

Utilisation and losses of nitrogen in grazed grassland

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Abstract

In the past, N response studies on grassland concentrated on cutting experiments. However, the applicability of the results of this work on commercial farms has been questioned and recent research has been more concerned with responses to applied N under grazing in comparison with cutting. The purpose of this paper is to discuss the results of experiments conducted on an old sandy soil near Wageningen and a recently reclaimed loamy soil in Flevoland to compare the response of herbage yield to applied N under grazing and cutting and to assess the fate of applied N. At both sites, rates of N application to the cut and grazed swards were about 250, 400, 550 and 700 kg ha⁻¹ year⁻¹; the cutting experiments also included control plots without N application.

In all cases, the response of herbage yield to applied N on the grazed plots was considerably smaller than on the cut plots. This resulted from a relatively high dry matter yield at the lowest rate of N application, a lower apparent recovery of applied N, a smaller effect of absorbed N on herbage yield and a considerably lower maximum herbage yield under grazing in comparison with cutting. These differences were caused by specific (positive and negative) effects of grazing as well as by the fact that net herbage yields under grazing (i.e. herbage intake) were compared with gross yields under cutting. The comparison of gross and net herbage yields and the too high lowest N rate on the grazed plots hamper a proper calculation and comparison of the optimum rates of N under grazing and cutting in these experiments.

Soil inorganic N was monitored on the grazed plots on the sandy soil during 5 years. There was a regular pattern of accumulation of inorganic N in the growing season and loss during the winter. In particular at the highest rates of N application, estimated nitrate losses in drainage water did not account for the

losses of inorganic N from the soil layer of 0 - 60 cm. Nitrogen losses by denitrification in the top soil amounted to 1 - 3% of fertilizer N applied.

Small-plot experiments were conducted on a sandy soil to assess the fate of animal excreta after application to grassland. The proportion of urinary and faecal N lost by volatilisation of ammonia was, on average, 13%. Other rapid losses of urine N were observed. These averaged about 30% of urine N and appeared to be associated with nitrification. The remaining part of urinary N, on average about 55%, was recovered in the herbage or as residual inorganic N in the soil layer of 0 - 60 cm.

The results obtained were used to draw up N balance sheets of the grazed plots on the sandy soil. Between 30 and 40% of applied fertilizer N was not accounted for. This suggests accumulation of organic N in the soil, denitrification in deeper soil layers and underestimation of nitrate leaching. Despite intensive research, it is not yet possible to account for all N losses from grazed grassland.

Introduction

In his inaugural lecture at the Wageningen Agricultural University, 't Mannetje (1983) mentioned three research priorities related to the application of nitrogen (N) to Dutch grasslands:

- To assess the optimum rate of N application in grazing or whole-farm experiments that should extend over a period of several years to take account of positive or negative effects on sward quality. Up to then, N response on grassland had been studied almost exclusively in cutting experiments, often during a short period on a selected good sward.
- To study the best distribution of N over the growing season. 't Mannetje stated that the recommended decrease of the N rate in the course of the season was not based on experimental evidence.
- To explore the possibilities to replace fertilizer N by legumes. In this respect, he advocated the use of lucerne to replace grass and maize silage.

These points indicate doubts about scientific evidence of common practices of N application to grassland in The Netherlands. 't Mannetje stressed the need to reduce variable costs on dairy farms in a situation of decreasing milk quota and milk prices. He expected to accomplish this by avoiding a too liberal use of artificial fertilizers and concentrates. More recently, concern about negative effects of intensive ruminant production systems on the environment has also stimulated a critical assessment of the levels of fertilizer and concentrate use (van

der Meer, 1982; Aarts et al., 1988; Aart et al., 1992; 't Mannetje and Paoletti, 1992; 't Mannetje, 1994; van der Meer and van der Putten, 1995).

In 1984, 't Mannetje initiated a joint research project to compare herbage yield response to applied N under grazing and cutting and to study N flows and losses in grazed grassland. This paper summarizes and discusses the main results obtained.

Herbage yield response to applied N under grazing and cutting

The research project mentioned in the Introduction was mainly carried out at two experimental farms of the Wageningen Agricultural University, viz. the A.P. Minderhoudhoeve at Swifterbant in Eastern Flevoland, and the Meenthoeve at Achterberg near Wageningen. The A.P. Minderhoudhoeve is situated on a calcareous silty loam, reclaimed in the 1950's. The experimental fields had been under grass for more than 20 years, but were resown with *Lolium perenne* cv. Wendy in 1985. Herbage yield response to applied N was assessed under continuous grazing by dairy cows, and under cutting at a dry matter yield of approximately 2000 kg ha⁻¹ and in the first year (1986) also under weekly cutting. Rates of N application were 250, 400, 550 and 700 kg ha⁻¹ year⁻¹. The cutting experiment included control plots without N application. The Meenthoeve is situated on a sandy soil with a moderate organic matter content. The old permanent grassland on the experimental fields had been resown in 1981 with a mixture of the *Lolium perenne* cvs. Splendor, Pelo and Vigor. At this site, herbage yield response to applied N was assessed under rotational grazing by steers, and under cutting at a dry matter yield of approximately 2000 kg ha⁻¹. Rates of N application were the same as in the experiments at the A.P. Minderhoudhoeve. Experimental methods at the two sites have been described extensively by Deenen (1994).

Effects of the grassland utilisation system on the response of herbage yield to applied N are conveniently analysed by means of the so-called three quadrants diagram, which interrelates supply of N, N uptake in the herbage and herbage yield (Figure 1). This method of analysis is based on the fact that the effect of applied N on herbage yield (quadrant II) depends on the proportion of applied N absorbed by the crop (N yield in the herbage, quadrant IV), and the effect of absorbed N on herbage yield (quadrant I). It has been shown elsewhere (van der Meer and van Uum-van Lohuyzen, 1986) that the shape of the N response curve depends on:

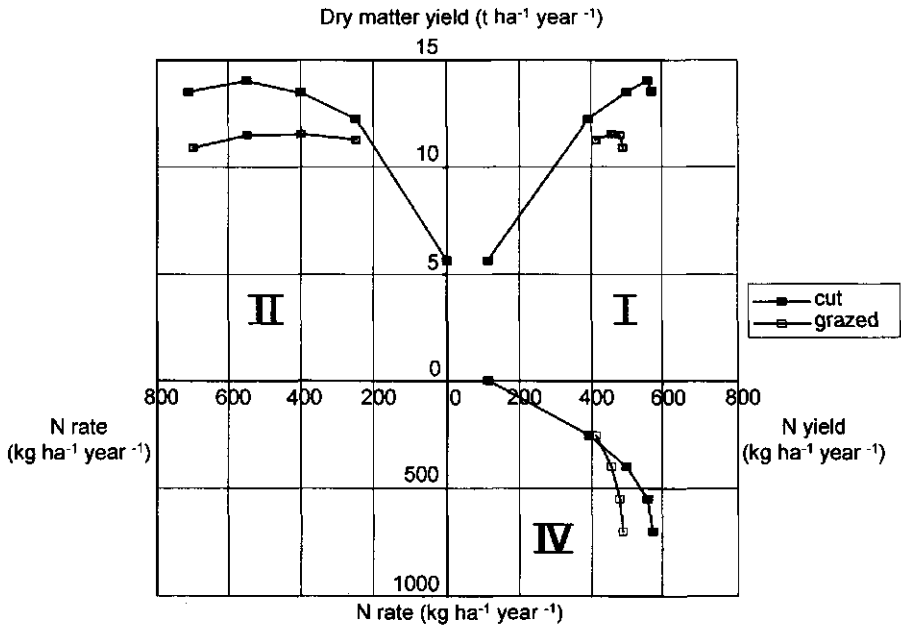


Figure 1. The effect of the rate of N application on N uptake and herbage yield under cutting and rotational grazing with steers on the sandy soil in 1987. For cutting, results are averages of 4 replicates, and for grazing of 2 replicates (Deenen, 1994).

- the uptake of N from other sources than fertilizer or slurry, viz. from soil reserves, atmospheric deposition, biological fixation and urine and dung from grazing animals (shown by the intercept of the lines in quadrant IV);
- the apparent recovery of applied N (ANR), i.e. the proportion of applied N harvested in the herbage (shown by the slope of the lines in quadrant IV);
- the N concentration in the harvested herbage, i.e. the reciprocal of the dry matter yield per kg N harvested (quadrant I), and
- the maximum dry matter yield (quadrant I).

Grazing, compared to cutting, probably affects these aspects as follows:

- Excreta of grazing animals increase N supply. This may enhance N yield in the herbage at nil fertilizer N, and also ANR because of the increasing production of urine N at increasing rate of N application.
- Grazing animals generally harvest a smaller part of the above-ground biomass than the cutting machine, because they leave longer stubbles and refuse herbage around dung pats. Longer stubbles and herbage residues may enhance regrowth, but most probably reduce the harvest index for N and, hence, N yield at nil fertilizer N as well as ANR. A part of the N in stubbles and herbage

residues will be utilised by regrowths later in the season, but another part will be added to the pool of organic N in the soil.

- Under grazing, herbage generally is harvested in a younger stage of growth and, consequently, with a higher N content (Sibma and Alberda, 1980). This, together with negative effects of the grazing animals on the sward by treading, poaching, fouling and urine scorching, will reduce dry matter yield at all levels of N uptake, as well as maximum dry matter yield.

In both experiments conducted by the former Department of Field Crops and Grassland Science of the Wageningen Agricultural University, herbage yields under grazing were lower and responded less to applied N than under cutting (Deenen, 1994). On the calcareous silty loam at Swifterbant, this was caused by a slightly lower N yield at the lowest rate of N application (250 kg ha⁻¹ year⁻¹), a considerably lower ANR, and a lower yield of dry matter or net energy for lactation at all levels of N uptake. Under grazing, average marginal ANR values over the years 1986, 1987 and 1988 were 63, 40 and 13% of the extra N applied for the increments 250 to 400, 400 to 550 and 550 to 700 kg N ha⁻¹ year⁻¹, respectively. Unfortunately, Deenen did not specify ANR values obtained in the cutting experiment, but only indicated that they varied between 80 and occasionally more than 100% for all rates of N application. However, the three quadrants diagrams presented in his thesis, show considerably lower values for the increment 550 to 700 kg N ha⁻¹ year⁻¹. The mean apparent nitrogen efficiency values (ANE; van der Meer *et al.*, 1987) for the increments of N application mentioned, were 9.7, 6.5 and 1.9 kVEM per extra kg N under grazing (VEM is the unit for net energy for lactation in the Dutch feed evaluation system; 1 VEM = 6.9 kJ), and 17.6, 10.6 and 5.8 kVEM per extra kg N under cutting (Deenen, 1994).

On the sandy soil at Achterberg, there was no response to N application above the lowest rate of 250 kg ha⁻¹ year⁻¹ under rotational grazing with steers, whereas under cutting herbage yields increased up to an annual application rate of approximately 550 kg N ha⁻¹ (Figure 1). Deenen (1994) attributed the lack of response to higher N rates under grazing to negative effects of poaching and urine scorching on the sward, which increased at the higher N rates. However, the rather small response in the cutting experiment to annual N rates of more than 250 kg ha⁻¹ (Figure 1) also indicates less favourable growing conditions at Achterberg than at Swifterbant. Figure 1 confirms the findings in the experiment at Swifterbant, viz. on the grazed fields a relatively high herbage yield at the lowest rate of N application, a lower ANR, and a smaller effect of absorbed N on herbage yield than under cutting.

With respect to the differences between cutting and grazing, it should be taken into account that in the experiments discussed here, the response to applied N has

been expressed in terms of harvested herbage. Under cutting, this was the gross herbage yield, i.e. all the herbage harvested by cutting at a height of 4 - 5 cm. Under farming conditions, there will be losses after cutting, associated with the field period, harvesting, conservation and feeding, and only a part of the gross yield will be utilised by the animals. There is no information on the effect of the rate of N application on these losses and on the proportion of cut herbage finally utilised by the animals. Under grazing, harvested herbage was the net yield, i.e. the herbage consumed by the animals. Here, "harvesting and feeding losses" were not included in the yield.

In other comparisons of herbage yield response to applied N under cutting and grazing, herbage yield under grazing has been assessed by measuring accumulated herbage at the start of grazing (e.g. Boxem, 1973; Benke, 1992). In these studies, strips were cut and sampled only before turning in the animals to the fields and no estimates were made of herbage growth during grazing and of herbage residues. As a consequence, these experiments did not provide information on the utilisation efficiency of accumulated herbage, which may be affected by grazing management, soil and weather conditions and rate of N application (Deenen, 1994). Therefore, it is difficult to draw practical conclusions from these comparisons.

In some studies, defoliation frequency may have affected the observed differences in N response between cutting and grazing. In the experiments at Swifterbant and Achterberg, harvesting frequencies on the cut and grazed fields were independent and determined by growth rate and target yields at harvest, assuring good management for each grassland utilisation system and N rate (Deenen, 1994). In the studies of Jackson and Williams (1979) and Benke (1992), all experimental treatments were harvested on the same dates, which will be sub-optimal for some treatments and affect the shape of the N response curves and the differences between cutting and grazing.

Deenen (1994) calculated "optimum" rates of N application, assuming a marginal profitability of 7.5 kg dry matter per kg N applied. However, because he determined gross herbage yield under cutting and net yield under grazing, it is not correct to use the same marginal profitability for both grassland utilisation systems. Hence, Deenen overestimated the difference in "optimum" N rate between cutting and grazing (about 200 kg ha⁻¹ year⁻¹). In addition, the lowest rate of N application to the grazed plots at Swifterbant and Achterberg was too high to calculate accurate N response curves and optimum N rates.

The marginal profitability of 7.5 kg dry matter per kg N applied, used in the calculations of Deenen, has been proposed by Prins (1983) as the average of

different values reported in literature. Several authors have used this value in later studies without taking account of the factors affecting marginal profitability, like herbage quality, grassland utilisation system, management factors affecting utilisation of gross yield, and farm structure and management (Mooij and Vellinga, 1992; Deenen, 1994). After determining herbage yield response to applied N, more attention should be given to a correct interpretation and use of the results.

The experiments at Swifterbant and Achterberg have shown a consistently smaller response of herbage yield to applied N under grazing than under cutting. Probably, this smaller response was partly caused by the fact that under grazing net herbage yield had been determined and under cutting gross herbage yield. However, the relatively high herbage yields at the lowest rate of N application, and the lower apparent recoveries of applied N and maximum herbage yields under grazing in comparison with cutting (Deenen, 1994), also point to specific positive and negative effects of grazing on the response to applied N. These specific effects may lead to a lower optimum N rate under grazing, even if a lower marginal profitability is observed than under cutting. Model calculations, as proposed by Mooij and Vellinga (1992), may be used for a further analysis of specific effects of grazing on the response of herbage yield to applied N and on the optimum rate of N application.

Nitrogen losses from grazed grassland

The experiments at Swifterbant and Achterberg, discussed in the preceding paragraph, also have been used to quantify N flows and losses ('t Mannetje and Jarvis, 1990). Here, the main results of the work on the sandy soil at Achterberg will be presented. In the grazing experiment, we monitored the amount of residual inorganic N in the soil layer of 0 - 60 cm at each rate of N application. For this purpose, soil samples were taken four times a year, viz. just before fertilizer application in spring, in the second half of June, in the second half of August, and immediately after the last grazing in late October or early November. Soil sampling in June and August was always carried out immediately after grazing and before fertilizer application. Each time, the experimental treatments were sampled in quadruplicate. The replicates consisted of 12 to 15 cores of about 4 cm diameter, taken at random from the field. These cores were divided into layers of 20 cm to give 3 sub-samples per replicate. Sub-samples were analysed individually for ammonium and nitrate N. The results are presented in Figure 2.

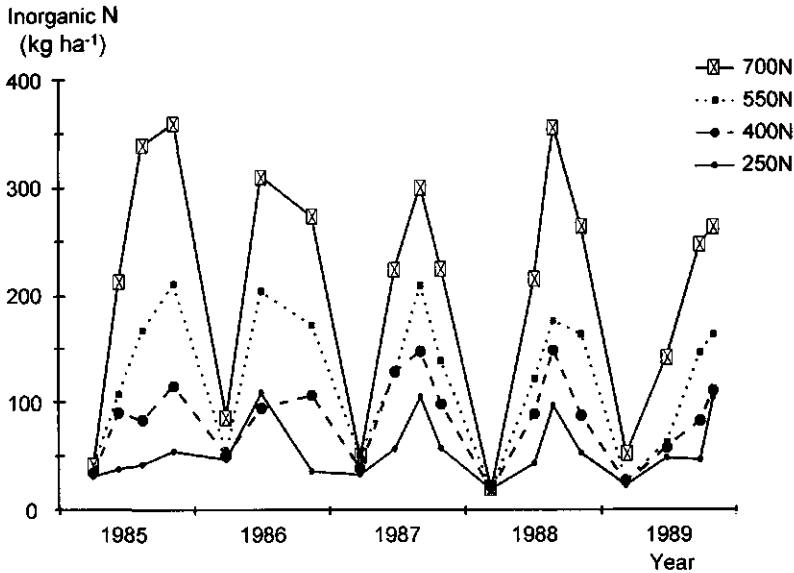


Figure 2. The effect of the rate of N application on the content of inorganic N in the soil layer of 0 - 60 cm under the grazed swards on the sandy soil (H.G. van der Meer and A.H.J. van der Putten, unpublished results).

Figure 2 shows a regular pattern of accumulation of inorganic N in the growing season and loss during the winter. Rate of N application had a large effect on accumulation, in particular in 1985 and 1988 when the additional N of the increment 550 to 700 kg ha⁻¹ year⁻¹ was completely recovered as soil inorganic N in late August and early November (Figure 2). Generally, the total content of inorganic N in the soil layer of 0 - 60 cm was highest in August and decreased in the last 2 months of the growing season. Probably, this decrease was caused by denitrification in the deeper soil layers and/or leaching losses associated with the precipitation surplus in autumn. Analysis of the distribution of inorganic N over the three sampled soil layers of 20 cm showed a downward movement of nitrate during the whole growing season in all the experimental years (H.G. van der Meer and A.H.J. van der Putten, unpublished results). In fact, there was already a significant increase of inorganic N in the layer 40 - 60 cm in the second half of June. This suggests that N leaching losses even may occur during the drier part of the growing season.

Estimates of nitrate leaching losses from the tile-drained experimental plots in the years 1986/1987, 1987/1988 and 1988/1989 (J.H.A.M. Steenvoorden, unpublished results; Macduff *et al.*, 1990), however, gave lower values than expected on the basis of the amounts of inorganic N in the soil layer of 0 - 60 cm. Nitrate leaching

was calculated as the product of nitrate concentration in tile effluent and drainage volume, estimated from a hydrological mass balance in time steps of 1 - 10 days. Calculated leaching losses from the grazed plots receiving 250, 400, 550 and 700 kg N ha⁻¹ year⁻¹ were 48, 61, 116 and 141 kg N ha⁻¹ year⁻¹, respectively. The corresponding amounts of inorganic N in the soil layer sampled in early November of these years were 48, 97, 157 and 253 kg ha⁻¹ (Figure 2). These data and presumed nitrate losses from the soil layer of 0 - 60 cm during the growing season indicate that only a fraction of accumulated inorganic N was accounted for in drainage water. The fate of the other fraction is not clear. Probably, denitrification in the deeper soil layers caused some N losses. In addition, it is possible that the rather complicated hydrological situation of the experimental fields hampered a correct calculation of drainage volume, causing underestimation of leaching losses.

During the growing seasons of 1989 and 1990, de Klein and van Logtestijn (1994) conducted denitrification measurements on the grazed experimental plots at Achterberg receiving 250 and 400 kg N ha⁻¹ year⁻¹. Estimated denitrification losses in the top soil layer of 11 cm were 2.7 and 5.4 kg N ha⁻¹ in 1989, and 7.6 and 8.4 kg N ha⁻¹ in 1990. So, these losses ranged between 1.1 and 3.0% of fertilizer N applied and were not significantly affected by N application rate (de Klein and van Logtestijn, 1994).

The return of N in excreta strongly affects N flows and losses in grazed pasture. Grazing ruminants generally excrete more than 75% of ingested N in faeces and urine (Whitehead, 1970; van der Meer, 1982). The steers grazing the experimental plots at Achterberg in 1986 and 1987 excreted approximately 80 - 90 kg N ha⁻¹ year⁻¹ in faeces, and 250 - 400 kg N ha⁻¹ year⁻¹ in urine (Deenen, 1994). Rate of N application hardly affected N excretion in faeces and moderately increased N excretion in urine.

The fate of urinary and faecal N, voided by grazing animals, was studied simultaneously with the experiments at Achterberg in small-plot experiments under similar soil and weather conditions at the experimental farm Droevendaal near Wageningen (Vertregt and Rutgers, 1988; van der Meer and van Uum-van Lohuyzen, 1989; van der Meer and Whitehead, 1990). This research was carried out with artificial urine, containing 12 g N l⁻¹, and fresh faeces, containing 13.8% dry matter and 0.392% total N. Urine and faeces were applied at rates of 5 l m⁻² and 32 kg m⁻², respectively, to plots of about 1 m² covered with a short dense sward dominated by *Lolium perenne*. Ammonia volatilisation was always measured continuously during the first 10 days after application of urine or faeces, and in some cases for longer periods. Soil inorganic N was measured immediately before and 10 days after urine application, and at the end of the growing season

after the last cut. The average results of 10 applications of artificial urine in 1986 are presented in Table 1.

Table 1. Apparent recovery of urinary N in (A) the first 10 days, or (B) the remaining part of the growing season after application to grassland on a sandy soil near Wageningen. The values are average results of 10 experimental plots, established between the end of April and early July, with application of artificial urine at a rate of 60 g N m⁻². The results of untreated control plots were used to calculate "net effects" of urinary N.

Flow or pool	Recovery of urinary N (%)	
	average	range
<i>(A) in the first 10 days:</i>		
Ammonia volatilisation	10	4 - 17
Crop uptake	4	-1 - 9
Soil inorganic N	58	42 - 74
Not accounted for	28	6 - 48
<i>(B) in the remaining part of the growing season:</i>		
Ammonia volatilisation	13	5 - 22
Crop uptake	44	22 - 60
Soil inorganic N	9	-1 - 39
Not accounted for	34	24 - 47

Table 1 shows that 10 days after urine application, on average, 62% of applied N was recovered in the herbage and as inorganic N in the soil profile of 0 - 60 cm, whereas 10% had been lost by ammonia volatilisation and 28% could not be accounted for. The proportion of inorganic N not accounted for after 10 days ranged from 6 to 48%. Analysis of this variation revealed a negative correlation between the amount of ammonium N in the soil profile after 10 days and the amount of N not accounted for (van der Meer and Whitehead, 1990). This may point to N losses associated with nitrification. De Klein and van Logtestijn (1994) measured denitrification and nitrous oxide (N₂O) emission after application of artificial urine (40 g N m⁻²) to a *Lolium perenne* sward on the sandy soil at Achterberg. During 14-day periods following urine applications in May and June, N losses by denitrification amounted in both cases to 18% of urine N applied, whereas N₂O emission losses were 16 and 8%, respectively. To estimate total N₂ + N₂O losses, the origin of the N₂O must be known. If denitrification was the only source of N₂O, total N₂ + N₂O losses would be 18%. However, if nitrification was the only source of N₂O, total N₂ + N₂O losses would range between 26 and 34%

of urine N. These values are of the same magnitude as the proportion of urinary N not accounted for in Table 1.

In the remaining part of the growing season after urine application, N recovery in the harvested herbage averaged 44% of urine N (Table 1). From urine applications after the beginning of June, N recovery in the harvested herbage decreased and inorganic N in autumn increased (van der Meer and Whitehead, 1990). Soil inorganic N in autumn was mainly nitrate N and was completely lost during the following winter, probably both by denitrification and leaching.

The average proportion of faecal N lost by ammonia volatilisation from 2 experimental "dung pats" of 1 m², established on 1 July 1987 at the experimental farm Droevendaal, was 13% (van der Meer and Whitehead, 1990). Topped grass tillers, planted on 27 August 1987 on these "dung pats", absorbed almost 8% of faecal N in the remaining part of the growing season of 1987 and 4.5% in the growing season of 1988. About 5.5% of faecal N was recovered as inorganic N in the soil profile in the autumn of 1987 and was probably lost during the following winter. The swards did not show a residual effect of applied dung N in 1989. This indicates that a large part of the dung N, probably about 70%, was rather stable organic N.

Nitrogen balance sheets of cut and grazed grassland

Based on information reported by Deenen (1994) and in the preceding paragraph, N balance sheets have been drawn up for the different experimental treatments at Achterberg (Tables 2 and 3).

Table 2. The effect of the level of fertilizer N on the N balance sheet of cut grassland at Achterberg. Figures are in kg N ha⁻¹ in 1987.

	Level of fertilizer N				
	N0	N1	N2	N3	N4
<i>N inputs:</i>					
Soil inorganic N in spring	25	33	38	51	48
Fertilizer	0	250	400	550	700
Total	25	283	438	601	748
<i>N outputs:</i>					
Harvested herbage	111	415	535	632	645
Soil inorganic N in autumn	20	19	32	67	96
Total	131	434	567	699	741
Not accounted for	-106	-151	-129	-98	7

Under cutting, N output in harvested herbage and as soil inorganic N at the end of the growing season exceeded N input as soil inorganic N in spring and in fertilizer, except at the highest rate of N application (Table 2). This is caused by the fact that the N supply by atmospheric deposition and mineralisation of soil organic N has not been included. At this site, these processes probably contribute about 160 kg N ha⁻¹ year⁻¹ to the N yield in the harvested herbage (Deenen, 1994). Addition of this N supply to the N inputs results in amounts of N not accounted for ranging from 9 to 167 kg N ha⁻¹. The amounts of N not accounted for, calculated in this way, indicate that considerable N losses during the growing season only occurred on the plots with the highest rate of N application. Probably, these losses were caused by denitrification and nitrate leaching.

The following assumptions were made to calculate the N balance sheets of the grazed plots (Table 3):

- Nitrogen in liveweight gain: 30 g N per kg gain (Lantinga *et al.*, 1987).
- Ammonia volatilisation: 13% of N excreted in faeces and urine (Vertregt and Rutgers, 1988; Table 1); amounts of excreted N were reported by Deenen (1994).
- Denitrification of fertilizer N: 3% of applied N (de Klein and van Logtestijn, 1994).
- Undefined losses of urine N: 30% of urine N produced (Table 1).

Table 3. The effect of the level of fertilizer N on the N balance sheet of grazed grassland at Achterberg. Figures are in kg N ha⁻¹, and averaged over 1986 and 1987.

	Level of fertilizer N			
	N1	N2	N3	N4
<i>N inputs:</i>				
Soil inorganic N in spring	40	44	52	66
Fertilizer	268	406	517	672
Total	308	450	569	738
<i>N outputs:</i>				
Liveweight gain	27	27	27	22
Removed herbage residues	15	14	12	17
Ammonia volatilisation	47	53	56	56
Denitrification of fertilizer N	8	12	16	20
Undefined losses of urine N	84	97	103	106
Soil inorganic N in autumn	46	102	155	249
Total	227	305	369	470
Not accounted for	81	145	200	268

Table 3 shows that on the grazed plots, N inputs exceeded measured plus estimated N outputs at all rates of N. This is a remarkable difference with the N balance sheets of the cut plots (Table 2). The positive values of N not accounted for indicate (net) accumulation of organic N in the soil and, probably, underestimation of N losses from the system, in particular at the higher rates of N application. Hassink and Neeteson (1991) reported an increase of organic N of about $50 \text{ kg ha}^{-1} \text{ year}^{-1}$ in the top 10 cm of the soil of the grazed plots. This amount was not affected by the rate of N application and is slightly lower than the stable fraction of faecal N, estimated at about $60 \text{ kg ha}^{-1} \text{ year}^{-1}$ (70% of faecal N). Because N inputs and N outputs in the Tables 2 and 3 only relate to the period between fertilizer application in spring and the last grazing in autumn, the amounts of N not accounted for also refer to this period. In the preceding paragraph, it was concluded that downward N losses from the soil occurred during the growing season (Figure 2 and related discussion). This points to denitrification in deeper soil layers and underestimation of nitrate leaching. However, it is difficult to accept that these losses explain the large values of N not accounted for.

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