



# Marginal increase in nitrate leaching under grass–clover leys by slurry and mineral fertilizer

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**Abstract** On dairy farms, fertilization of grass–clover swards ensures stable grass yields but may increase the potential for nitrate leaching on light-textured soils. The aim of this study was to quantify the N use efficiency and nitrate leaching under fertilized grass–clover leys. The study was conducted over 2 years at two sites, with increasing applications of mineral fertilizer (0–480 kg available N ha<sup>-1</sup>) alone or in combination with a basic application of cattle slurry. For plots fertilized with mineral N, the N soil surface balance was independent of the application rate and in the same range as for unfertilized plots (–11 to 51 kg N ha<sup>-1</sup>). However, when plots were fertilized with slurry N (+mineral N), the surplus was substantially increased owing to the fraction of organic N applied in slurry (95–100 kg N ha<sup>-1</sup>) and higher biological N<sub>2</sub> fixation inputs (55–228 kg N ha<sup>-1</sup>). The type of fertilizer had no effect on nitrate leaching across the full range of application rates. Nitrate leaching increased quadratically as a function of application rate, with a range of 3–117 kg N ha<sup>-1</sup>

(0.33–17 mg l<sup>-1</sup> in soil solution sampled with suction cups) in the first year and less in the second year, when clover proportion was lower due to the self-regulatory nature of grass–clover mixtures. Importantly, the rate of marginal leaching increased with fertilization level: below 150 kg N ha<sup>-1</sup> there was no additional leaching from fertilization and at 200 kg N ha<sup>-1</sup> around 5% of additional fertilizer-N was leached. This is less than generally found for arable crops and thus even in intensive dairy systems, grass–clover leys are an environmentally favorable crop.

**Keywords** Red clover · White clover · Festulium · Ryegrass · Nitrogen balance · Surplus

## Introduction

Grass–clover leys are important for dairy farming, and furthermore grassland is predicted to expand for a variety of reasons, including carbon sequestration to counteract climate change, raw materials for higher-value goods (green bio processing and biochemical manufacturing), and energy production (Conant et al. 2001; Odgaard et al. 2019). Mixtures of grass and clover species improve yield stability (Eriksen et al. 2014; Frankow-Lindberg et al. 2009) and quality as feed for ruminants (Phelan et al. 2015) compared to fertilized grass-only leys due to niche complementarity (Nyfeler et al. 2009) and clover protein content (Søgaard 2009; Suter et al. 2015). As a result,

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appropriately managed grass-clover combinations out-yield pure grass and clover stands (Kirwan et al. 2007; Nyfeler et al. 2011).

Plant biomass response to N application varies between years and sites but it is generally found that N application increases grass production partly at the cost of clover but with an overall productivity advantage (Frame and Newbould 1986; Schils and Snijders 2004; Elgersma et al. 2000; Moloney et al. 2021; Trott et al. 2004; Thers et al. 2022; Kristensen et al. 2022; Nyfeler et al. 2009). Nielsen (2015) estimated the net profit of fertilizer addition to forage production by valuing legume proportion and protein content for feeding of lactating cows and subtracting the cost of N fertilizer. With a high clover content in the forage (~50%), N fertilizer application was uneconomic, but at low clover content (~20%) the economic optimum was more than 240 kg N ha<sup>-1</sup>. On dairy farms, slurry is collected and utilized as a fertilizer in systems where cows are fed partly or year-round indoors (Kayser et al. 2015). Farmers typically apply slurry in the early season and mineral fertilizer later (Kristensen et al. 2022).

The sources of nitrate leaching from agricultural land is excess fertilizer N or mineralization of crop residues and soil organic matter. In dairy farming, the nitrate leaching potential is further increased by mixed rotations of arable crops, where symbiotic N<sub>2</sub> fixation by grass-clover leys can import large amounts of N. In comparison to cereals or maize (Vogeler et al. 2020; Wachendorf et al. 2006), grass-clover leys typically exhibit low levels of N leaching even under fertilized conditions (Eriksen et al. 2015; Loiseau et al. 2001; Wachendorf et al. 2004). However, literature across fertilizer levels and especially type (mineral or organic), as well as sward botanical compositions is scarce in the context of practical dairy farming.

Estimating the increase in nitrate leaching for a crop by adding extra N, the 'marginal leaching', is a good measure when evaluating crop N use efficiency and associated losses. Zhao et al. (2022) computed marginal leaching as the derivative of the nitrate leaching as function of N application, which represents the percentage of N leached for each extra unit of N applied. Vogeler et al. (2020) recently determined a marginal N leaching rate of roughly 10–25% under cereal crops treated at the recommended rate for 3 years. Zhao et al. (2022) used a marginal leaching model and predicted mean values in Denmark

and Europe of 21 and 15% marginal leaching, respectively, at the recommended rate of N fertilizer application. However, for grass-clover marginal leaching across a broader range of N fertilizer application levels is needed for appropriate evaluation in a context of current farming practices.

This study's goal was to determine the N use efficiency and nitrate leaching, including marginal leaching, in fertilized grass-clover leys. The experiment was carried out on-farm at two sites over 2 years, with increasing mineral fertilizer treatment alone or in combination with a basic application of cattle slurry.

## Materials and methods

### Experimental sites description

Details on the experimental setup, parameters and management can be found in Kristensen et al. (2022), where yields and N<sub>2</sub>-fixation are also presented. Briefly, the experiments started in 2018 at two farmers' fields located in south-western Jutland, Denmark (55° 32' N, 8° 29' E, hereafter 'SW') on a soil containing 67 g clay kg<sup>-1</sup>, 59 g silt kg<sup>-1</sup>, 470 g fine sand kg<sup>-1</sup>, 377 g coarse sand kg<sup>-1</sup> and 27 g organic matter kg<sup>-1</sup> in 0–25 cm, and in mid-western Jutland (56° 10' N, 8° 46' E, hereafter 'MW') on a soil containing 43 g clay kg<sup>-1</sup>, 42 g silt kg<sup>-1</sup>, 344 g fine sand kg<sup>-1</sup>, 521 g coarse sand kg<sup>-1</sup> and 5 g organic matter kg<sup>-1</sup> in 0–25 cm (Kristensen et al. 2022). The ley at SW was under-sown spring barley in April 2017 and consisted of 10% white clover (*Trifolium repens* L.) and 90% different varieties of perennial ryegrass (*Lolium perenne* L.). At MW site, the ley consisted of a mixture of 11% red clover (*Trifolium pratense* L.), 7% white clover, 37% perennial ryegrass and 45% festulolium (*Festulolium braunii*, K.A.) that was under-sown winter wheat in August 2016. At SW, before seeding, fertilizer was applied as 30 Mg cattle slurry ha<sup>-1</sup> and 40 kg mineral N ha<sup>-1</sup> (NS 27–4). Barley was harvested green and 15 Mg cattle slurry ha<sup>-1</sup> were applied afterwards. The ley was cut once at the end of September 2017 and removed from the field. At MW, in spring 2017, fertilizer was applied as 100 kg mineral-N ha<sup>-1</sup> (NPKS 19–0–15–5). Winter wheat was harvested green and the ley was cut 4 times afterwards in that year. The grass was removed from the field after cutting. About 150 kg cattle slurry

available-N ha<sup>-1</sup> in total were distributed prior the cuts in 2017.

At both sites, ten treatments in four replicates were arranged in randomized plot design. For two consecutive years (2018–2019), plots (12×3 m) were fertilized with increasing N levels from 0 to 480 kg plant available N ha<sup>-1</sup>, either as mineral fertilizer (NS 27–4, 13.5% ammonium-N, 13.5% nitrate-N) or combined with a basic application of acidified cattle slurry (120 kg available N ha<sup>-1</sup>) surface-applied with 12 m wide trailing hose farm-scaling equipment. These were applied in spring and after each cut (Table 1). Characteristics of the cattle slurry can be found in Kristensen et al. (2022). Available N refers to the ammonium content of the slurry, which constituted 51–70% of total N.

The quantity of symbiotically fixed N (qBNF) by clovers in the ley mixtures was estimated using the <sup>15</sup>N isotope dilution method (Fried and Middelboe 1977; McNeill et al. 1994). Details regarding the harvesting method, determination of the botanical composition and measurements of N<sub>2</sub>-fixation activity can be found in Kristensen et al. (2022). The middle row of 1.5 m width in each plot was cut 5 times per year with a grass harvester (Haldrup, Denmark). The harvest dates in 2018 were 21st May, 26th June, 30th July, 3rd September and 15th October at the MW site, and 22nd May, 19th June, 30th July, 6th September and 10th October at the SW site. In 2019 the harvest dates were 25th May, 27th June, 29th July, 18th September and 23rd October at MW, and 20th May, 18th

June, 30th July, 6th September and 10th October at SW. The total weight of the biomass for each main plot was measured directly on the harvester. The biomass collected in the sub-plots were hand sorted into red clover, white clover, grass (including ryegrass and festulolium fractions), and weed, dried at 60 °C for 48 h and weighed to determine the botanical composition.

Similarly for weather information can be found in Kristensen et al. (2022), but briefly, the cumulative precipitation in the period April–August was 284 mm (2018) and 234 mm (2019) at SW, and 262 mm (2018) and 352 mm (2019) at MW; in the period September–March, it was 543 mm (2018–19) and 878 mm (2019–20) at SW, and 576 mm (2018–19) and 882 mm (2019–20) at MW. At both sites, the average temperature in the period April–August was 15.1 (2018) and 13.5 (2019); in the period September–March it was 7.2 (2018) and 6.6 (2019). The 2 years differed in climatic conditions with spring and summer 2018 being markedly warmer and dryer than normal.

#### Nitrate leaching

Before the experiment started, three suction cups were installed at a depth of 1 m in each plot in February and March 2018. During the period September to March, soil water was sampled approximately every second week by exerting a negative pressure of 80 kPa about 3 days prior of sampling. Equal volumes

**Table 1** Nitrogen application treatments distribution during the growth season in the experiment with grass-clover leys at two sites. Mineral fertilizer was applied alone or combined with a basic application of cattle slurry

	Annual N rate	Spring		After 1st cut		After 2nd cut	After 3rd cut
		Slurry	Mineral	Slurry	Mineral	Mineral	Mineral
		<i>kg inorganic N ha<sup>-1</sup></i>					
Control	0						
Mineral N	60		60				
	120		80		40		
	240		120		80	40	
	360		150		120	60	30
	480		150		120	120	90
Slurry + mineral N	120	60		60			
	240	60	60	60	20	40	
	360	60	90	60	60	60	30
	480	60	90	60	60	120	90

of the solutions from the three replicates were mixed and analyzed for nitrate concentration using a Technicon Auto Analyzer (Eriksen et al. 2015). The water balance was calculated using the model EVACROP (Olesen and Heidmann 1990) for which inputs were daily precipitation, temperature, crop type, time of sowing and cutting and soil physical parameters. Nitrate leaching was calculated from the average nitrate concentrations in the soil water samples between two sampling dates multiplied by the model-calculated drainage during this period. The accumulated leaching was measured from April 1st to March 31st in 2018–19 and 2019–20.

### Nitrogen balance

The N balance was calculated from measured and estimated values, as previously done by Eriksen et al. (2015). Input corresponded to the fertilizer amendment (mineral N and/or slurry available and organic N),  $N_2$  fixation and atmospheric deposition. Output corresponded to the removed N fraction measured in the harvested biomass.  $N_2$ -fixation was estimated from the amount of clover N and %Ndfa in herbage shoot (Kristensen et al. 2022) multiplied by three factors that took into account fixed N in roots and stubbles, N transfer to companion grass and immobilized N in soil organic matter for white and red clovers in sandy soils (Høgh-Jensen et al. 2004). Atmospheric deposition was estimated at about 11–12 kg N ha<sup>-1</sup> y<sup>-1</sup> (Ellermann et al. 2018). N input from irrigation, equivalent to 160 mm (2018) and 30 mm (2019) at SW, and 175 mm (2018) and 50 mm (2019) at MW, was negligible due to the low N concentration in the ground water (<0.26 mg N l<sup>-1</sup>). Gaseous losses ( $N_2O$ ) were not measured and probably negligible to be considered in the balance since denitrification from grassland was expected to be minimal (Reinsch et al. 2021). Ammonia losses from plant tissues and mineral fertilizers were also neglected (Sommer and Jensen 1994; Wang and Schjoerring 2012). Nonetheless, some ammonia losses may have occurred since slurry was only moderately acidified upon application (pH 5.6–7).

### Curve fitting and statistical analysis

We explored different functions to model the nitrate leaching (cubic, quadratic and exponential; Table S1

and Fig. S1). Although the cubic equation presented the best fit, in certain cases, the model was illogical predicting multiple decreases and increases, that did not conform with “common sense”, while the exponential model had the lowest match ( $R^2$ ). Exponential has been used to describe nitrate leaching under cereal crops (Børgesen et al. 2020; Vogeler et al. 2020). However, under perennial crops, we found that a quadratic model was more adapted.

A quadratic Eq. (1) was used to describe the nitrate leaching ( $NL$ ) as a function of fertilizer N applied,  $X$  (kg N ha<sup>-1</sup>), as follows:

$$NL(\text{kgNha}^{-1}) = \gamma + \alpha X + \beta X^2 \quad (1)$$

where  $\alpha$ ,  $\beta$ , and  $\gamma$  are model parameters.

Marginal N leaching ( $NL_{\text{marg}}$ ), which corresponds to the percentage N leached per additional N fertilizer applied at a specific N rate, was calculated from the derivative of the quadratic function (Eq. 2):

$$NL_{\text{marg}}(\%) = (\alpha + 2\beta X) \times 100 \quad (2)$$

Statistical analysis was performed with the R program version 3.5.1 (R Core Team, 2018). A linear mixed model (lmer function in the lme4 package) with the restricted maximum likelihood (REML) method was implemented to analyze effects of year, location, fertilizer types, rates and all interactions, on the N input, output and surplus. Block was considered as random variable. The same linear mixed model was used to analyze nitrate leaching but the soil water sampling dates were also included as repeated measurements. When the results from the ANOVA were significant ( $p \leq 0.05$ ), a posthoc Tukey test was performed using the glht function in the multcomp package. Model assumptions, i.e., normality (Shapiro–Wilk test) and homoscedasticity (Bartlett test), were tested prior to analysis. Data that did not meet these assumptions were log transformed. For all statistical tests, significance is defined by  $p \leq 0.05$ .

## Results

### Nitrogen balance (input/output)

N input varied between 361–583 kg N ha<sup>-1</sup> in SW 2018, 273–749 kg N ha<sup>-1</sup> in MW 2018, 297–519 kg N ha<sup>-1</sup> in SW 2019 and 278–745 kg N ha<sup>-1</sup> in MW

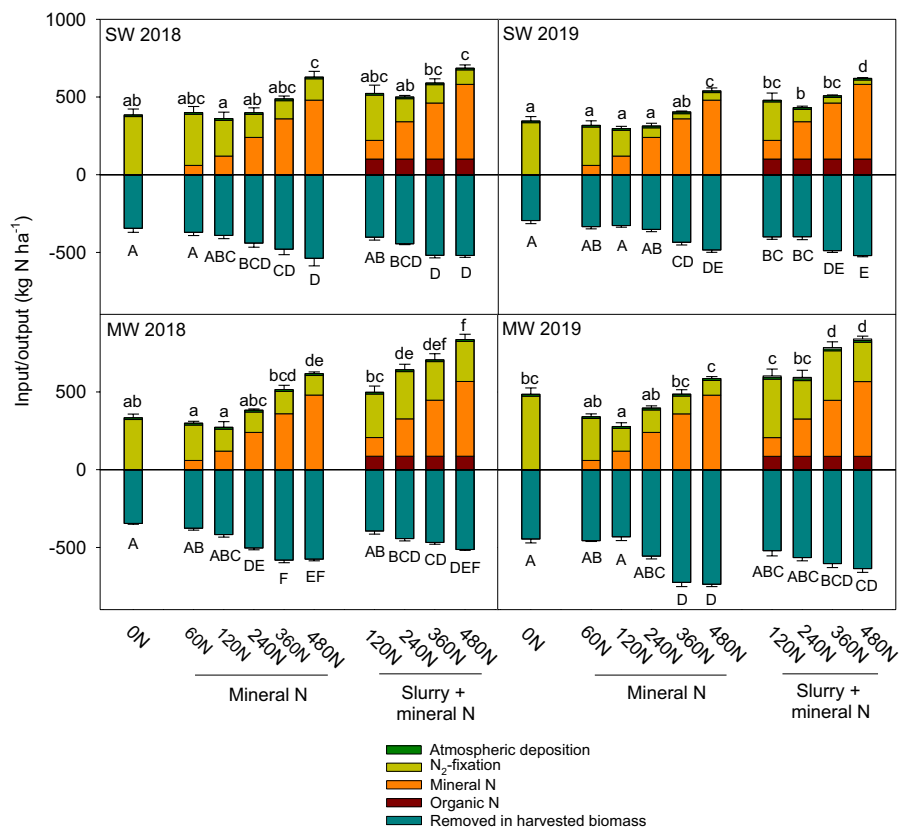
2019 (Fig. 1). Fertilizer application and biological N<sub>2</sub> fixation in clovers were the major inputs of the grassland. The increase of N input was a net effect of the increase of fertilization being larger than the reduction of biological N fixation. The N output with the harvested biomass ranged between 344 and 538 kg N ha<sup>-1</sup> in SW 2018, 346–582 kg N ha<sup>-1</sup> in MW 2018, 294–520 kg N ha<sup>-1</sup> in SW 2019 and 431–736 kg N ha<sup>-1</sup> in MW 2019. Thus, the surplus calculated in Fig. 2, was in the range –11 to 51 kg N ha<sup>-1</sup> in the control. Application of mineral N did not significantly increase N surplus in most cases (*p* > 0.05), except in MW2019, where it was actually lower. Also, at SW2019, application of 240 or 360 kg mineral fertilizer significantly reduced N surplus compared to the control. When plots were fertilized with slurry N, however, surplus was higher than the control (*p* ≤ 0.001) in the case of high N application rates (> 240 kg N in MW or even > 360 kg N in MW2019), owing to the fraction of organic N applied. Without organic N included in the balance, surplus for fields fertilized with slurry were not statistically different

from the control (data not shown) except for one treatment (plots fertilized with 480 kg slurry + mineral N ha<sup>-1</sup> at MW in 2018) (*p* ≤ 0.01). For each treatment, a similar surplus was determined in both years.

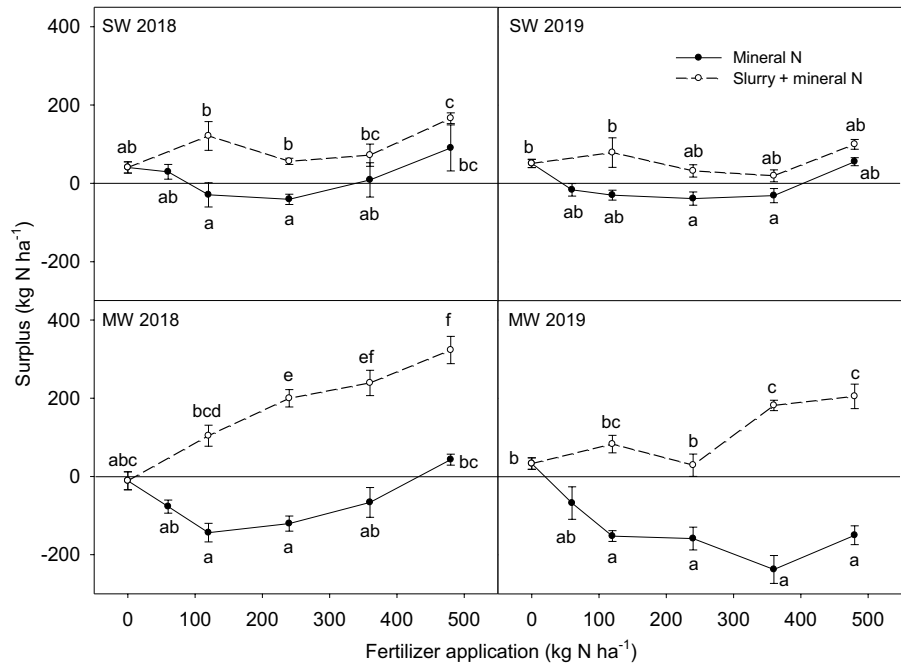
### Nitrate leaching

The cumulative nitrate leaching throughout the drainage period increased linearly in the unfertilized plots between September and April (Fig. 3). By April, cumulative leaching in the unfertilized plots was 37 (SE: ± 14) kg N ha<sup>-1</sup> in SW 2018, 23 (± 2) kg N ha<sup>-1</sup> in MW 2018, 13 (± 3) kg N ha<sup>-1</sup> in SW 2019 and 51 (± 12) kg N ha<sup>-1</sup> in MW 2019. Note that the 5th cut of the biomass was performed around mid-October in both years, but nitrate-N was already detected in soil water on September 1st as drainage occurred. Only at MW in 2018–19 was cumulative leaching in the control the lowest throughout the wet season. In the second year, cumulative leaching was much lower than in the first year (*p* ≤ 0.01). This was noticeable for plots

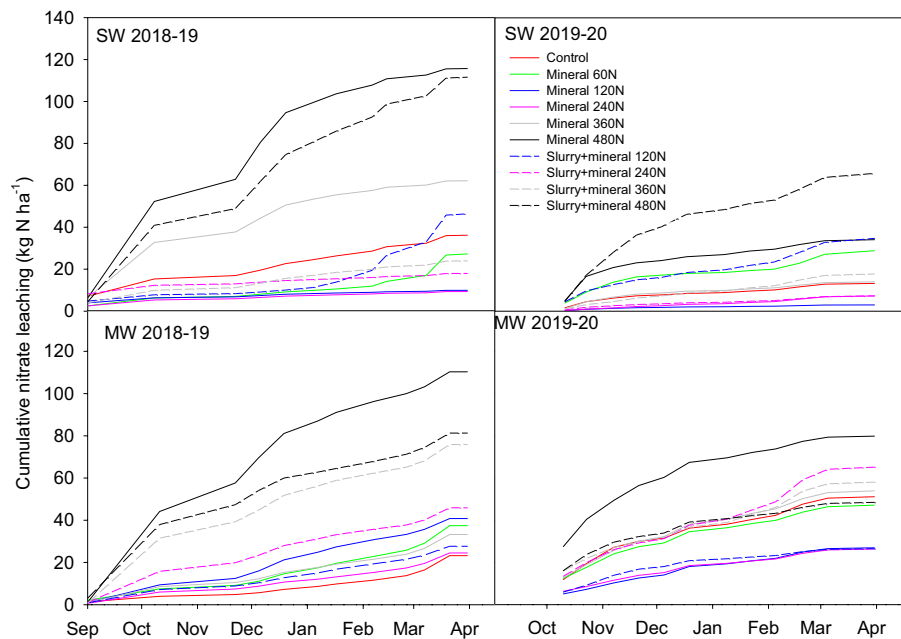
**Fig. 1** Nitrogen balance at two sites (SW and MW) and years. Error bars: SE (n = 4). Different letters indicate significant differences between treatments for the input (small letters) and for the output (capital letters) within the same year and site. Numbers from Kristensen et al. (2022)



**Fig. 2** Nitrogen surplus (total N) at two sites (SW and MW) and years as function of N fertilizer rate (inorganic N), calculated as the sum of input and output shown in Fig. 1. Symbols indicate mean values. Error bars: SE (n=4). Different letters indicate significant differences between treatments



**Fig. 3** Cumulative nitrate leaching measured throughout the autumn–winter period for each treatment at two sites (SW and MW) and years



fertilized with high N rates ( $\geq 360$  kg N ha<sup>-1</sup>). Cumulative leaching increased linearly in all treatments in the second year, while in the first year there were some irregularities. High leaching in the 1st year for plots fertilized with 480 kg N ha<sup>-1</sup> was due to high nitrate concentration ( $> 20$  mg

N-NO<sub>3</sub> l<sup>-1</sup>) until early December 2018 (Fig. S2). Hence, more than 70% of the cumulative leaching was reached on January 1st 2019 for those treatments. For few treatments (application of 60 kg mineral N ha<sup>-1</sup> and 120 kg slurry + mineral N ha<sup>-1</sup> in SW 2018), nitrate concentration increased in the

2nd part of the season (Fig. S2) resulting in a steep increment of the accumulated leaching after January 1st, 2019 (Fig. 3).

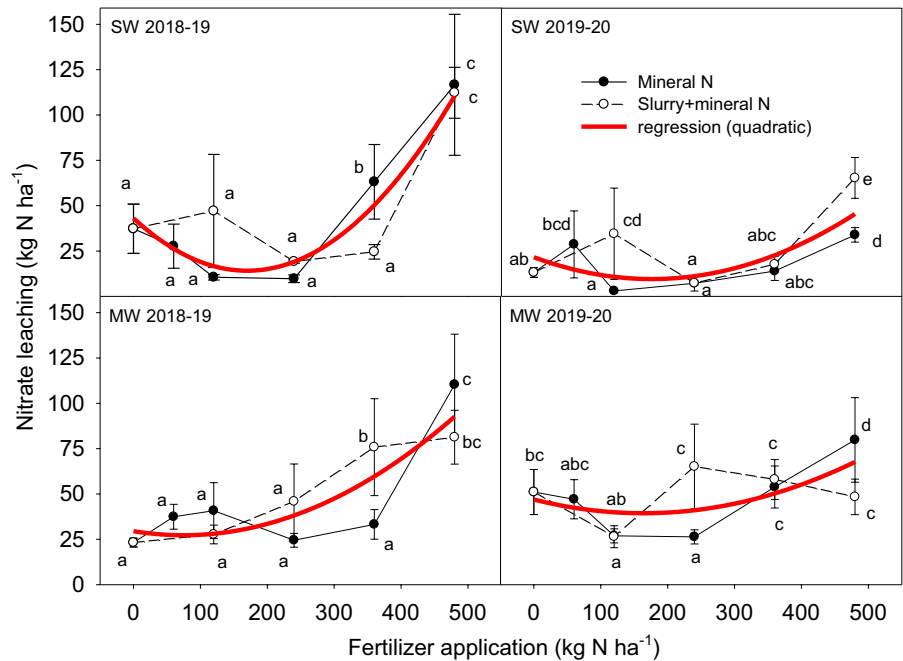
Nitrate leaching was fertilizer rate dependent ( $p \leq 0.001$ ) but independent of fertilizer type ( $p = 0.242$ ) (Fig. 4). Although leaching was significantly affected by the location ( $p \leq 0.01$ ), some similarities could be observed between sites such as the range of values and the curve shape. There was a poor correlation between the N surplus and nitrate leaching (Fig. 5). Nitrate leaching ranged between 3

and 117 kg N ha<sup>-1</sup>, whereas the surplus ranged from -144 to 323 kg N ha<sup>-1</sup>.

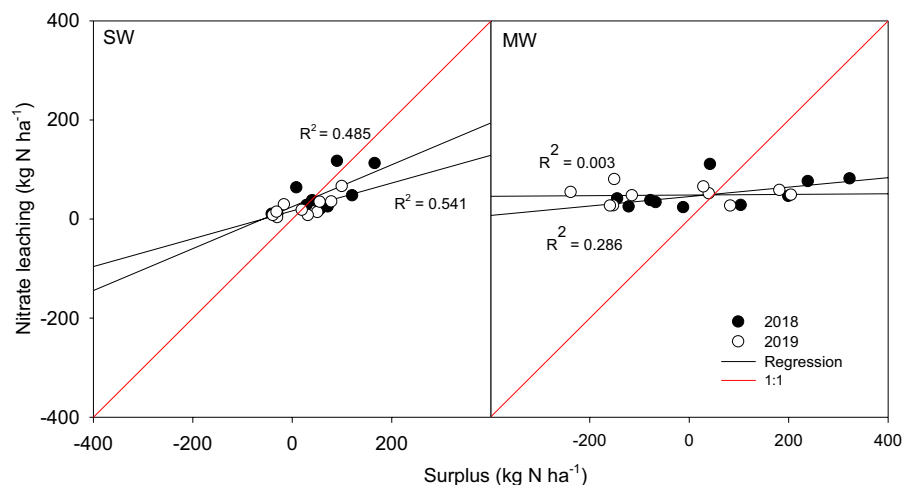
Descriptive model of nitrate leaching and marginal leaching

Leaching curves as a function of fertilizer application rate had a slight decrease from 0 to 120–240 kg N ha<sup>-1</sup> and an increase above these N application rates (Fig. 4). This was confirmed by the fit of the quadratic Eq. ( $0.37 \leq R^2 \leq 0.86$ ) (Table 2).

**Fig. 4** Nitrate leaching at two sites (SW and MW) during the drainage period (Sep–Apr) and years as function of N fertilizer rate (inorganic N) and fitted regression. Error bars: SE (n=4). Different letters indicate significant differences between treatments, based on repeated measurements ANOVA



**Fig. 5** Correlation between N surplus and nitrate leaching at two sites (SW and MW) and years



**Table 2** Parameters obtained by fitting Eq. (1) and (2) to the nitrate leaching and marginal nitrate leaching curves at two sites and years as function of N fertilizer rate

Regression			$\gamma$	$\alpha$	$\beta$	$R^2$	RMSE
Quadratic (leaching)	2018–19	SW	43.0908	-0.3406	0.001	0.86	15.7787
		MW	29.4979	-0.0594	0.0004	0.76	15.7678
	2019–20	SW	21.7836	-0.1376	0.0004	0.50	14.3186
		MW	46.9641	-0.0924	0.0003	0.37	14.9664
Derivative-Linear (marginal leaching)	2018–19			-0.2	0.0007	0.84	11.2106
	2019–20			-0.115	0.00035	0.98	1.8576

Marginal leaching was calculated from the derivative of the leaching equations and therefore was represented by linear regressions (Fig. 6). Marginal leaching did not differ significantly between sites but only between years where the slope in the 2nd year was half that in the 1st year (0.14 in 2018–19 and 0.07 in 2019–20). For both years, marginal leaching became positive for fertilizer rates higher than approximately 150 kg N ha<sup>-1</sup>.

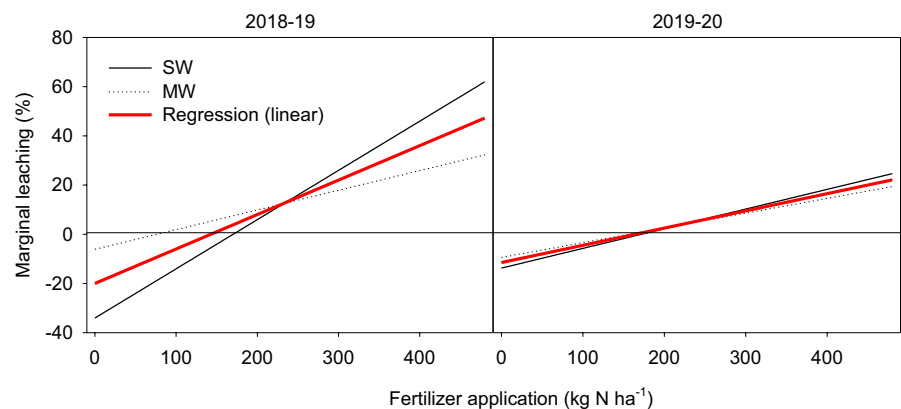
## Discussion

### Nitrogen balance unable to predict leaching

Farmers, governments, and consumers have been increasingly focused on improving farm efficiency and lowering environmental impacts (McLellan et al. 2018; Oenema et al. 2003). Thus, one main goal of this study was to evaluate N use efficiency in grass-clover ley and the link to nitrate leaching. The nitrogen balance is a basic indicator that may be used to estimate the risk of N loss from agricultural systems

at various scales (Eriksen et al. 2015; Nevens et al. 2006; Reinsch et al. 2021). It is often assumed that N surplus is a good predictor of N leaching under annual crops, particularly in sandy soils (Wachendorf et al. 2004). However, this was not the case under the perennial crops in this experiment (Fig. 5), since the range of nitrate leaching was narrow (3–117 kg N ha<sup>-1</sup>) compared to the large range of the surplus (-144 to 323 kg N ha<sup>-1</sup>). We believe the surplus is quite representative for production grassland, as Wachendorf et al. (2004) reported similar ranges in grassland in northern Germany. Thus, a simple soil surface N balance based on input and output was unable to predict nitrate leaching for the perennial crop. One reason may be that variations in soil organic N stocks are not considered in the N balance, although manure application results in an increase in soil organic N (Bhagal et al. 2018; Yang et al. 2016; Jensen et al. 2022).

Organic N accounted for a significant portion of the N surplus, but it was likely immobilized in the soil and did not raise a risk of leaching during the next autumn–winter season (Sørensen et al. 2019). At both sites and years, nitrate leaching was unaffected

**Fig. 6** Marginal N leaching at two sites (SW and MW) and years as function of N fertilizer rate (inorganic N) and year- average regression



by fertilizer type. Similar findings have been made by others. Thus, after applying slurry or mineral N fertilizer to a spring barley crop, Thomsen et al. (1997) traced the fate of organic N component in sheep feces and found no significant variation in nitrate leaching throughout the first and second winters. Also, in the first 2 years following application, Pedersen et al. (2021) found no significant effect of fertilizer type, either deep litter or mineral N fertilizer, on nitrate leaching after spring barley. Similarly, Delin and Stenberg (2020) found no significant increase in nitrate leaching in grass-clover swards fertilized with slurry in the spring compared to mineral N in a Swedish study, where soil organic matter mineralized at the same rate in fields fertilized with slurry or mineral N. For some treatments, leaching exceeded the surplus, particularly at the MW site (Fig. 5.). Leaching produced by mineralization of organic matter was the most obvious reason for leaching exceeding the N surplus since the sites were both suited for high  $N_2$ -fixation and had a long-term grassland history that supplied fertile soils. Long-term dairy crop rotations with temporary grasslands accumulate considerable stocks of both C and N even in sandy soils (Jensen et al. 2022) which may continue to mineralize for decades.

#### Nitrate leaching in grass-clover leys

One main goal was to study the effect of fertilization on nitrate leaching. Fertilizer rates of less than  $240 \text{ kg N ha}^{-1}$  did not lead to greater leaching than those in the unfertilized control, explaining the negative marginal leaching (Figs. 4 and 6). In fact, when the fertilizer rate was  $120\text{--}240 \text{ kg N ha}^{-1}$ , nitrate leaching was lowest. This corresponds to Reinsch et al. (2021), who recently reported an average leaching of  $25 \text{ kg N ha}^{-1}$  in temporary grass-clover treated with  $292 \text{ kg N ha}^{-1}$  on sandy loam in northern Germany over a 2-year period. Similarly, Wachendorf et al. (2004) employed a quadratic fit to explain the drop in nitrate leaching followed by a re-increase in response to increased total N input in a cutting system. Also, these authors made no distinction between fertilizer types because both treatments using mineral fertilizer with and without slurry fit the same quadratic curve. Although it is expected that high clover content and/or high fertilization rates increase leaching risk (Lüscher et al. 2014), clover content is mostly

reduced when fertilizer rates rise due to grass competition for available N in the soil (Kristensen et al. 2022; Nyfeler et al. 2011). Clearly, there was a self-regulatory mechanism where a rise in fertilization was compensated by a decline in biological nitrogen fixation activity in the current study, at least until a particular fertilization rate threshold. The first-year thresholds were set at  $240 \text{ kg N ha}^{-1}$ , while the second-year thresholds were set at  $360 \text{ kg N ha}^{-1}$ . Above those levels, fertilizer exceeded the ability of the grasses to extract soil-available N, resulting in a significant rise in nitrate leaching. For application rates  $\geq 360 \text{ kg N ha}^{-1}$ , the proportion of clover decreased significantly from 20% (SW 2018) to 10% (SW 2019), and from 40% (MW 2018) to 20% (MW 2019) during the 2 years (Kristensen et al. 2022). Despite the cumulative application of more than  $700 \text{ kg N ha}^{-1}$ , the abundance of ryegrass helped to limit leaching in the second year.

#### Marginal increase in nitrate leaching by fertilizer application

The rate of marginal leaching increased with the fertilization level (Fig. 6). When the fertilizer rate was less than  $150 \text{ kg N ha}^{-1}$ , there was no additional leaching from swards. Herbage yields ranged between  $11$  and  $17 \text{ Mg DM ha}^{-1}$  at this rate, with clover accounting for 30–50% of total DM at both sites and years (Kristensen et al. 2022). The marginal N leaching rate for both years was roughly 5% at a fertilization rate of  $200 \text{ kg N ha}^{-1}$ , which is less than the marginal leaching of an arable crop when applied the recommended rates. For example, Vogeler et al. (2020) calculated a marginal leaching in winter cereals fertilized at the recommended application rate ( $170 \text{ kg N ha}^{-1}$ ) from 4 to 11% in the 1st year and 12–20% in the 2nd year. Thus, the replacement of arable crops by grassland could be an effective targeted N-regulation strategy that might be implemented in nitrate vulnerable areas.

#### Conclusion

A significant finding of this study was that across a broad range of N application rates, nitrate leaching was unaffected by whether mineral fertilization was applied alone or in combination with a basic dressing of cattle slurry. Interestingly, there

was no relationship between the N surplus and leaching losses. This implies a buildup of N in soil organic matter pools, which may or may not result in increased leaching losses in the long run depending on crop rotation and management or losses in gaseous form. Continued high doses of N fertilizer over 2 years did not result in similar increase in nitrate leaching due to the strong self-regulatory nature of grass-clover mixtures where increased soil available N was absorbed by the grasses given a competitive advantage over clover.

Nitrate leaching increased quadratically as a function of application rate and the marginal leaching, the increase by adding extra N, increased linearly. Importantly, the marginal leaching depended on application rate: Below 150 kg N ha<sup>-1</sup> there was no additional leaching from fertilization and at 200 kg N ha<sup>-1</sup> around 5% of additional fertilizer-N was leached. This is less than generally found for arable crops and thus even in intensive dairy systems, grass-clover leys are an environmentally favorable crop. Future research should focus on the fate of accumulated soil organic N when cultivating the grass-clover swards in terms of legacy effect and leaching losses.

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#### Declarations

**Competing interests** The authors declare no competing interests.

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