de relatie tussen het initiële DON gehalte van een grond en de snelheid van N mineralisatie, alvorens het initieel DON gehalte gebruikt kan worden om de hoeveelheid N te schatten die in een grond tijdens het groeiseizoen gemineraliseerd kan worden.

In de hedendaagse landbouwpraktijk wordt SOM soms extreem gemineraliseerd, wat tot uitputting van de bodem kan leiden. Echter, zoals in dit proefschrift beschreven is, kunnen bij een lager SOM gehalte van de bodem grotere schommelingen in de concentratie totaal oplosbaar N optreden, wat vervolgens tot meer uitspoeling zou kunnen leiden, waardoor het milieu meer verontreinigd kan worden met N. Het opbrengen van mest met een hoger DM gehalte op de bodem kan leiden tot een verhoging van het SOM gehalte van de bodem, waardoor de nutriënt- en de waterbindingscapaciteit van de bodem en de bodemstructuur kan verbeteren.

Dankwoord



Dankwoord

Met het afronden van dit proefschrift komt het einde inzicht van mijn promotiestudie. Het was zonder meer een leuke en leerzame periode en ik wil in het volgende gedeelte dan ook de vele mensen bedanken die bij dit project betrokken waren.

Mijn promotor Willem van Riemsdijk wil ik bedanken voor zijn hulp bij het schrijven van de verschillende artikelen. Willem, je constructieve opmerkingen hebben dit proefschrift sterk verbeterd. Verder wil ik je bedanken voor de snelheid waarmee je reageerde en voor de mailtjes met interessante literatuur, die ik af en toe van je mocht ontvangen.

Mijn co-promotor Erwin Temminghoff wil ik bedanken voor zijn dagelijkse begeleiding. Erwin bedankt voor je hulp bij het opzetten van de experimenten en voor je hulp bij het schrijven van de verschillende hoofdstukken van dit proefschrift.

Min of meer tegelijkertijd met mijn aio-schap begon Petra van Vliet aan haar Post-doc project binnen het VEL-VANLA project, waarbij haar onderzoek vooral betrekking had op 'biologische mest- en bodemkwaliteit', terwijl mijn onderzoek meer gericht was op de chemische aspecten ervan. Petra, bedankt voor je meedenken aan proefopzetten, het becommentariëren van manuscripten en je hulp bij de uitvoering van de statistiek (vaak in je vrije tijd!). 'Weer of geen weer', onze werkbezoeken in Friesland waren altijd een soort van uitje; van je koekjes, cake, oliebollen, taarten en baksels waarvan ik nog steeds niet weet hoe ze te benoemen heb ik enorm gesmuld.

Nicole Wrage, jou wil ik bedanken voor je hulp en het wegwijs maken op de vakgroep tijdens het begin van mijn promotie.

Veel dank ben ik Jaap Nelemans verschuldigd. Jaap, niet alleen dacht je mee met het opzetten van proeven. Wanneer iets niet mogelijk/goed was zei je dat niet alleen, maar dacht je ook mee aan mogelijke alternatieven. Met veel plezier heb ik dan ook mijn werkzaamheden op jullie (ja Willeke jij ook bedankt!) lab uitgevoerd. Dat Willeke van muziek hield wist ik, maar jouw enorme literaire kennis heeft me verbaasd. Niet alleen bleek je een expert in visserslatijn, maar ook in spreekwoorden bleek je uitstekend onderlegd.

Natuurlijk wil ik ook iedereen van het chemisch biologisch laboratorium bodem bedanken voor hun hulp bij het uitvoeren van experimenten en analyses.

Het was altijd wel mogelijk om nog een gaatje in de agenda te vinden, of het aantal metingen aan te passen.

Bedankt 'VEP-ers' voor al jullie hand- en spandiensten gedurende al die jaren van onderzoek, het waren er teveel om allemaal op te noemen.

Ook wil ik alle medewerkers (!) van de sectie Bodemkwaliteit bedanken voor de gezellige en leerzame tijd. De atmosfeer van een kelder is meestal bedompt, maar dat was beslist niet het geval in de kelder van de westvleugel van gebouw 316!. Naast het contact tijdens het werk, kwamen we elkaar regelmatig tegen met uitjes, borrels, lunches, e.d. Vooral het zeilweekend vond ik erg geslaagd, zelfs met twee kapiteins op één boot.

During my PhD period many people from abroad worked at the department. I hope you liked your visit, I know I did.

Mijn kamergenoot op de Dreijen was Erwin Kalis. Mijn eerste gedachte tijdens onze kennismaking was: 'wat een vreemde vogel'. Maar Erwin, je bleek een zeer gezellige collega met wie ik het goed kon vinden. Inmiddels ben je werkzaam bij het Laboratorium voor Fysische Chemie en Kolloïdkunde, maar ik weet zeker dat we contact zullen houden. Alvast bedankt dat je mijn paranimf wilde zijn.

Na de verhuizing naar het Atlas-gebouw kwam ik bij Ellen Fest op de kamer. Volgens velen was ik dan ook degene die het meeste erop vooruit ging, waarvoor mijn dank uitgaat naar Winnie en Esther.

Ellen, het was niet alleen erg gezellig (!) met je op de kamer, maar je bleek ook nog eens een uitstekend secretaresse te zijn. Je hebt me ontzettend geholpen met de lay-out van het boekje. Ook jij bedankt dat je mijn paranimf wilde zijn.

Verder wil ik Jaap Bloem, An Vos en Meint Veninga bedanken voor hun hulp bij het maken van de bacteriepreparaten.

Ook wil ik alle mensen bedanken die meegeholpen hebben met de veldwerkzaamheden tijdens één van de bezoeken aan het VEL-VANLA gebied.

Twee studenten hebben binnen dit promotieonderzoek een afstudeervak gevolgd. Abdenna, you worked very hard for your thesis, and it was rewarding to see the progress you made during your study. I wish you all the best for the future. Daarnaast kwam er op een zekere morgen ene Erwin van Boekel (Aaahhh nee, niet nog een Erwin ☺) informeren naar de mogelijkheden voor een afstudeerproject. Erwin, je appelflap was niet nodig om tot een positief oordeel te komen, want je was een ijverig student waarmee goed was samen te werken. Hoofdstuk 5 is met name op jouw onderzoeksresultaten gebaseerd.

Ook wil ik alle mensen bedanken die mij tijdens mijn promotie geholpen hebben, terwijl ze niet (altijd) direct bij het onderzoek betrokken waren: Agnes van de Pol, Marthijn Sonneveld, Lijbert Brussaard, Ron de Goede, Jan Huismans, Gerard Monteny, Jeroen Groot, Hugo van de Meer, Nick Hutchings, Jan Dijkstra, Ellis Hoffland, Joan Reijs, Phillip Ehlert, Liping Weng, Thom Kuiper en Frank Verhoeven. Jullie opmerkingen, meedenken en spontane (re)acties vormden voor mij een stimulans.

Verder wil ik de deelnemers aan het VEL-VANLA project, in het bijzonder de boeren die betrokken waren bij het veldexperiment (Hoofdstuk 6), bedanken voor het mogelijk maken van het onderzoek.

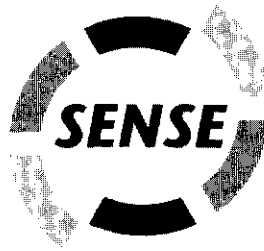
Arno de Haas wil ik bedanken voor de tekening van de DMT-mestcel.

'Peer' (Peter) Schermers bedankt voor je hulp bij het tekenen van figuur 1.2 en bij het ontwerpen van de voorkant. Het kostte iets meer tijd dan ik verwachtte, maar het resultaat is er dan ook naar.

Tenslotte wil ik mijn familie en vrienden bedanken voor al hun steun. Af en toe heb ik het gevoel gehad dat ik door mijn soms drukke werkzaamheden en lange dagen te weinig tijd voor jullie had en wat traag op mailtjes reageerde (he Nicole?). Het stappen, musiceren, en kletsen zorgden voor een zeer welkome afleiding. Tevens wil ik me bij jullie verontschuldigen voor het telkens weer moeten afslaan van jullie vrijwillige 'donaties'.

Levensloop

Berdus (Bert) van der Stelt werd op 22 mei 1976 geboren te Almkerk (NB). In 1994 behaalde hij het VWO diploma aan het Christelijk Altena College te Sleetwijk. Van 1994 tot 1998 studeerde hij Milieukunde aan de hogeschool West-Brabant. Voor zijn afstudeerscriptie onderzocht Bert de mogelijkheden tot preprecipitatie op rioolwaterzuiveringsinstallatie Dongemond. In september 1998 ging Bert scheikunde studeren aan de Universiteit Utrecht. Zijn onderzoeksspecialisatie heeft hij uitgevoerd bij de vakgroep Enzymologie & Proteïne Engineering, alwaar hij de mogelijkheden onderzocht tot het *in vitro* vouwen van fragmenten van een buitenmembraan eiwit (OMPLA) tot een functioneel eiwit. Na de afronding van zijn studie Scheikunde werd Bert in januari 2002 aangesteld als assistent in opleiding aan de Wageningen Universiteit. Hiervoor heeft hij een onderzoek uitgevoerd naar de bijdrage van mestkwaliteit en bodemkwaliteit aan een verbeterde nutriëntenbenutting en verminderde N-verliezen in de melkveehouderij. De belangrijkste resultaten van dit onderzoek staan in dit proefschrift beschreven. In maart 2007 is het onderzoek afgerond met de verdediging van dit proefschrift.



Netherlands Research School for the
Socio-Economic and Natural Sciences of the Environment

CERTIFICATE

The Netherlands Research School for the
Socio-Economic and Natural Sciences of the Environment
(SENSE), declares that

Berdus van der Stelt

Born on: *22 May 1976* at: *Almkerk The Netherlands*

has successfully fulfilled all requirements of the
Educational Programme of SENSE.

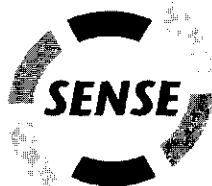
Place: *Wageningen* Date: *30 March 2007*

the Chairman of the
SENSE board

Prof. dr. R. Leemans

the SENSE Director
of Education

Dr. C. Kroeze



The SENSE Research School declares that Mr. Berdus van der Stelt has successfully fulfilled all requirements of the Educational PhD Programme of SENSE with a work load of 37 ECTS, including the following activities:

SENSE PhD courses:

- Environmental Research in Context
- Research Context Activity: Member of organizing committee SENSE Summer Symposium 2005 'Kyoto and Beyond: A Good Climate for Responding to Climate Change?'
- Basic and Advanced Statistics
- Soil and Plant Analysis and Data Handling
- Speciation and Bioavailability

Other advanced MSc and PhD courses:

- Speciation and Transport
- Chemical Interactions in Soil, Water and Sediment
- Dynamic Presentation
- Scientific Writing

Presentations:

- Oral presentation: Contestable Nature; Claims on the Countryside, Utrecht, The Netherlands, June 2004
- Poster presentation: 14th Nitrogen Workshop, Maastricht, The Netherlands, October 2005

Deputy director SENSE

Dr. A. van Dommelen

