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Lower nitrate leaching from dairy cattle slurry compared to synthetic fertilizer calcium ammonium nitrate applied to grassland☆

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ABSTRACT

Nitrate leaching from agriculture can be reduced by the choice of fertilizer and a proper timing of its application. For permanent grassland grown under temperate conditions, nitrate leaching was hypothesized to be lower from dairy cattle slurry (CS) compared to synthetic fertilizer calcium ammonium nitrate (CAN), based on differences in chemical composition, consequential effects on nitrogen (N) conversion processes in soil, and resulting differences in synchronization of (nitrate) N availability and plant N uptake. We tested the hypothesis in a two-year field experiment on cut grassland on a leaching-sensitive sandy soil, fertilized each year with 320 kg ha^{-1} of plant-available N from either 100% top-dressed CAN or a combination of 40% from CAN and 60% from sod-injected CS, and measured effects on grass herbage yield, herbage N uptake, and nitrate concentration in pore water at 1.0 m depth. Our results show a comparable level of herbage N uptake for both treatments, allowing for a proper comparison of nitrate leaching at a similar level of plant-available N. Average nitrate concentration in pore water in the main leaching period (over winter) was after the first (dry) growing season 44% lower for CS + CAN (41 mg l^{-1}) compared to CAN only (73 mg l^{-1}), and after the second (wet) growing season 35% lower for CS + CAN (32 mg l^{-1}) compared to CAN only (49 mg l^{-1}). Nitrogen application increased nitrate concentration at 1.0 m depth not only in winter but also in the growing season. We conclude that for permanent grasslands in temperate regions, nitrate leaching from timely applied CS may be considerably lower than from CAN, which is different from previous assumptions.

1. Introduction

Reduction of nitrate leaching from agriculture is important, to increase the nitrogen (N) efficiency of its production cycles and improve the quality of ground- and surface water. Nitrate is worldwide a common groundwater contaminant causing a serious risk for drinking water quality and wetlands. In Europe, the maximally permitted nitrate concentration for the production of drinking water is often exceeded in surface waters and shallow aquifers, including those recharged by water from areas with agricultural activity. High nitrate concentrations can also result in eutrophication of groundwater-fed ecosystems, both directly as well as indirectly by mobilizing sulphate in deeper aquifers (Smolders et al., 2010). Nitrate leaching to groundwater is therefore a serious threat for wetlands and there is an urgency to reduce it (Smolders et al., 2010).

Nitrate leaching from agriculture is influenced by many factors (Cameron et al., 2013), among which crop type has a large influence. Under temperate growing conditions, nitrate leaching is on average lower from perennial crops, such as permanent grassland, than from arable crops (Cameron et al., 2013; Van Duijnen et al., 2023). However, after a dry growing season, nitrate leaching from grassland may on drought-sensitive soils still exceed the set limits (Van Duijnen et al., 2023). Within crop type, efforts to reduce nitrate leaching can include measures such as the choice of fertilizer and a proper timing of its application.

In dairy milk producing countries, a large part of N application on grasslands is often through the recycling of N excreted by dairy cattle, either excreted during grazing, or excreted in the barn, stored, and later applied as cattle slurry (CS) to fields. An application of CS is usually supplemented with synthetic fertilizer, in Northern and Western Europe often in the form of calcium ammonium nitrate (CAN). Organic CS and synthetic CAN differ in chemical composition, which influences N

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conversion processes in soil, the synchronization of N availability and plant N uptake, and nitrate leaching loss as a result. In CAN, half of the N is present as ammonium and the other half as nitrate. In CS, usually half of the N is present as ammonium and the corresponding other half as organic N, although proportions may vary. The highly mobile nitrate-part of CAN is easily leached to below the rooting zone when its application is followed by heavy or prolonged rainfall (De Boer et al., 2016; Esala and Leppänen, 1998), and is then lost for plant uptake.

Nitrogen application with CS is largely based on its ammonium concentration, because the short-term availability of its organic N for plant uptake is low, with an estimated 17–21% of organic N mineralized in the first year after application (Sørensen et al., 2017). Direct leaching of ammonium is very low, and ammonium has to be nitrified before it can leach as nitrate, a process that under temperate conditions takes two to ten weeks to completion (Nair et al., 2021; Rahman et al., 2021). The continuous and relatively high uptake of both ammonium and nitrate (Bailey, 1998) by the grass plants during the ongoing nitrification should result in an on average lower nitrate concentration in soil after application of ammonium when compared to nitrate, and in lower nitrate leaching.

Organic N from CS first has to be mineralized into ammonium and then to be nitrified before any nitrate can leach. The resulting slow release of small amounts of nitrate, countered by a large plant uptake potential, should also result in low nitrate leaching from this fraction. In addition, 20–25% of the ammonium in CS can be immobilized in organic form after its application, to be slowly mineralized later (Sørensen, 2004; Sørensen et al., 2017). Integration of the above processes suggests a relatively low level of nitrate leaching from CS applied to permanent grasslands when compared to nitrate-containing fertilizers such as CAN, a novel hypothesis earlier presented and substantiated by De Boer (2017).

Experimental evidence to support this hypothesis is scarce, and results from available field experiments appear conflicting. Most of the relevant studies conclude that there are no differences in leaching between CAN and CS when applied to grassland (Kayser et al., 2015; Schröder et al., 2010; Ten Berge et al., 2002), but results from a longer-term study by Jarvis et al. (1987) do show lower nitrate leaching from combinations of CS and CAN compared to CAN only. A review of the relevant studies, given later in this paper, suggests that the reported absence of differences may have been caused by technical challenges during experimentation, e.g. by measuring at times or on locations that are less suitable to establish potential differences between CS and CAN. Given the limited availability of relevant or reliable data, new experimental work was necessary.

We therefore initiated a field experiment, with specific attention given to the representativity of its set-up and conditions, and tested the novel hypothesis that nitrate leaching from cut grassland on drought-sensitive sandy soil is lower for sod-injected CS than for top-dressed CAN, at a similar level of plant N uptake.

2. Materials and methods

2.1. Site properties

The experimental site was located in the east of the Netherlands (52°02′18.4 N, 6°33′11.5 E) on a sandy soil consisting of 30 cm of topsoil (91% sand, 7% silt, 2% clay) overlaying white sand. At start of the experiment, soil in the 0–10 cm layer had a pH-KCl of 5.5, a CEC of 49 mmol + kg^{-1}, contained 33 g kg^{-1} organic matter, 1.46 g kg^{-1} total N, 0.23 g kg^{-1} P–Al, 89 mg kg^{-1} plant-available K (0.01 M $CaCl_2$), and 4.3 mg kg^{-1} plant-available S (0.01 M $CaCl_2$) (all properties expressed on a DM basis, except pH). Soil bulk density was 1.2, 1.5, and 1.6 kg l^{-1} at 5, 15, and 25 cm depth, respectively. Gravimetric water content in soil layer 0–10 cm was 6% at irrigation point (pF = 3.3) and 20% at field capacity (pF = 2.0). The site was naturally drained and groundwater level varied from field level in winter to −156 cm below in summer. Given the limited capillary rise on this soil type (up to 0.5 m; Bloemen, 1980), grass growth depended on rainfall, water stored in the 0–80 cm soil layer, and additional irrigation when the groundwater level dropped below −80 cm. The grass sward was 12 years old, and species composition was in March 2020 dominated by perennial ryegrass (*Lolium perenne* L.) (47%), *Poa annua* L. (33%), *Poa trivialis* L. (6%), and *Stellaria media* L. (6%). The sward was free of (N-fixing) clover during the first experimental year and an ingression of clover during the third growth period of the second experimental year was stopped by chemical treatment. In previous years, the grassland had been cut five times each growing season and had received N fertilization of up to 320 kg plant-available N ha^{-1} $year^{-1}$, of which 40% came from CS and the rest from CAN.

2.2. Experimental design

In the experiment, five treatments were replicated four times in four randomized blocks. Treatments were either unfertilized (control) or fertilized with CAN, CAN and CS, CAN and zeolite, and CAN, CS, and zeolite. Results of the treatments with zeolite are not reported here, but the random variance of these treatments was used in the statistical analysis (see paragraph 2.8). Per block, treatments were randomly assigned to 9 m wide and 10 m long plots, situated perpendicular to the long direction of the field, behind each other. The experiment was conducted from January 2020 until April 2022.

2.3. Grassland fertilization

The experiment included five harvest cycles (growth periods) per full growing season, fertilized either with CAN or with CS supplemented with CAN. Plots with CAN only received N applications for the five growth periods of 120, 80, 60, 40, and 20 kg N ha^{-1}, respectively, for an annual total of 320 kg N ha^{-1}. The N distribution over the growth periods was based on grass growth potential during the year. Plots with CS received CS applications for the first four growth periods of 30, 20, 20, and 20 m^3 ha^{-1}, for an annual total of 90 m^3 ha^{-1}, with the final application in the last week of July. Amounts of plant-available N from CS were estimated for four growth periods following an application, based on application level, concentrations of NH_4–N and organic N (Table 2) and default N availability coefficients for NH_4–N and organic N (Anonymous, 2020), and were supplemented with N from CAN to match the level of plant-available N per growth period applied to the CAN only plots. In the second growing season, supplementary CAN for the first and second growth period was reduced by 10 and 5 kg N ha^{-1}, respectively, to compensate for an expected residual mineralization of organic N from CS applied in the first growing season. Supplementary N from CAN to plots with CS + CAN was 40% and 38% of N applied to the CAN only plots for the first and second growing season, respectively. During the fifth growth period of the first growing season, on 18 September 2020, all plots were erroneously fertilized with 35 kg N ha^{-1} (NH_4–N) and 48 kg S ha^{-1} from air scrubber solution. An overview of the applied N per growth period per year, for total N and estimated plant-available N, is for both treatments given in Table 1. Field application of CS results in a higher emission of ammonia when compared to CAN, which may reduce the amount of plant-available N and consequently also nitrate leaching. In the present study, the used N availability coefficients included such losses, for the application of CS by sod-injection.

Amounts of phosphorus (P) and potassium (K) applied with CS were considered sufficient for optimal grass growth, and plots with CS application therefore did not receive P or K with mineral fertilizer. Plots fertilized with only CAN received P and K with mineral fertilizer, per growth period based on estimated P and K application on plots with CS application. Phosphorus applications with fertilizer were 17, 11, 11, and 11 kg P ha^{-1} for the first four growth periods of 2020, respectively, and 16, 11, 11, and 11 kg P ha^{-1} for the first four growth periods of 2021, respectively. Potassium fertilizer applications were 149, 98, 98, and 0

Table 1
Applied amounts of total N and estimated plant-available N (kg N ha^{-1}) with CAN or CS for treatments CAN only and CS + CAN, for each growth period in 2020 and 2021.

Year	Growth period	Total N				Estimated plant-available N			
		CAN only	CS + CAN			CAN only	CS + CAN		
			From CS	*From CAN*	*Total*		*From CS*	*From CAN*	*Total*
2020	1	120	112	75	187	120	43	75	118
	2	80	79	38	116	80	43	38	81
	3	60	80	15	96	60	42	15	58
	4	40	72	0	72	40	40	0	40
	5	55[a]	35[a]	0	35[a]	55[a]	48[a]	0	48[a]
	Total	355	378	128	506	355	217	128	345
2021	1	120	119	66	185	120	33	66	100
	2	80	79	32	111	80	34	32	66
	3	60	75	18	93	60	36	18	55
	4	40	70	0	70	40	37	0	37
	5	20	0	7	7	20	13	7	20
	Total	320	343	123	466	320	154	123	277

[a] Including 35 kg NH_4–N erroneously applied with air scrubber solution.

Table 2
Dairy cattle slurry characteristics for each application in the field experiment, expressed in g kg^{-1} fresh product except for pH (–) and EC (mS cm^{-1}).

Year	Application nr.	Characteristics									
		pH	*EC*	*DM*	*OM*	*Total N*	*Total P*	*Total K*	*NH_4–N*	*DON*	*Total C*	*DOC*
2020	1	7.5	23	75	55	3.79	0.55	5.09	2.30	1.06	26.1	12.1
	2	7.3	24	80	59	3.94	0.56	5.37	2.44	1.20	30.9	14.2
	3	7.5	23	76	55	3.94	0.53	5.18	2.19	1.19	31.2	13.1
	4	7.3	21	76	58	3.55	0.53	4.34	1.92	0.61	30.6	10.0
2021	1	7.5	21	82	62	3.98	0.67	4.22	1.71	0.47	33.8	8.2
	2	7.4	24	81	65	3.93	0.62	4.51	1.84	0.59	32.5	7.9
	3	7.2	22	73	56	3.69	0.62	4.19	1.76	0.52	29.2	8.3
	4	7.2	23	72	54	3.45	0.56	4.29	1.65	0.52	29.2	7.3

kg K ha^{-1} for the first four growth periods of 2020, respectively, and 152, 108, 104, and 95 kg K ha^{-1} for the first four growth periods of 2021, respectively. In both years, all plots received an S application for the first and second growth period of 15 and 15 kg S ha^{-1}, respectively. Sulphur fertilization can increase N utilization and uptake by the grass and thus reduce the general level of nitrate leaching (Brown et al., 2000).

Mineral N fertilizer was applied as CAN (13.5% NH_4–N, 13.5% NO_3–N), mineral P fertilizer as triple superphosphate (20% P), mineral K fertilizer as KCl (50% K), and mineral S fertilizer as kieserite (20% S). All (granular) mineral fertilizers were top-dressed with a pneumatic fertilizer row spreader (Swincosem, Mierlo, The Netherlands). Mineral fertilizers were applied in 2020 on March 26, May 18, June 23, July 28, and September 2; and in 2021 on March 26, May 18, June 18, July 22, and September 3. Mineral fertilizers were applied two to ten days (on average six days) after the previous harvest.

Slurry was applied with a Vervaet Hydrotrike (Vervaet, Biervliet, The Netherlands), fitted with a Vredo sod-injector with 45 double cutting discs at a row distance of 17.6 cm, for a working width of 7.9 m (Vredo, Dodewaard, The Netherlands). The slots were 2 cm wide at the top and maximally 6 cm deep. During slurry application, the Hydrotrike passed over the middle of each 9-m wide plot, leaving an unfertilized strip of on average 55 cm on either side. Driving speed during application was 4 km h^{-1}, and tire pressure was usually between 2.5 and 2.8 psi, sometimes 3.2 psi during application later in the growing season, on dry soil. Slurry was applied in 2020 on March 23, May 15, June 26, and July 29; and in 2021 on March 24, May 19, June 18, and July 26. Slurry was applied five to seven days (on average seven days) after the previous harvest.

2.4. Manure measurements

Prior to each slurry application, a sample was taken from the tank of the Hydrotrike, after mixing its contents. Samples were stored at −18 °C and analyzed later by the Chemical Biological Laboratory in Wageningen (The Netherlands). Slurry density was determined volumetrically; pH and EC directly in slurry with standard electrodes, at a temperature of 20 °C; total N and total P with segmented flow analysis (SFA) (SAN++, Skalar, Breda, The Netherlands) (after destruction of fresh slurry with sulphuric acid and salicylic acid, and the addition of selenium and hydrogen peroxide, at a temperature of 100 °C); total K with ICP-AES (Thermo iCAP 6500 DUO, Thermo Fisher Scientific, Waltham MA, USA) (after the destruction method described before); NH_4–N with SFA (after extraction with 1 M KCl); dissolved organic N (DON: total dissolved N – NH_4–N – NO_3–N) with SFA after extraction with 0.01 M $CaCl_2$; and dissolved organic C (DOC: total dissolved C – inorganic C) also with SFA after extraction with 0.01 M $CaCl_2$. Dry matter (DM) was determined by drying at 105 °C for 24 h, and organic matter (OM) by incineration of dried slurry (70 °C) at 550 °C for 3 h. Total C was determined using a CN analyzer (FlashSmart, Thermo Fisher Scientific) after grinding of dried slurry (70 °C) to 50 μm. Slurry characteristics for each application are given in Table 2.

2.5. Weather measurements and irrigation

Weather measurements were of rainfall and evapotranspiration. Rainfall data (daily recorded) were obtained from weather station Lichtevoorde (KNMI, De Bilt, The Netherlands), located at 6 km distance from the experimental site, and evapotranspiration data (calculated per day) from station Hupsel, located at 5 km distance from the experimental site. Long-term averages (1991–2020) of monthly rainfall and evapotranspiration were from stations Lichtevoorde and De Bilt, respectively.

The experimental site was irrigated in 2020 during periods of prolonged drought, with 30 mm water on May 25, 30 mm on June 1, 20 mm on June 29, 25 mm on August 1, 22 mm on August 8, and 22 mm on

August 9, for an annual total of 149 mm.

2.6. Sward measurements

Sward measurements were of herbage fresh yield, herbage DM concentration, and herbage N concentration. Herbage fresh yield was determined by cutting the grass with a Haldrup GR grass plot harvester (Haldrup GmbH, Ilshofen, Germany) to a height of 6 cm, from two 1.5 m wide and 10 m long strips at a 2-m distance from each plot border, for a total harvested area of 30 m^2. Harvested material was weighed, and samples were oven-dried for 48 h at 70 °C to determine dry weight and DM concentration. Total N concentration in the dried samples was determined using a CNHS analyzer (vario MICRO cube; Elementar Analysesysteme GmbH, Langenselbold, Germany) after grinding the material to 0.5 mm. Plots were in 2020 harvested on May 8, June 19, July 23, August 31, and October 16; and in 2021 on May 14, June 11, July 19, August 24, and October 1.

2.7. Pore water nitrate measurement

Water measurements were of nitrate concentration in pore water and of groundwater level. Before the experiment started, two permanent ceramic suction cups were installed in each plot, on January 29, 2020, at a depth of 1.0 m below field level. The ceramic cups (655X01-B1M3, ø 22 mm, 70 mm length; Soil Moisture Corp., Santa Barbara, CA, USA) were fitted to a PVC pipe (ø ¾") and connected with a tube (22 NSR Nylo-seal) running through the PVC pipe up to the surface. The two cups were installed in the middle of the 9-m plot width, each cup in the 10-m length direction at 1 m distance of either side of the plot center. The top of the PVC-pipe was covered with a plastic cover (20 x 20 × 15 cm) which recessed a few cm into the grass sward.

Pore water from the cups was frequently sampled, every two to three weeks in the main leaching period (winter period) of 2020 and 2021, every two to three weeks in the growing season of 2020, and a few times in the growing season of 2021. At sampling, the plastic cover was removed, a syringe (60 ml) was connected to the Nylo-seal tube and its plunger was pulled back, resulting in suction to the cup and a slow filling of the syringe with pore water. A day later, syringes were disconnected and the samples from the two cups were mixed, subsampled, stored (−20 °C), and analyzed within two weeks for nitrate concentration, colorimetrically determined with SFA (SEAL AutoAnalyser 3; SEAL Analytical, Norderstedt, Germany) after the addition of a color reagent in an imidazole buffer.

Fluctuations in groundwater level were measured in two groundwater measuring tubes (2 m length; 0.45 μm filter), installed at the border between plotnr. five and six, and plotnr. 15 and 16, in the middle of the plot length. When pore water was sampled, groundwater level was also measured, using a plumb line and a tapeline. Between July 26, 2021 and January 28, 2022, all measurements of groundwater level, except one, were skipped.

2.8. Calculations and statistical analysis

Monthly rainfall deficit or surplus was calculated by subtracting evapotranspiration from rainfall. Herbage DM yield was calculated per plot per harvest by multiplying fresh herbage yield by DM concentration, and herbage N uptake was calculated by multiplying DM yield by N concentration in DM. Herbage DM yield and N uptake of the five harvests were for each individual year analyzed with the REML-procedure for repeated measurements in statistical package Genstat (19th edition; VSNI, Hemel Hempstead, UK), using 'Plotnr.' as *Subjects* and 'Harvest nr.' as *Timepoints*. Fixed model was 'Fertilizer type * Zeolite * Harvest nr.', random model was 'Block', option *Equally spaced time points* was disabled, and the used correlation model was *Unstructured*. Factor 'Fertilizer type' had levels CAN only and CS + CAN, and levels of factor 'Zeolite' were the presence or absence of zeolite application. Results from the zeolite treatments were included in the statistical analysis for a better estimation of the random variance of measurements and thus increase the power of the statistical tests. Results of the unfertilized control treatment were excluded from statistical analysis because of lack of orthogonality and level differences when compared to fertilized treatments. Prior to statistical analysis, amounts of N uptake of the five harvests in 2021 were logtransformed (LN) because residuals were level-dependent. Annual DM yield and N uptake were analyzed for each individual year using the ANOVA-procedure in Genstat. For all statistical analyses, the significant F-value was determined at the 95% level ($p \leq 0.05$), and in the event of F-significance, means of treatments were compared using the F-protected least significant difference (LSD) test ($p \leq 0.05$).

The pore water measurement period can be split into a 'growing season leaching period' and a 'winter leaching period'. In the growing season, the leached volume of water is relatively small, whereas over winter the leached volume is very large and has the largest contribution to the annually leached amounts of nitrate to groundwater. The winter period was set to start half November, when the rainfall surplus starts to build up, and to end when nitrate concentration in pore water started to decrease, in February/early March. At that moment, nitrate leaching is largely finished and nitrate concentrations can decrease due to dilution with additional rainfall. Nitrate concentrations in pore water at 1.0 m depth were analyzed for each winter leaching period individually, using the above REML-procedure and replacing 'Harvest nr.' by 'Measurement nr.'. Prior to analysis, nitrate concentrations of the winter leaching period of 2020/2021 were logtransformed.

3. Results

3.1. Rainfall, rainfall surplus, and groundwater level fluctuation

Annual rainfall in 2020 was 666 mm, 152 mm lower than the 30-year average (Fig. 1). Monthly rainfall in the growing season (April until and including September) was in all months, except June, lower than the 30-year average, with the largest differences in April, May, and August. In 2021, annual rainfall was 816 mm, 2 mm lower than the 30-year average. Monthly rainfall in the growing season was in August and September lower, but in May, June, and July considerable higher than the 30-year average.

Annual rainfall surplus in 2020 was 19 mm, 218 mm lower than the 30-year average. Monthly rainfall deficit was in April, May, and August considerably higher than the 30-year average. The 2020 growing season was considerably drier compared to the 30-year average, with a rainfall deficit of 316 mm compared to 79 mm for the average. In 2021, annual rainfall surplus was 217 mm, 21 mm lower than the 30-year average. In May and July there was a rainfall surplus instead of a deficit, and in August and September there was a deficit instead of a surplus. The 2021 growing season was slightly wetter than the 30-year average, with a rainfall deficit of 57 mm compared to 79 mm for the average.

In the winter leaching period of 2020/2021 (November 16, 2020 until and including March 3, 2021), rainfall was 282 mm and evapotranspiration 44 mm, resulting in a rainfall surplus in this period of 238 mm. In the winter leaching period of 2021/2022 (November 22, 2021 until and including February 7, 2022), rainfall was 191 mm and evapotranspiration 19 mm, resulting in a rainfall surplus of 172 mm.

Groundwater level decreased rapidly after February 2020, and the lowest level measured was −156 cm on August 31 (Fig. 2). After this measurement, groundwater level started to increase again, until a level of −10 cm on February 3, 2021. Groundwater level decreased less rapid and was in general higher in the 2021/2022 season compared to the 2020/2021 season, with a lowest level measured in the 2021/2022 season of −73 cm on July 26, 2021.

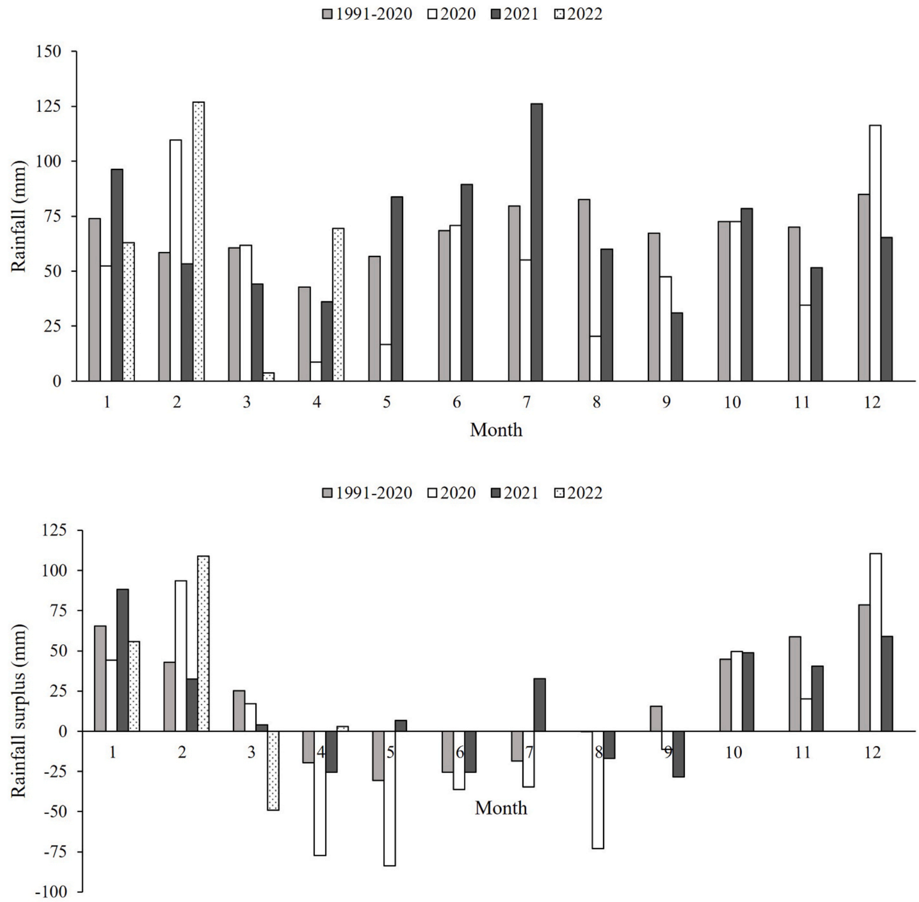

Fig. 1. Monthly rainfall and rainfall surplus/deficit for the years 2020 and 2021, the first months of 2022 (until the end of the experiment), and the 30-year average (1991–2020).

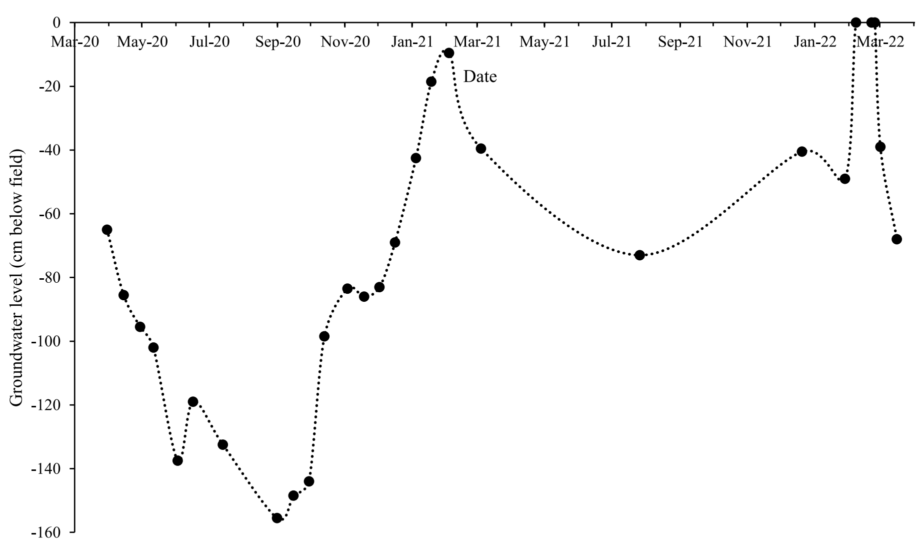

Fig. 2. Groundwater level during the experimental period.

3.2. Grass herbage yield and N uptake

Grass herbage yield in 2020 tended to be significantly influenced by fertilizer type, depending on growth period ($p = 0.06$), with a significantly higher yield of the first growth period for CS + CAN compared to CAN only (Fig. 3). Annual yield was in 2020 significantly lower for CS + CAN compared to CAN only ($p = 0.05$), and was 5.3, 12.1, and 11.8 t ha^{-1} for the control, CAN only, and CS + CAN, respectively. In 2021, herbage yield was significantly influenced by fertilizer type, depending on growth period ($p < 0.01$). Yields of the second and fourth growth period were significantly lower for CS + CAN compared to CAN only, but yield of the fifth growth period was significantly higher. Annual yield was in 2021 significantly lower for CS + CAN compared to CAN only ($p < 0.05$), and was 4.9, 15.6, and 15.1 t ha^{-1} for the control, CAN

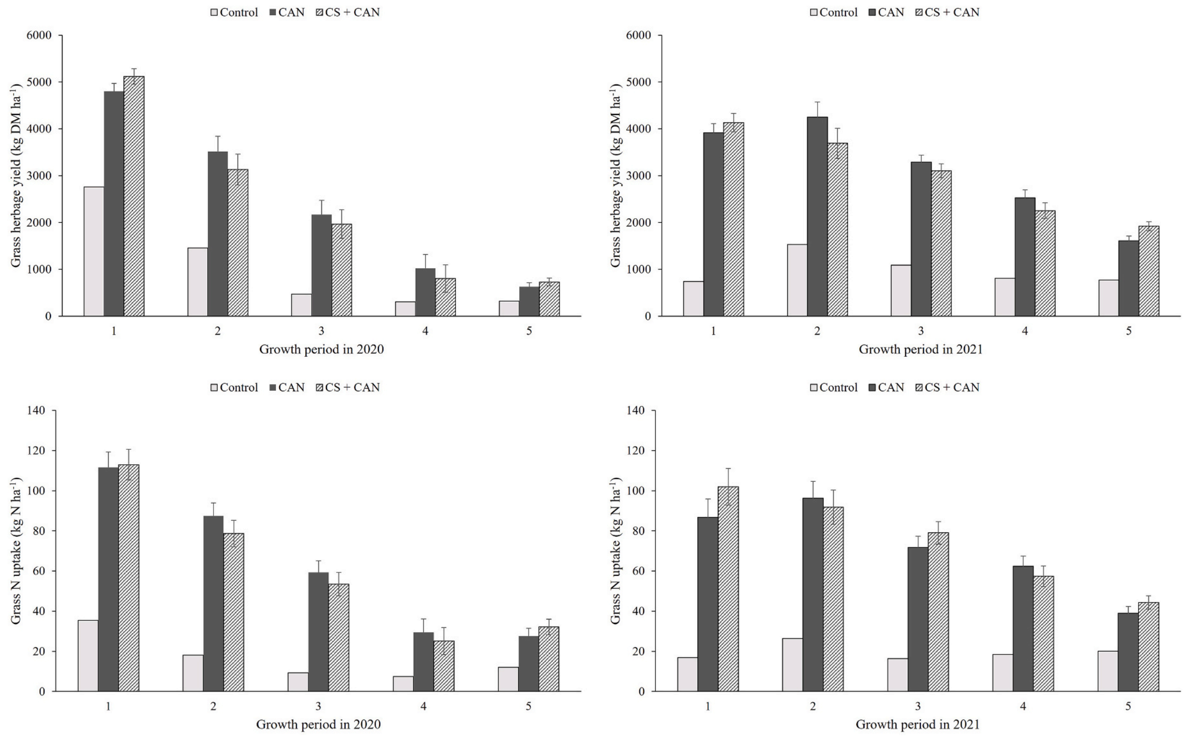

Fig. 3. Herbage yield and N uptake per growth period in 2020 and 2021 for a permanent grassland without N fertilization (control), fertilized with calcium ammonium nitrate (CAN), or when 60% of N from CAN was replaced by plant-available N from cattle slurry (CS). Error bars represent 2 x standard error within each growth period.

only, and CS + CAN, respectively.

Grass N uptake in 2020 was significantly influenced by fertilizer type, depending on growth period ($p < 0.001$). Nitrogen uptake of the second growth period was significantly lower for CS + CAN compared to CAN only, but uptake of the fifth growth period was significantly higher (Fig. 3). Annual N uptake was in 2020 not significantly different for CS + CAN compared to CAN only ($p > 0.05$), and was 82, 315, and 302 kg N ha^{-1} for the control, CAN only, and CS + CAN, respectively. In 2021, N uptake was also significantly influenced by fertilizer type, depending on growth period ($p = 0.02$). Uptake of the third growth period was significantly higher for CS + CAN compared to CAN only, but uptake of the fourth growth period was significantly lower. Annual N uptake was in 2021 not significantly different between CS + CAN and CAN only ($p > 0.05$), and was 98, 356, and 374 kg N ha^{-1} for the control, CAN only, and CS + CAN, respectively.

3.3. *Nitrate concentration in pore water*

Nitrate concentration in pore water at 1.0 m depth started in 2020 to clearly differentiate between treatments in June (Fig. 4). Nitrate concentration was higher for CAN only compared to CS + CAN, especially during July and August 2020, and from November 2020 until April 2021. After the measurement on March 3, 2021, nitrate concentrations decreased for all treatments and differences between treatments had disappeared at the measurement on April 14, marking the measurement on March 3 as the end of the winter leaching period. Nitrate concentration for CS + CAN was usually higher than for the control, and both treatments showed an increase in concentration between January 18,

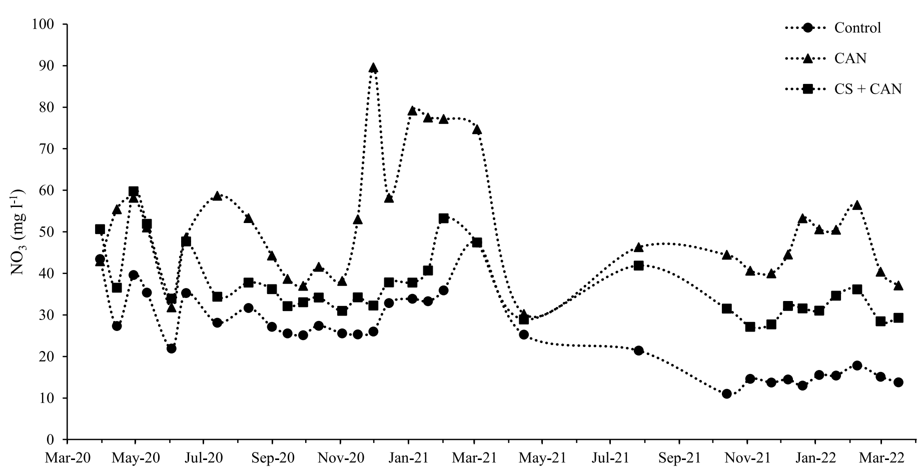

Fig. 4. Nitrate concentration in pore water at 1.0 m below field level (n = 4 per measurement) during the experimental period for a permanent grassland without N fertilization (control), fertilized with calcium ammonium nitrate (CAN), or when 60% of N from CAN was replaced by plant-available N from cattle slurry (CS).

2021 and March 3, 2021.

In the winter leaching period of 2020 (November 16, 2020 until and including March 3, 2021; n = 7 measurements), there was a significant effect of fertilizer type on nitrate concentration ($p < 0.001$). Average nitrate concentrations during this period were 34, 71, and 41 mg l^{-1} for the control, CAN only, and CS + CAN, respectively.

In 2021, nitrate concentration at 1.0 m depth was on July 7 and October 13 higher for CAN only compared to the control and CS + CAN, and for CS + CAN higher than for the control (Fig. 4). These differences continued in the winter leaching period. After the measurement on February 7, 2022, nitrate concentration decreased for all treatments and especially for CAN only, marking the measurement on February 7 as the end of the winter leaching period on February 7. Fluctuations in nitrate concentrations were in the leaching season of 2021/2022 smaller than in the leaching season of 2020/2021.

In the winter leaching period of 2021 (November 22, 2021 until and including February 7, 2022; n = 6 measurements), there was no significant effect of fertilizer type on nitrate concentration ($p = 0.15$). Average nitrate concentrations were 15, 49, and 32 mg l^{-1} for the control, CAN only, and CS + CAN, respectively.

4. Discussion

4.1. Plant-available N and nitrate leaching

Replacement of 58–60% of plant N uptake from CAN by CS resulted in a 35–44% reduction of nitrate concentration in pore water at 1.0 m depth during winter. These results support the original hypothesis by De Boer (2017), further refined in this paper, that nitrate leaching from CS applied to permanent grassland should be lower than from CAN, at a similar level of plant N uptake.

Large differences in plant N uptake, and thus in its precursor plant-available (~leachable) N, may contribute to differences in nitrate leaching between treatments. In the present experiment, annual N uptake was in 2020 4% higher but in 2021 5% lower for CAN only compared to CS + CAN. If these differences would have had an influence on nitrate leaching, they would have resulted in a small overestimation of the difference between CAN only and CS + CAN in 2020 and a small underestimation in 2021, but the overall conclusions would not have changed.

4.2. Drought effects

The largest difference in nitrate concentrations between fertilized treatments occurred after the dry growing season of 2020. Irrigation may have stimulated nitrate leaching in the growing season by preferential or bypass flow (Esala and Leppänen, 1998) after the irrigation on June 1, because this irrigation was followed by an increase in nitrate concentrations (Fig. 4). After later irrigations, there was no increase.

The difference in nitrate leaching between CS and CAN could theoretically be smaller on soils with lower drought-sensitivity. With ample water supply during the growing season, plant N uptake is higher and nitrate leaching lower, which could in particular reduce leaching from fertilizers with a higher nitrate leaching potential, such as CAN. Occurrence of capillary rise could further reduce potential differences, because nitrate leached below the rooting zone can then be transported back into that zone and still be taken up by the plant. In our study, water supply and growing conditions were very good in 2021, evidenced by a high herbage yield and N uptake. Groundwater level during this growing season was much higher than in 2020, and capillary rise (40–50 cm for sandy soils) could therefore have contributed to a relatively higher (nitrate) N uptake for CAN. Despite these favorable conditions for CAN, nitrate leaching over the winter of 2021 was still considerably lower for CS + CAN compared to CAN only, suggesting that lower leaching from CS relative to CAN could also occur on soils that are less or not drought-sensitive. Additional experimental work should be done to test this hypothesis.

Severe summer drought, in the absence of irrigation, can result in the death of grass plants and potentially increase nitrate leaching from unused fertilizer once rainfall starts again and grassland N uptake capacity is still limited (Kayser et al., 2020; Klaus et al., 2020). In case of such relative overfertilization, and therefore a lack of synchronization between a slow nitrate release and plant nitrate uptake, differences in leaching between CS and CAN could be smaller. Results reported by Jarvis et al. (1987), on a site with similar drought-sensitivity as our site, but without irrigation, still showed lower nitrate leaching from combinations of CS and CAN compared to CAN only, averaged over a five-year period and including two dry growing seasons (Fig. 3 in Jarvis et al., 1987).

4.3. Timing effects

In the present experiment, attention was given to a proper timing of CS application in relation to the start and end of the growing season. Because the experimental site dried up slowly at the end of winter, CS could not be applied earlier than in the second half of March, which in most years is just before the start of the growing season and therefore at an appropriate time. The time of the final CS application, at the end of July, was chosen to provide ample time for unstable organic N in CS to be mineralized and taken up by the grass before the end of the growing season. A later application may result in a larger soil mineral N surplus at the end of the growing season and higher nitrate leaching for the treatment with CS. The appropriate time for the final CS application is not known, and neither is the relative contribution of a late application to leached amounts of nitrate. Additional research is necessary to identify the latest date when CS could be applied without increasing nitrate leaching over winter.

Cattle slurry and CAN were both applied within on average a week after the harvest of the previous growth period. Proper timing of application can also reduce nitrate leaching from nitrate-containing fertilizer, especially when incidences of heavy or prolonged rainfall, irrigation events, or drought periods, can be avoided (De Boer et al., 2016; Esala and Leppänen, 1998). Such timing would potentially reduce nitrate leaching from CAN more than from CS, because of the directly available nitrate given with CAN. However, the advice of 'proper timing' is difficult to follow up, because farmers often do not have the option to apply fertilizer at the 'right' time. Rainfall forecasts may be inaccurate, and farmers' timing decisions depend on other activities at the farm and/or the contractor's planning. In practice, CS appears to have a lower risk of nitrate leaching compared to CAN when applied at a similar time.

4.4. Other field data

Only few field experiments have been performed to study differences in nitrate leaching between CS and CAN on permanent grassland, and their results appear conflicting, at least partly because experimental setups, technical challenges, and lack of representative conditions may have prevented an accurate comparison to establish potential differences. Missing in all studies is a hypothesis stating which differences would be expected based on cause-effect relationships. A resulting lack of direction may have affected the choices made for experimental designs and conditions.

Jarvis et al. (1987) reported a lower average nitrate leaching from combinations of CAN and injected CS compared to CAN only, at a similar level of plant N-uptake (more details in Wadman and Sluijsmans, 1992). However, all CS was applied at one time, by deep injection in late winter (March), which does not represent the current situation in the Netherlands, where CS is applied several times, by shallow sod-injection, until September. Nevertheless, results (averaged over a 5-year period) were largely similar to results of the present study, on a largely similar soil type and with comparable groundwater fluctuations (the site was located at 5 km distance from our experimental site).

Ten Berge et al. (2002) concluded that nitrate leaching from CS was likely similar to CAN, at the same level of plant N uptake. Their modelling study was based on differences in mineral N concentration in the soil profile in autumn, but did not take into account that nitrate concentration in the upper groundwater in late winter is regularly poorly explained by soil nitrate in autumn (e.g. 19% explained variance in Schröder et al. (2010) and 65% in Kayser et al. (2015)). The only data used by Ten Berge et al. (2002) with actual measurement of effects of CS and CAN on nitrate concentrations in upper groundwater came from Jarvis et al. (1987), but the analysis by Ten Berge et al. (2002), after data selection, did not show the differences that were present when the whole dataset of Jarvis et al. (1987) was analyzed stand-alone.

Schröder et al. (2010) found no differences in nitrate leaching between top-dressed CAN and combinations of CAN and sod-injected CS in a two-year experiment on two different sites, but these sites lacked representativity and the experimental procedures had weaknesses which may have affected the results, as described in the following. On the dry site, average nitrate concentrations for normally fertilized plots (340 kg N ha^{-1} $year^{-1}$ from CAN) were 109 and 183 mg l^{-1} for 2007 and 2008, respectively, much higher than the average concentrations found in the upper meter of groundwater under drought-sensitive sandy soils on Dutch dairy farms in these years (70 and 55 mg NO_3 l^{-1}, respectively) (Van Duijnen et al., 2023). Nitrate concentrations of unfertilized plots were also very high, 75 and 69 mg l^{-1} for 2007 and 2008, respectively. On the wet site, N uptake of the unfertilized plots was already very high (226–247 kg N ha^{-1} $year^{-1}$), making additional fertilization less efficient and potentially resulting in relatively high nitrate leaching. Nevertheless, nitrate leaching from the fertilized plots was lower than would be expected, possibly due to relatively high denitrification deeper in this wet soil. On both sites, nitrate concentrations were measured only twice during the entire experimental period, in late winter/spring, and the first time (in May) when the leaching of the previous growing season was already over for two or three months and the new growing season well underway. Moreover, our results show that by half April, differences in nitrate concentration between treatments may have disappeared (Fig. 4) at or below the depth in groundwater where Schröder et al. (2010) measured. In addition, De Boer (2004) found that differences between fertilized treatments in nitrate concentration in the upper 1.1 m of groundwater, which were present on January 18, had disappeared on March 18. These results provide evidence that conclusions on differences between treatments may be unreliable when nitrate concentrations are measured after the leaching season is over, and also demonstrate the risk of measuring at only one time in the leaching season.

Kayser et al. (2015) could not establish differences in nitrate leaching between top-dressed CAN and sod-injected CS in a four-year experiment, but their results also lack representativity. Until and including an application of 240 kg total N ha^{-1} $year^{-1}$, and with the last N application before the third growth period, annual nitrate leaching was unusually low (av. 6 kg N ha^{-1} $year^{-1}$) and not different between unfertilized and fertilized plots. However, annual leaching more than doubled following an additional N application of 80 kg total N ha^{-1} for a fourth growth period, and Kayser et al. (2015) suggested a carry-over of N applied with CS to the next spring. These results suggest that the fourth N application was too high and/or applied too late given the prevailing conditions, and increased nitrate leaching due to overfertilization. In our experiment, we gave the highest N application during spring, the most productive grass growth period in the Netherlands, and reduced N application progressively throughout the growing season towards an application of only 20 kg N ha^{-1} at the beginning of September. This way, the earlier applied N could be fully taken up by the grassland going towards the end of the growing season.

4.5. Long-term effects

Recurring application of CS increases the soil organic N stock and organic N mineralization over time. This increased mineralization has to be taken into account in order to prevent overfertilization of the grassland and an increase in nitrate leaching. In the Netherlands, a methodology is applied by which every four years organic N in soil is determined, annual soil N mineralization estimated, and the N fertilization regime can then be adapted accordingly (Anonymous, 2020).

With arable cropping, mineralization of soil organic N in late summer and autumn may result in increased nitrate leaching in the absence of a catch crop as an N sink (Nouri et al., 2022). For a perennial crop such as grassland, N mineralized during this period can be fully taken up by the crop, provided that N fertilization is kept limited. Because soil N mineralization and plant N uptake (through root activity) both mainly depend on temperature and soil gas exchange, these processes are largely synchronized, i.e. when conditions become favorable for N mineralization, plant N uptake is also increased. No evidence was found for the occurrence of soil N mineralization out of sync with grass N uptake during late summer, autumn or winter. On the contrary, Woods et al. (2016) showed that in winter the maximal grass N uptake capacity (in the presence of high N fertilization) was much higher than the N uptake from soil N mineralization (in the absence of N fertilization). The results by Jarvis et al. (1987), averaged over a five-year period and thus including longer-term effects, still showed lower nitrate leaching from combinations of CS and CAN compared to CAN only. Given the above, it seems unlikely that a lower nitrate leaching from CS compared to CAN would disappear when long-term effects are included. Rather, when nitrate-containing fertilizer is over the years gradually replaced by slowly released plant-available N from accumulating slurry organic N, nitrate leaching could be further reduced.

5. Conclusion

After earlier presenting the hypothesis that on cut grassland nitrate leaching from timely applied CS should be lower than from CAN, we measured in a field experiment under temperate conditions that nitrate concentrations in pore water at 1.0 m depth were indeed lower, both after a dry as well as after a wet growing season. Our results further show that nitrate was not only leached after the growing season had ended, but also during the growing season, to a depth of at least 1.0 m. Our work should be replicated on other sites, different in leaching-sensitivity, and additional experimental work should be done on the long-term effects of mineralization of residual organic N from CS. We conclude, based on our hypothesis and the presented experimental evidence, that for permanent grasslands in temperate regions, nitrate leaching from timely applied CS may be considerably lower than from CAN, and may contribute much less to the negative effects of nitrate groundwater pollution than previously assumed.

CRediT authorship contribution statement

Herman C. de Boer: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Supervision, Writing – original draft, Writing – review & editing, Investigation, Project administration. **Mark van Mullekom:** Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing – review & editing, Project administration. **Alfons J.P. Smolders:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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