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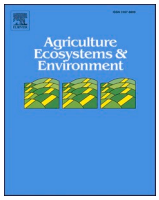

Potential measures to reduce nitrate and nitrous oxide losses from renovated grasslands

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ABSTRACT

Grassland renovation (grassland renewal or temporary conversion to arable land) is a common practice employed by farmers to reverse declining yields in agricultural grasslands. Renovation practices may lead to substantial nitrate (NO_3) leaching and nitrous oxide (N_2O) emissions. Farmers lean towards renovating grasslands in autumn instead of spring because of better sward establishment and low weed infestation. Limited nitrogen (N) uptake of renewed grassland during autumn may, however, increase the potential risk of NO_3 leaching and N_2O emission. Potential options to mitigate these N losses are reduction in tillage intensity and N application rate, or the application of nitrification inhibitors. We assessed the effects of these measures as well as timing of renewal and the conversion to grassland to maize on NO_3 leaching and N_2O emissions after one growing season. Five replicated field experiments were conducted of one year each at five locations in the Netherlands. We measured crop yield, soil mineral N, NO_3 concentrations in groundwater and N_2O emission. Averaged over all experiments, the measures had an effect on NO_3 concentration in groundwater, although not significant. The highest NO_3 concentrations in groundwater were observed after autumn renewal (17.2 mg NO_3-N L^{-1}), autumn renewal combined with mitigation strategies (12.8–19.4 mg NO_3-N L^{-1}) and conversion to maize (12.5–12.8 mg NO_3-N L^{-1}), with all of these treatments exceeding the European norm of 11.3 mg NO_3-N L^{-1}. Concentrations after renewal in spring were lower (3.1 mg NO_3-N L^{-1}) and did not lead to an increase compared to the control (4.4 mg NO_3-N L^{-1}). N_2O emissions increased significantly directly after grassland renewal relative to the reference grassland and N_2O fluxes were similar in spring and autumn. We conclude that autumn renewal leads to significant increases in the risk of NO_3 leaching which cannot be mitigated by the technical measures in this study.

1. Introduction

Grassland plays a major role in North West-European agriculture because it is an important source of feed in the intensively managed dairy and beef farming systems. It is one of the most important types of land use in North West-Europe and its share of agricultural land is 91 % in Ireland, 63 % in the United Kingdom, 28 % in Germany and 42 % in the BENELUX countries (EUROSTAT, 2016). To counteract declines in yield and quality of intensively managed grassland that occur over time due to sward deterioration (i.e. compaction, weed invasion) and extreme weather conditions, farmers may apply various management strategies (Vellinga et al., 2004). First, renewal of grassland by tillage and reseeding (jointly referred to as grassland renewal) within an interval of five to ten years (Buchen et al., 2017; Schils et al., 2002; Velthof et al., 2010). Second, rotation of grassland and arable crops every few years. Third, conversion of grassland to continuous arable land (referred to as grassland conversion) for the increasing production of arable crops (Nitsch et al., 2012), such as maize for biogas production (Osterburg et al., 2010; Taube et al., 2014). Renovation techniques used in the renewal or conversion of grasslands often include a combination of the chemical destruction of the old sward and soil cultivation (ploughing, rotary hoe, subsoiling or spaders) before reseeding. Other grassland management strategies are more conservative and include practices like reseeding without prior destruction of the sward or under cover crops

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(Ammann et al., 2020).

The grassland management strategies that include destruction of the sward may however lead to considerable environmental losses. As soil organic matter content is usually higher in grassland than in arable soils (Buchen et al., 2017), ploughing techniques often increase degradation of soil organic matter and loss of carbon due to elevated microbial decomposition (Reinsch et al., 2018a). This also leads to a large increase in readily available N through mineralization of organic N, which provides N substrates for both nitrification and denitrification (Six et al., 2004; Velthof et al., 2010). Due to the high organic N content of grassland soils, renovation could temporarily increase the mineral N content of the soil to more than 200 kg N ha^{-1} (Davies et al., 2001). As a growing crop is absent just after ploughing, there is a period of several weeks with little or no N uptake. During this period, the mineralized N accumulates in the soil and can be lost through nitrate (NO_3) leaching and/or the gaseous (by)products of nitrification and denitrification processes, such as nitrous oxide (N_2O) or dinitrogen gas (N_2) (Buchen et al., 2017). Leaching of NO_3 to the surface water may cause eutrophication of these waters with potential harmful algal blooms, fish kills and health risks as results (Glibert, 2020). Additionally, contamination of drinking water sources with NO_3 can make it unsuitable for human consumption (i.e. exceeding the European environmental standards of 11.3 mg NO_3-N L^{-1} as established by directives like the Water Framework Directive, the Drinking Water Directive and the World Health Organization) (WHO, 2017).

N_2O is a potent greenhouse gas that contributes to climate change and is identified as one of the major greenhouse gases emitted from agricultural land (IPCC, 2023). Emissions may occur directly from soil, but may also occur indirectly, as a result from NO_3 leaching into aquatic systems and subsequent denitrification (IPCC, 2019). Reducing N losses resulting from grassland management strategies is therefore important to mitigate climate change, ensure good quality of ground and surface waters and increase N efficiency on farm scale.

A few studies have already studied the effect of grassland ploughing on both NO_3 leaching and N_2O emission and have been summarized by Biegemann (2014). The risk of both increased NO_3 leaching and N_2O emissions coincides with wet climatic conditions in combination with high soil mineral N concentrations. Most N losses occurs within a year and could be as high as 132 kg N ha^{-1} $year^{-1}$ in the first year when soil is left fallow after ploughing of grassland (Adams and Jan, 1999; Davies et al., 2001; Hansen and Eriksen, 2016; Lloyd and Society., 1992; Seidel et al., 2009; Buchen et al., 2017). Renewal in autumn in a temperate climate leads to strong NO_3 leaching, up to 36–106 kg N ha^{-1} $year^{-1}$ (Francis, 1995; Seidel et al., 2009) and conversion to arable land results into even higher losses of 28–254 kg N ha^{-1} $year^{-1}$ (Francis, 1995; Kayser et al., 2008; Lloyd and Society., 1992; McLenaghen et al., 2010). For N_2O, grassland renewal can temporarily increase emissions after ploughing for about 1–2 months, after which the reseeded grassland has taken up most of the mineralized mineral N (Buchen et al., 2017; Mori and Hojito, 2007; Velthof et al., 2010). However, N_2O emissions vary largely from 0 to 29 kg N ha^{-1} $year^{-1}$ in the first year after ploughing (Reinsch et al., 2018b).

Losses of N can be mitigated by improving grassland management factors, such as the timing of ploughing and N application, 4 R factors of N fertilization (right source, right rate, right time and right place) (Banger et al., 2020), and application of nitrification inhibitors (Huérfano et al., 2016). The timing of the year in which ploughing occurs can have a substantial effect of the N losses due to different climatic conditions in autumn and spring in North-West Europe. The highest growth rates of grass, and therefore the highest N demand, occur in spring and summer, while N demand of grass in autumn and winter is limited. Therefore, the risk of NO_3 leaching and N_2O emissions is likely to be higher when grassland ploughing is conducted later in the year (Kayser et al., 2018). Indeed, Seidel et al. (2009) found an increase in NO_3 leaching when renewal occurred in autumn compared to renewal in spring. Emissions of N_2O can be mitigated with several measures. Examples of mitigation options are reducing the N application after renovation, accounting for the mineralization of organic N; changing of timing of N application (autumn vs. winter vs. spring) considering the effect of freeze-thaw events, or differences in rainfall and soil temperatures; and considerations in the application technique (injection vs broadcasting) in which broadcasting is the preferred application method as injection creates an anaerobic environment abundant in readily oxidizable C resulting in enhanced N_2O emission.(Maris et al., 2021). Reducing the soil N surplus (i.e. balancing the N input and N output) is important to reduce N losses, as farmers often apply more N than needed by plants (Guenet et al., 2021). Lower N application rates, the splitting of N doses and application over time (Schils et al., 2010), the application of N stabilizers (Harty et al., 2016), slow release fertilisers (Bella et al., 2017; Subbarao et al., 2006) and nitrification inhibitors (Harty et al., 2016) are other potential mitigation options mentioned in the literature. Nitrification inhibitors are chemical compounds that are added to mineral fertilizers and manure or sprayed onto the soil directly. They inhibit the transformation of ammonium (NH_4) to nitrite (NO_2) by Nitrosomonas bacteria, and thereby the production of N_2O during nitrification (Harty et al., 2016). This inhibition results in lower soil NO_3 concentrations and consequently in reduced NO_3 leaching. In addition, N_2O production through denitrification of nitrate released during nitrification is also reduced when nitrification is inhibited. Meta-analyses show that nitrification inhibitors added to fertilizers reduce N_2O emission from fertilisers, varying from 20 % to 80 % (Akiyama et al., 2013; Gilsanz et al., 2016).

Several studies focus on mitigation of N losses from grassland renewal, including effects of renovation technique (Buchen et al., 2017; Krol et al., 2016; Reinsch et al., 2018b; van Groenigen et al., 2010), time of renewal (Velthof et al., 2010), use of a nitrification inhibitor (Baral et al., 2020) or measures focussed on mitigation of either NO_3 leaching or N_2O emission (e.g. N application rate) (Banger et al., 2020). It is very well possible that there are interactions between these measures. For a realistic assessment of their potential it is needed to combine them in one field experiment. However, there is no study available that combines all three aspects (renovation technique, timing of grassland renewal and N application rate) in one experiment. Furthermore, there are very few studies that assess the effect of reducing the N application rate after renewal (Seidel et al., 2007). In addition, it is common that nitrification inhibitors are added to manure, but studies in which nitrification inhibitors are directly added to the soil instead are scarce (Baral et al., 2020).

Here, we report a study quantifying the effect of the timing of grassland renewal (spring vs autumn); conversion to arable land; N loss abatement measures including several renovation techniques (tillage, restricted tillage rotary hoe, subsoiling and mechanical destruction); N application rate; and the addition of a nitrification inhibitor (3,4-dimethyl pyrazole phosphate (DMPP)) added to the soil on NO_3 leaching and N_2O emissions. Five identical field experiments of one year each were carried out on intensively managed grasslands on sandy soil at five locations in the Netherlands, during which we studied the effects of abovementioned mitigation strategies as well as timing of renewal and conversion to arable land (forage maize). The experiments were set up to test the following hypotheses:

- H1: Renewal in autumn increases the soil mineral N content in autumn compared to spring renewal and therefore increases NO_3 leaching;
- H2: Mitigation strategies after autumn renewal (reduced tillage, reduced N application rate, nitrification inhibitor) decrease the soil mineral N content and risk of NO_3 leaching;
- H3: Conversion to forage maize increases the soil mineral N content in autumn compared to both continuous grassland and spring renewal, leading to increased NO_3 leaching; and
- H4: Grassland renewal increases N_2O emission, most prominently following renewal in autumn.

2. Material and methods

2.1. Field sites and management

2.1.1. Research sites

A total of five identical experiments were carried out throughout 2019, 2020 and 2021 on various grassland sites on sandy soils in eastern and southern part of the Netherlands; Vredepeel, Bennekom and Wageningen (Supplementary data Table A). The experiments in Vredepeel were carried out during the three consecutive years. During the first two years two experiments were located on the same field (VP_19, VP_20) next to each other, and the third year (VP_21) on a field nearby. The experiments in Bennekom (BEN_20) and Wageningen (WAG_21) were both carried out for one year. Prior to the study, all sites had been managed by local farmers as continuous grassland for eight and nine, five, three and three years for Vredepeel (first two years), Vredepeel (third year), Bennekom and Wageningen, respectively (Supplementary data Table A). All sites were sandy soils with a pH ranging from 5.1 to 5.8 and an organic matter content between 3.1 % and 7.7 % (Supplementary data Table B). Precipitation data was collected from nearby stations of the Royal Netherlands Meteorological Institute (KNMI) (Supplementary Table C). The air temperature was recorded at 2 m height with a hourly temporal resolution (Supplementary Table D).

2.2. Experimental set-up

The experiments comprised a randomized block design with 11 treatments and four replicates per experiment (Table 1) and a plot size of 9 m × 12 m. Factors were timing of grassland renewal (spring vs. autumn), conversion to forage maize crop, intensity of tillage management, N rate application and use of a nitrification inhibitor:

- Reference treatment, i.e. no renovation (control);
- Renewal in spring, grassland chemically destroyed, ploughed to 0–25 cm and cultivated with a rotary hoe (standard tillage), reseeded in March-April (S_R);
- Grassland chemically destroyed, ploughed to 0–25 cm and cultivated with a rotary hoe, conversion to maize crop in April-May (S_M);
- Grassland chemically destroyed, ploughed to 0–25 cm and cultivated with a rotary hoe, conversion to maize crop in May-June after first cut of grass (S_M_1);
- Renewal in autumn, grassland chemically destroyed, ploughed to 0–25 cm or subsoiling (0–30 cm) and cultivated with a rotary hoe (standard tillage), reseeded in September (A_R_ST);
- Renewal in autumn, grassland chemically destroyed, shallow subsoiling of 0–15 cm and cultivated with a rotary hoe (restricted tillage), reseeded in September (A_R_RT);
- Renewal in autumn, grassland chemically destroyed, no tillage (zero tillage), reseeded in September (A_R_ZT);
- Renewal in autumn, mechanical grassland destruction for weed control through intensive tillage, reseeded in September (A_R_MD);
- Renewal in autumn, grassland chemically destroyed, ploughed to 0–25 cm or subsoiling (0–30 cm) and cultivated with a rotary hoe, reduced N application by 55 kg N ha^{-1}, reseeded in September (A_R_N55);
- Renewal in autumn, grassland chemically destroyed, ploughed to 0–25 cm or subsoiling (0–30 cm) and cultivated with a rotary hoe, reduced N application by 105 kg N ha^{-1}, reseeded in September (A_R_N105);
- Renewal in autumn, grassland chemically destroyed, ploughed to 0–25 cm or subsoiling (0–30 cm) and cultivated with a rotary hoe, application of a nitrification inhibitor (3,4-Dimethylpyrazole phosphate, DMPP, Vizura) by spraying the soil directly after tillage in September and October (A_R_NI).

Table 1
Summary of experimental treatments: control, renewal in spring (S_R), conversion to maize (S_M and S_M_1) and renewal in August with or without mitigation strategies (all other treatments). Target N supply of calcium ammonium nitrate (CAN) and cattle slurry for each treatment.

Code	Treatment			Target N supply[2]	
	System	Renovation period[1]	Soil treatment	CAN (kg N ha^{-1})	Cattle slurry (kg N ha^{-1})
Control	Grassland			215	180
S_R	Grassland	Spring	Standard tillage	155	80
A_R_ST	Grassland	Autumn	Standard tillage	230	180
A_R_RT	Grassland	Autumn	Restricted tillage	230	180
A_R_ZT	Grassland	Autumn	Zero tillage	230	180
A_R_MD	Grassland	Autumn	Mechanical weed control	230	180
A_R_N55	Grassland	Autumn	Reduced N −55 kg ha^{-1}	175	180
A_R_N105	Grassland	Autumn	Reduced N −105 kg ha^{-1}	125	180
A_R_NI	Grassland	Autumn	Nitrification inhibitor	230	180
S_M	Maize	Spring		30	60
S_M_1	Maize	Spring, after first cut		110	160

[1] Exact renovation date is given in the supplement (Supplementary data Table L)

[2] Timing of application of distribution of CAN and cattle slurry is given in supplement (Supplementary data Table F and G)

For the treatments with chemical destruction of the grassland, the sward was destroyed by application of a broadband herbicide (300 L ha^{-1} water, 3 L ha^{-1} glyphosate solution; Round up, Monsanto). The target N application was based on common agricultural practice in the Netherlands in line with the guidelines by the Commission Fertilization Grassland and forage crops (CBGV) (Bemestingsadvies, n.d.). The target total N fertilization with calcium ammonium nitrate (CAN) and cattle slurry was 395 kg N ha^{-1} $year^{-1}$ for control and 235 kg N ha^{-1} for renewal in spring (Table 1). Moreover, for autumn renewal treatments the target N fertilization varied from 410 kg N ha^{-1} $year^{-1}$ for all tillage and weed control treatments to 355 and 305 kg N ha^{-1} $year^{-1}$ for the reduced N application treatments. The sward was managed by taking 4–5 cuts per year, depending on the grass growth and environmental conditions. CAN and cattle slurry were applied on four or five occasions each year dependent on the number of cuts. Total N target supply for the maize treatments was 90 (S_M) and 270 (S_M_1) kg N ha^{-1} $year^{-1}$. The composition of manure varied per application (Supplementary data Table E) and therefore the N application rate for each treatment slightly differed from the target (Table 1) due to variation in cattle slurry composition throughout the year (Table 2). Following dressings were not corrected with respect to the previous cut. N application data (Supplementary data Table F and G) as well as harvest date (Supplementary data Table H) were dependent on sward growth and weather conditions. N application rate per cut is described in Tables J and K (Supplementary data) and agricultural management in Table L (Supplementary data).

2.3. Soil and groundwater sampling and analysis

Soil samples for determining the soil mineral nitrogen (N_{min}) concentrations were taken from plots at 0–30, 30–60 and 60–90 cm depth, during spring (May-July), summer (July-August), autumn (September-October), winter (November-December) and spring in the following year (February-March of the following year) (Supplementary data Table M).

Table 2
Nitrogen fertilization, average yield, N content and N uptake. Small letters indicate differences between yield ($p < 0.05$). Numbers between brackets represents the standard deviation ($n = 20$).

Treatment[1]	N fertilization (kg N ha^{-1})	Yield (t DM ha^{-1})		N content (g N kg DM^{-1})		N uptake (kg N ha^{-1})	
Control	397	11.5	$(3.1)^{b}$	28	$(3)^{b}$	320	$(56)^{d}$
S_R	234	8.1	$(2.0)^{a}$	37	$(4)^{c}$	293	$(46)^{cd}$
A_R_ST	409	10.3	$(2.9)^{b}$	27	$(3)^{ab}$	268	$(56)^{bcd}$
A_R_RT	409	10.4	$(3.1)^{b}$	27	$(2)^{ab}$	285	$(74)^{bc}$
A_R_ZT	409	10.2	$(3.1)^{b}$	27	$(2)^{ab}$	271	$(69)^{abc}$
A_R_MD	409	9.9	$(2.6)^{b}$	27	$(2)^{ab}$	261	$(62)^{ab}$
A_R_N55	351	9.9	$(3.0)^{b}$	26	$(2)^{ab}$	260	$(69)^{ab}$
A_R_N105	308	9.7	$(3.0)^{b}$	25	$(2)^{a}$	243	$(68)^{a}$
A_R_NI	409	10.3	$(3.2)^{b}$	28	$(2)^{ab}$	282	$(72)^{bc}$
S_M[2]	91	17.7	(4.0)	12	(2)	213	(47)
S_M_1[3]	276	19.1	(6.0)	15	(2)	286	(51)

[1] Treatment code is shown in Table 1
[2] Yield, uptake and N content from maize. Maize yield was not included in statistical analysis.
[3] Yield, uptake and N content from both grass (5.1 t ha^{-1}; 118 kg N ha^{-1}) and maize (14.0 t ha^{-1}; 168 kg N ha^{-1}).

The control and all treatments that were renewed or converted in spring (S_R, S_M and S_M_1) were sampled during all five periods, whereas all autumn renewal treatments were only sampled during autumn, winter and the following spring. Sampling was performed with a gouge auger with an inner diameter of 2 cm. For each sampling fifteen subsamples were taken per plot, which were mixed and stored in a freezer until further processing. For analysis, the soil samples were extracted with a 0.01 M $CaCl_2$ at a soil:solution ratio of 1:2 (V:V) (Houba et al., 2008). Concentrations of NH_4 and NO_3 were measured using a segmented-flow analysis (SFA, Skalar, SAN++) (Houba et al., 2008). The concentrations per volume of soil (mg L^{-1}) were then converted to mineral N content (kg ha^{-1}) assuming that the soil density did not change during the analysis. The mineral N content in the 0–90 cm layer in autumn, winter and the following spring were used only as an indicator for potential nitrate leaching to groundwater as there are more pathways for N losses aside from NO_3 losses, such as transformation to organic N and loss as N_2 through complete denitrification.

The groundwater of each plot of each experiment was sampled during the following spring. Groundwater samples were taken from the upper meter of groundwater at the centre of all individual plots of each experiment as described by (Fraters et al., 1998). A vertical hole was made with an auger up to 0.8 m below the groundwater table. The water was then sampled with a well-screen and a suction pump through a polyethylene tube. The groundwater was filtered with a 0.45-µm cellulose nitrate membrane filter and then samples were acidified to $pH < 2$ (using concentrated H_2SO_4) to conserve the samples according to NEN_ISO 5667–3. Each individual sample was analyzed for NO_3 and NH_4 content using SFA.

2.4. Plant sampling and analysis

The aboveground biomass of the grassland treatments was harvested 4 or 5 times per year, from a representative area of 15 m^2 (3 × 5 m) for each plot, by mowing to a height of 5 cm using a Haldrup plot harvester. The fresh yield of each subplot was determined in the field with the Haldrup plot harvester. A grass subsample of 1 kg was taken for analyses of dry matter (DM) and N content. Maize yields were determined via a harvest of two rows and 10 m length per plot, using a tractor and shredder (New-Holland 1870), which was also used to determine fresh yield. Two subsamples of 1.5 kg each were taken for analysis. For grass and maize, DM was determined by drying the samples at 60 °C for 72 h. Subsequently, total N concentration was analyzed using Dumas (Saint-Denis and Goupy, 2004). The N surplus was defined as the total N applied (from cattle and CAN) minus total N uptake.

2.5. N_2O measurements

N_2O measurements were only performed at the Bennekom and Wageningen site. The N_2O fluxes were measured weekly from March 2020 to March 2021 in Bennekom and from March 2021 to March 2022 in Wageningen. Treatments A_R_MD and A_R_N55 were excluded from the measurements due to practical constraints. The measurements were performed with the closed chamber method as described by Velthof et al. (2010). The sampling started before renovation, and the sampling frequency was on average once per week during the growing season. This frequency was increased to two times a week during the first week after renovation and after N application. Outside the growing season, the frequency decreased to once per 2–3 weeks on average. This resulted in an average of 30–35 measurements per year. At the beginning of each experiment, a PVC collar of 20 cm diameter and 10 cm height was installed permanently into the soil to a depth of 5 cm at the center of each plot to provide a base for the measurements (Velthof et al., 2010). These collars were only removed during renovation, harvesting and N application for a short period of time and were afterwards re-installed in the same place. During N_2O measurements, gas-tight chambers with a height of 20 cm were placed on top of these collars to create a closed system of 4.7 L chamber volume. To allow gas sampling after a 10-min incubation period, the chambers were equipped with a rubber septum and vent tubes on the top. N_2O concentration analyses were performed with a PICARRO G2508 cavity ring-down spectroscopy gas analyzer (PICARRO, USA). The N_2O fluxes were then calculated assuming a linear concentration increase over the 10-min incubation period (Supplementary data on methodology of N_2O calculation and Figure A). The accumulated annual N_2O-N emission was calculated by linear interpolation between the measured daily fluxes. The period for comparing the N_2O fluxes on an annual basis was set from March to March of next year because renewal started halfway of March. In addition, emissions were also translated into yield-scaled by dividing the cumulative N_2O emission by total crop N uptake (van Groenigen et al., 2010).

2.6. Statistical analysis

Statistical analysis was performed using R Version 4.2.1 (R Core Team, 2022). A linear mixed model was applied to assess the effect of different treatments on soil mineral N, crop yield, NO_3 concentration and cumulative N_2O fluxes, using the nlme package (Pinheiro et al., 2023). In the models, treatment was included as a fixed factor, whereas the experiment (differing in year and location) and the experimental block were included as random factors, with the experimental block nested in the experiment. For the model in which analyses were done per experiment the experimental block was included as random factor. The effect of treatment was determined with the R function anova (linear mixed model). To assess the effect of grassland renewal on N_2O emission, daily fluxes were analyzed using the aforementioned steps. Normality and homogeneity of the residuals were checked visually by means of a histogram and q-q plot prior the analysis to meet the requirements of the analysis. When residuals were not normally distributed the response variable data was transformed using log or box-cox transformations. Significant differences between treatments were determined by Tukey's honestly significant difference posthoc test (HSD) with the function emmeans (Lenth, 2021) and CLD from the multcomp package (Hothorn et al., 2008).

3. Results

3.1. Meteorological data

At the Vredepeel site, the annual precipitation was 703 mm in 2019, 707 mm in 2020 and 780 mm in 2021 compared with the 30-year (1990–2020) average of 766 mm (Supplementary Table C). The

average temperature was 11°C, 11.8 °C and 10.4°C in 2019, 2020 and 2021, respectively (Supplementary Table D). The annual precipitation at the sites in Bennekom and Wageningen was 866 mm in 2021 and 782 mm compared to the 30-year average (864 mm between 1990 and 2020). During the summer period (June-August), monthly precipitation was relatively low in 2020, but higher in 2021 in comparison with the 30-year (1990–2020) average (Supplementary data Table C).

3.2. Nitrogen fertilization and yields

Averaged over the five experiments, spring renewal (S_R; 8.1 t DM ha^{-1}) led to statistically ($P < 0.05$) lower yield (Table 2) than all treatments with renewal in autumn (ranging from 9.7 to 10.4 t DM ha^{-1}) and the control (11.5 t DM ha^{-1}). The N content of the dry matter ranged from 25 to 28 g N kg^{-1} for the autumn renewal treatments, compared with 37 g N kg^{-1} for the S_R treatment. The N content of the S_R treatment led, despite the significantly lower DM yield, to a higher total N uptake (293 kg N ha^{-1}) compared to the treatments that were renewed in autumn (243 – 286 kg N ha^{-1}). Highest DM yield and total N uptake (320 kg N ha^{-1}) were observed for the control. Moreover, treatments with mitigation strategies (A_R_RT, A_R_ZT, A_R_N55, A_R_N105, A_R_NI) in autumn generally showed lower DM yields and total N uptake in comparison to the autumn treatment without any measures (A_R_ST), but they were not significant.

3.3. Soil mineral N

Averaged over all experiments, the soil mineral N of the 0–30 cm layer during spring sampling (after destruction of the sward; Supplementary data Table M) averaged from 12 to 156 kg N ha^{-1} across the control, S_R, S_M and S_M_1 treatments (Fig. 1). The soil mineral N of this layer after spring renewal (S_R) and conversion to maize (S_M and S_M_1) increased significantly ($P < 0.05$) compared to the control for most of the sites. During summer and autumn sampling the soil mineral N of S_R, S_M and S_M_1 decreased over time. For all sites the soil mineral N in autumn of S_R was similar to the soil mineral N of the control. The soil mineral N in autumn for conversion to maize (S_M and S_M_1) was significantly ($P < 0.05$) higher than the control for most of the sites except for VP_20.

Averaged over all experiments, the soil mineral N of the 0–90 cm layer during autumn sampling averaged from 45 to 223 kg N ha^{-1} across all treatments (Table 3). The soil mineral N of the S_R treatment (61 kg N ha^{-1}) did not significantly increase ($P < 0.05$) in comparison to the control treatment (45 kg N ha^{-1}; Table 3), but for all treatments with autumn renewal soil mineral N was elevated (115–223 kg N ha^{-1}) compared to spring renewal (S_R) and the control. Converting grassland

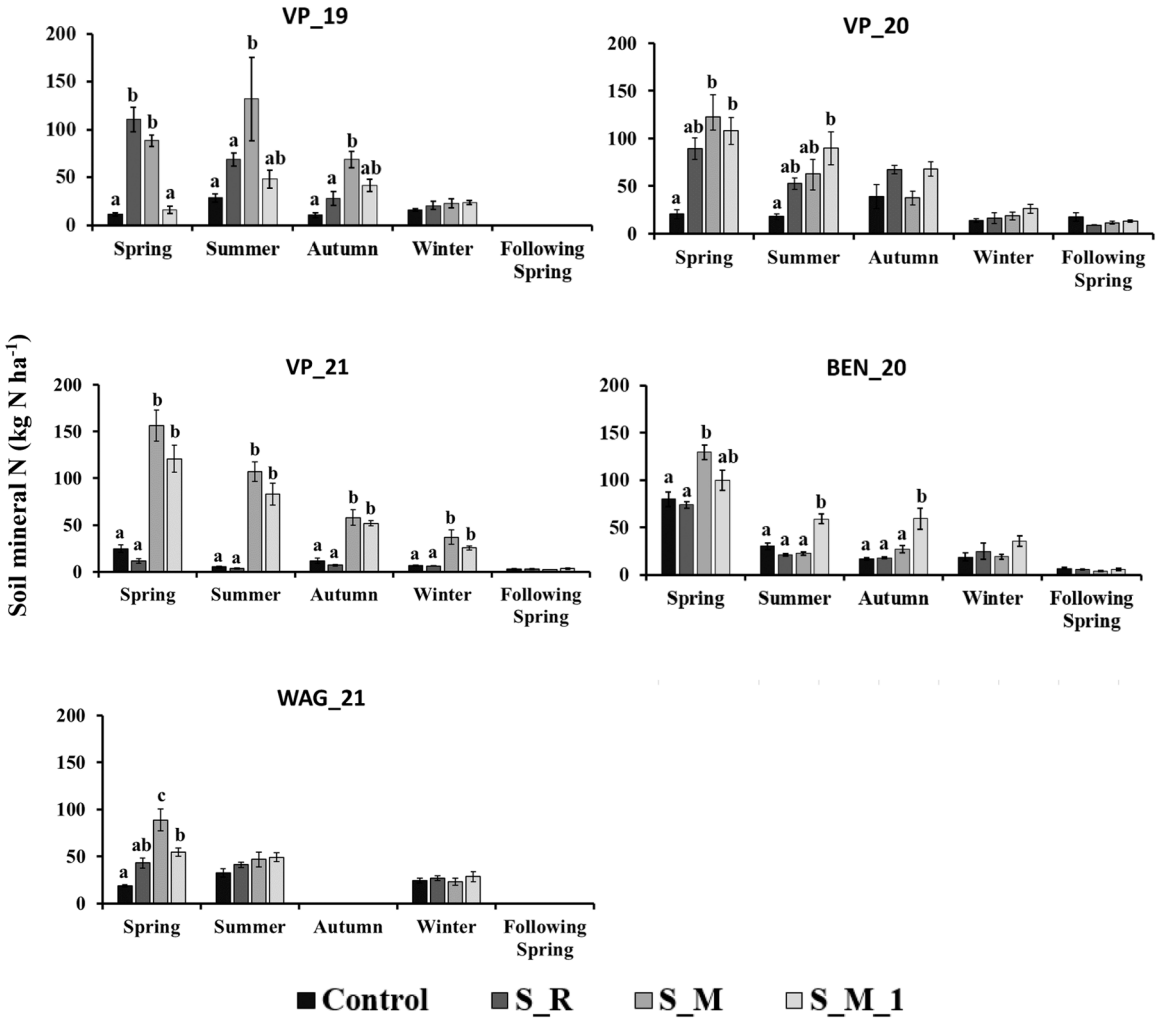

Fig. 1. Soil mineral N on five sampling dates for the control and all spring renovation treatments (S_R, S_M and S_M_1) for the 0–30 cm layer. VP = Vredepeel, BEN = Bennekom and WAG = Wageningen. Soil sampling date is shown in Supplementary data Table M. Treatment code is shown in Table 1. Statistical analyses was only performed for the four treatments. No data available for VP_19 at the following spring and for WAG_21 at autumn and the following spring. Small letters indicate significant differences between treatments per season. No significant difference was observed for columns without letters. Error bars represent the standard error.

Table 3
Average soil mineral N content (kg N ha^{-1}) in layer 0–90 cm of each treatment during autumn, winter and the following spring averaged over all available experiments. Small letters indicate significant differences between treatments and should be read vertically ($p < 0.05$). Numbers between brackets represent the standard deviation. No significant difference was observed for columns without letters.

Treatment[1]	Autumn		Winter		Following Spring	
Control	45	(25)^{a}	41	(18)^{a}	19	(10)^{a}
S_R	61	(33)^{a}	56	(23)^{a}	17	(9.7)^{a}
A_R_ST	160	(49)^{bc}	125	(48)^{bc}	29	(5.2)^{b}
A_R_RT	146	(37)^{b}	113	(35)^{bc}	33	(3.8)^{b}
A_R_ZT	137	(16)^{b}	101	(36)^{bc}	37	(20)^{b}
A_R_MD	223	(39)^{c}	154	(70)^{c}	37	(24)^{b}
A_R_N55	130	(42)^{b}	110	(45)^{bc}	35	(23)^{b}
A_R_N105	115	(32)^{b}	92	(34)^{b}	37	(16)^{ab}
A_R_NI	165	(40)^{bc}	124	(55)^{bc}	32	(14)^{b}
S_M	126	(40)^{b}	100	(26)^{bc}	27	(5.2)^{b}
S_M_1	122	(33)^{b}	101	(36)^{bc}	39	(3.8)^{b}

[1] Treatment code is shown in Table 1

to maize (S_M and S_M_1) increased the soil mineral N significantly with approximately 60–70 kg N ha^{-1} compared to the control and spring renewal (S_R). The soil mineral N content for mechanical sward destruction for weed control (A_R_MD) was significantly higher than almost all treatments. The soil mineral N content decreased over time and was lower at the winter sampling than in autumn. Sampling in the following spring showed a further reduction of soil mineral N in the 0–90 cm layer to 17–39 kg N ha^{-1} on average, with significantly lower soil mineral N for both control and S_R compared with the autumn renewal treatments, except for A_R_N105 (Fig. 2; Table 3). In the individual experiments, the mineral N concentration for the different treatments shows a similar pattern, although there are differences in terms of absolute soil mineral N content values (Supplementary data Tables O-P-Q). Comparison of the VP sites for each year shows that the soil mineral N content was higher in 2019 and 2020 than in 2021.

The decrease in the average soil mineral N content of the 0–90 cm layer between autumn and the following spring is relatively limited for the control (35 kg N ha^{-1}) and S_R (42 kg N ha^{-1}) (Fig. 2 and Supplementary data Table N). Autumn renewal without mitigation (A_R_ST) resulted in a decrease of 131 kg N ha^{-1}. Autumn renewal with mitigation strategies led to a lower loss of 111, 107, 105 and 84 kg N ha^{-1} for restricted tillage (A_R_RT), zero tillage (A_R_ZT), reduction of 55 (A_R_N55) and 105 (A_R_N105) kg N, respectively. Addition of a nitrification inhibitor (A_R_NI) did not affect the decrease in mineral N (128 kg N ha^{-1}) compared to standard tillage (131 kg N ha^{-1}). The highest decrease in soil mineral N was observed for mechanical sward destruction for weed control (A_R_MD; 172 kg N ha^{-1}). The maize treatments showed a higher average N decrease in mineral N (79 and 99 kg N ha^{-1} for S_M and S_M_1, respectively) than the control and spring renewal treatment (S_R), but mostly lower than autumn renewal. The strongest decrease in mineral N content for autumn renewal and maize was observed for the layers 0–30 and 30–60 cm (Supplementary data Table N).

3.4. NO_3 concentration in groundwater

Averaged over all experiments, the NO_3 concentrations in groundwater the year after renewal ranged from 3.1 to 29.0 mg N L^{-1} for all renewal treatments (Table 4) and for the control treatment the average NO_3 concentration was 4.4 mg N L^{-1}. Despite the relatively large differences of average NO_3 concentration among treatments, few significant differences were observed between treatments due to large variation between locations and years. There was no significant difference ($P > 0.05$) between S_R (3.1 mg N L^{-1}) and the control (4.4 mg N L^{-1}), but the NO_3 concentration for A_R_ST (17.2 mg N L^{-1}) and A_R_RT (19.4 mg N L^{-1}) were significantly higher ($P < 0.05$) than for S_R. Most of the mitigation strategies to reduce nitrate leaching, except for A_R_MD and A_R_RT, did slightly decrease the NO_3 concentration in groundwater, but no significant differences were found with the standard tillage treatment (A_R_ST). In contrast, the NO_3 concentration of the reduced tillage (A_R_RT) and mechanical destruction for weed control (A_R_MD) treatments increased significantly in comparison to control with concentrations of 19.4 mg N L^{-1} and 29.0 mg N L^{-1}, respectively.

Converting grassland to maize resulted in average NO_3 concentrations of 12.5 and 12.8 mg N L^{-1} for S_M and S_M_1 respectively, which were not significantly different from the other treatments (Table 4). The groundwater NO_3 concentration for the same treatments in the individual experiments followed mostly the same pattern: NO_3 concentrations for control and grassland renewal in spring (S_R) were lowest, followed by conversion to maize (S_M and S_M_1), and NO_3 concentration in treatments with autumn renewal was highest. However, absolute NO_3 concentration varied a lot among the experiments. For example, the NO_3 concentrations in 2021 were lower than in 2019 and 2020 and even among the same location (VP) the variation was relatively high over the years (Table 4). A significant relationship was found between soil mineral N and both N surplus ($R^2 = 0.25$) and NO_3 in groundwater ($R^2 = 0.23$) in the following spring. The relation between N surplus and NO_3 in groundwater was less clear (Supplementary data Figure B).

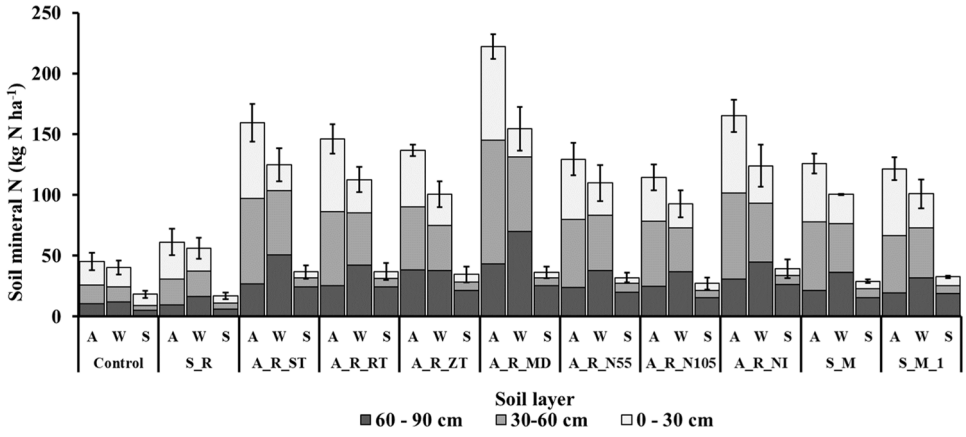

Fig. 2. Average soil mineral N content (kg N ha^{-1}) and standard error of the 0–30, 30–60 and 60–90 cm layer 0 – 90 cm layer of all locations in autumn (A), winter (W) and the following spring (S). Error bars represent the standard error for the full 0–90 cm layer. WAG_21 was not sampled in autumn and spring. VP_19 was not sampled in the following spring. Treatment codes are given in Table 1.

Table 4
Average NO_3 (mg N L^{-1}) in groundwater during the following spring for each treatment for all locations. Small letters indicate significant differences between treatments per site ($p < 0.05$). No significant difference was observed for columns without letters.

Treatment[1]	2019 VP[2] 11 Mar		2020 BEN[2] 5 Mar		VP[2] 9 Mar		2021 WAG[2] 19 Mar		VP[2] 8 Mar		Av	
Control	5.0	(7)[a]	13.0	(8)[ab]	2.6	(0)	1.0	(2)	0.4	(0)	4.4	(7)[a]
S_R	4.9	(3)[a]	3.4	(2)[a]	6.2	(1)	0.8	(1)	0.4	(1)	3.1	(4)[a]
A_R_ST	33.9	(13)[ab]	37.3	(6)[d]	9.9	(1)	1.6	(3)	3.3	(3)	17.2	(18)[b]
A_R_RT	18.4	(20)[a]	40.1	(8)[d]	22.6	(1)	5.6	(9)	10.6	(4)	19.4	(21)[b]
A_R_ZT	30.3	(15)[ab]	17.5	(10)[abc]	0.4	(1)	0.0	(0)	1.7	(1)	10.0	(14)[ab]
A_R_MD	82.7	(41)[b]	31.1	(5)[bcd]	25.2	(1)	3.7	(4)	2.5	(1)	29.0	(39)[b]
A_R_N55	29.1	(14)[ab]	17.7	(7)[abc]	8.7	(1)	1.3	(2)	4.9	(3)	13.4	(15)[ab]
A_R_N105	29.3	(18)[ab]	24.9	(7)[bcd]	10.4	(1)	3.1	(5)	4.5	(2)	14.4	(16)[ab]
A_R_NI	34.7	(30)[ab]	22.7	(9)[abcd]	0.7	(0)	4.1	(6)	2.0	(2)	12.8	(20)[ab]
S_M	29.9	(20)[ab]	14.6	(2)[abc]	4.6	(1)	0.8	(1)	13.9	(10)	12.8	(15)[ab]
S_M_1	15.6	(10)[a]	33.1	(6)[cd]	3.5	(1)	3.8	(7)	6.3	(8)	12.5	(13)[ab]

[1] Treatment code is shown in Table 1
[2] Locations of the experiments. VP = Vredepeel, BEN = Bennekom and WAG = Wageningen. Exact location is given in supplementary data (Table A)

3.5. N_2O emissions

Averaged over the two experiments where N_2O emissions were monitored, grassland renovation increased N_2O emission in all renovation treatments (Table 5), although this increase was only significant for the A_R_RT treatment. The observed peaks in N_2O fluxes are associated with agricultural management practices such as the chemical destruction of the sward, fertilization and ploughing (Supplementary data Figure D). Nitrous oxide fluxes were relatively higher in the experiment in 2021 (WAG_21) with peaks up to 3.60 mg N m^{-2} h^{-1} in comparison to maximum fluxes of 0.25 mg N m^{-2} h^{-1} in the experiment of 2020 (BEN_20), resulting in total N_2O emissions ranging from 15.4 to 46.0 kg N_2O-N ha^{-1} in WAG_21 and from 2.4 to 4.3 kg N_2O-N ha^{-1} in BEN_20 (Table 5). The large variation within treatments and between experiments resulted in little significant differences of the cumulative emission among treatments over the whole experimental period. However, analysis of daily fluxes showed significant differences between treatments directly after agricultural management (Fig. 3). The N_2O fluxes of the control treatment directly after spring renewal and subsequent fertilization were significantly higher than the S_R treatment at the BEN_20 site, but after mid-April the flux for S_R was significantly higher. For the WAG_21 site, the S_R treatment showed significantly higher fluxes than the control treatment after renewal without fertilization and after fertilization. Similarly, significant fluxes of the A_R_ST treatment were observed directly after autumn renewal without fertilization at the BEN_20 site and after renewal with fertilization at the WAG_21 site. The application of a nitrification inhibitor (A_R_NI) did not result in significant differences and results of the effect of reduced (A_R_RT) and zero tillage (A_R_ZT) were inconsistent (both not shown).

Yield-scaled N_2O emissions from grassland ranged from 11.7 to 18.4 g N_2O-N kg N in BEN_20 and from 45.8 to 134 g N_2O-N kg N in WAG_21 (Table 5). At WAG_21 the YSNE was significantly higher for mitigation treatment A_R_RT compared to standard tillage (A_R_ST). Averaged over all experiments, there was no significant difference in yield-scaled N_2O emission shown between control, renewal in spring and autumn renewal ($P > 0.05$).

4. Discussion

4.1. Effect of grassland renovation on DM Yield and N uptake

DM yields on fertilized grasslands observed in the Netherlands in 2019–2021 are in the range of 8.2–10.9 t DM ha^{-1} and maize yield in the Netherland ranged from 15.7 to 17.6 t DM ha^{-1} (Agrimatie, 2025). Typical DM yields on fertilized grasslands observed in Western Europe are in the range of 8.1–12.4 t DM ha^{-1} (Buchen et al., 2017; Reinsch et al., 2018b). The average DM yield for grassland in the present study ranged from 8.1 to 11.5 t DM ha^{-1} (Table 2) for all treatments, within the range of the previous studies. The average DM yield for maize in the present study ranged from 17.7 to 19.1 t DM ha^{-1} and was slightly above

Table 5
Average cumulative emission and yield scaled N_2O- emission (YSNE) per year and averaged over two years (BEN_20 and WAG_21). Small letters indicate significant differences between treatments. No significant difference was observed for columns without letters. Numbers between brackets represents the standard deviation (n = 4).

Treatment[1]	Cumulative emission kg N_2O-N ha^{-1}				YSNE[2] g N_2O-N kg^{-1} N				Average Cumulative emission kg N_2O-N ha^{-1}		YSNE g N_2O-N kg^{-1} N	
	BEN_20[3]		WAG_21[3]		BEN_20[3]		WAG_21[3]					
Control	2.7	(0.3)[ab]	15.4	(7.0)	11.7	(1.0)	45.8	(28)[a]	9.1	(8.6)[a]	28.8	(27.7)[a]
S_R	4.1	(1.7)[ab]	23.0	(7.2)	18.4	(7.1)	75.1	(21)[abc]	13.6	(11.5)[ab]	46.8	(34.8)[ab]
A_R_ST	3.1	(0.4)[ab]	19.6	(4.3)	16.5	(3.7)	63.6	(21)[ab]	11.4	(9.4)[ab]	40.1	(30.0)[ab]
A_R_RT	3.2	(0.5)[ab]	46.0	(9.3)	17.0	(1.8)	134	(42)[c]	24.6	(23.9)[b]	75.5	(70.4)[b]
A_R_ZT	2.4	(0.3)[a]	40.1	(3.9)	12.6	(1.8)	117	(23)[bc]	21.3	(20.4)[ab]	64.8	(58.1)[ab]
A_R_N105	2.8	(0.5)[ab]	21.3	(8.8)	16.5	(3.3)	72.6	(24)[abc]	12.1	(11.9)[ab]	44.6	(35.1)[ab]
A_R_NI	3.3	(0.2)[ab]	27.2	(9.1)	17.9	(1.3)	85.7	(34)[abc]	15.2	(14.5)[ab]	51.8	(44.4)[ab]
S_M[4]	2.8	(0.3)[ab]	34.4	(33)	-	-	-	-	18.6	(30.4)[ab]	-	
S_M_1[4]	4.3	(0.8)[b]	33.7	(15)	-	-	-	-	19.0	(19.3)[ab]	-	

[1] Treatment code is shown in Table 1
[2] YSNE is calculated as the cumulative N_2O-N emission divided by the above ground N uptake.
[3] Locations of the experiments. BEN = Bennekom and WAG = Wageningen. Exact location is given in supplementary data (Table A)
[4] YSNE statistics was not performed for the maize treatments.

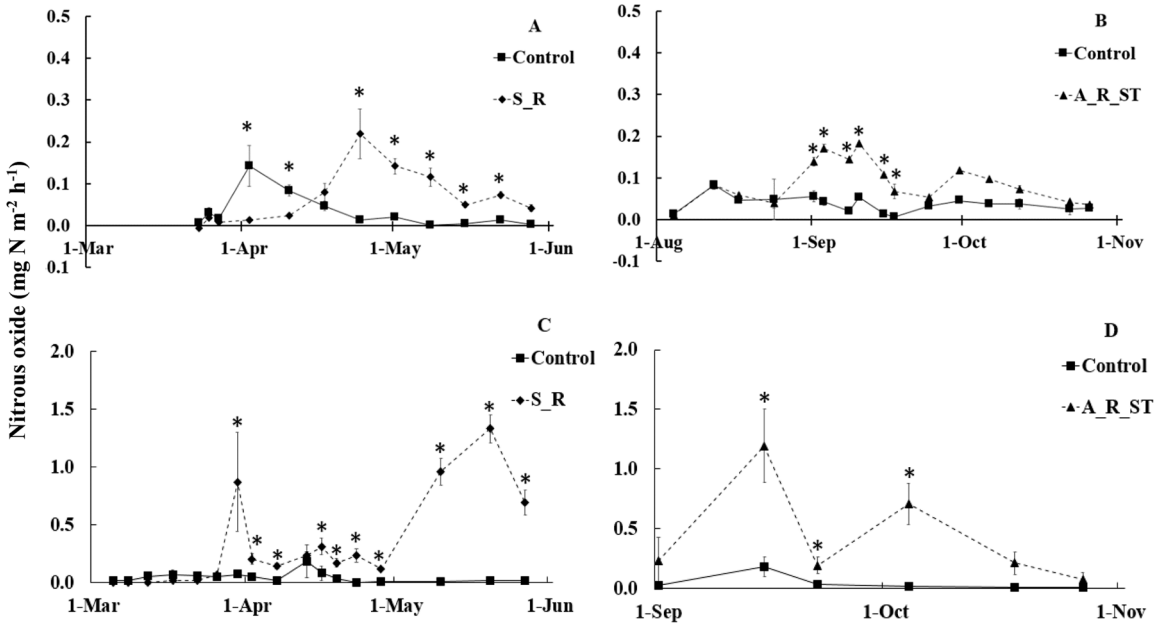

Fig. 3. Average N_2O flux (n = 4) for control, S_R and A_R_ST at BEN_20 (Fig. A and B) and WAG_21 (Fig. C and D) location before and after grassland renewal. For BEN_20, the S_R treatment was chemically destroyed on the 18th of March, ploughed on the 23rd of March and fertilized on the 16th of April. The A_R_ST treatment was chemically destroyed 19th of August, ploughed on the 7th of September and fertilized on the 8th of September. For WAG_21, the S_R treatment was chemically destroyed on the 18th of March, ploughed on the 15th of April and fertilized on the 30th of April. The A_R_ST treatment was chemically destroyed on the 3th of September, ploughed on the 8th of September and fertilized on the 9th of September (Supplementary data Table L). Asterisks show significant differences between daily fluxes. Note differences in scale of the Y-axes. Treatment codes are given in Table 1.

the previous studies. Our results show evidence that grassland renewal in spring (S_R) results in significantly lower DM yield compared to all other treatments. In the present study, the reduction in total yield in the S_R treatment can be explained by the absence of the first cut after spring renewal. The yield of the first cut is the most productive yield compared to all other cuts and can contribute up to 24–68 % of the total yield production in the whole year (Supplementary data Figure C), leading to a lower total DM yield production of the S_R treatment. However, the absence of the yield of the first cut of the S_R treatment was partly compensated by the following cuts (Supplementary data Figure C). Nevertheless, the results show that grassland renewal in spring (S_R) leads to a significant lower DM yield compared to grassland renewal in autumn (A_R_ST). Most farmers lean towards renewal in autumn because losing a period of grass production in autumn is also considered to have a smaller impact on yield than forgoing the first cut in spring (Velthof et al., 2010). The results of the present study supports this, showing that there are therefore agronomic arguments in favor of autumn renewal. The relatively low contribution of the grass cuts after autumn renewal explains why there is no difference in DM yield between control and A_R_ST.

In this study, we assessed effects of yield in the year of the renovation practices. These short-term effects may, however, be unrepresentative of the overall effect on a 5–10 year period, as grassland renewal may lead to improved yields (in both quantity and quality) than an older continuous grassland on the longer term. For example, a study of Terlikowski and Barszcxewski (2015) shows an increase of 39 % in DM yield during the first year following grassland renewal. Other studies, however, show neither an increase nor a decrease in DM yield over a two-year period (Buchen et al., 2017) or longer timeframes (Velthof et al., 2010; Biegemann, 2014).

Reducing the N application (A_R_N55 and A_R_N105) showed no significant DM yield loss compared to standard practice after renewal in autumn (A_R_ST). However, the N application planning throughout the growing season reveal that the N application rate for the first two cuts are identical for A_R_ST, A_R_N55 and A_R_N105 and from the third cut onwards the N application changes (Supplementary data Table K. Figure C (Supplementary data) shows that the contribution of the first two cuts (especially the first cut) are very high compared to the total yield. The contribution of the remaining cuts are small, but this is also when the N application rate changed. Therefore, due to the low contribution of the remaining cuts, the present study show only a small decrease in DM loss with no significant difference. According to the results of the present study, the N application may be reduced in the year of renewal as no effect significant effect on yield was observed.

Despite the significantly lower yield and lower N application rate of the S_R treatment compared to all other treatments, the N uptake was significantly higher than most of the autumn renewal treatments (Table 2), due to a higher N concentration in the grass. The high N uptake combined with a low N application rate could indicate a low loss of N through leaching (Table 4) or gaseous emissions (Table 5). Autumn renewal combined with a high N application rate (A_R_ST) resulted in similar N uptake, despite a difference of N fertilization of 175 kg N ha^{-1}, suggesting higher N losses in the treatments with autumn renewal (Table 4). Similar N uptake was also found for reduced N application rates (A_R_N55, A_R_N105), illustrating that the standard N fertilization rate is relatively high. However, as already mentioned, this could be explained by the N application planning throughout the growing season.

4.2. *Effect of grassland renovation on soil mineral N*

The soil mineral N content of the 0–30 cm layer strongly increased after renovation for several weeks to months as indicated for the S_R, treatment (Fig. 1). It was hypothesized (H1) that renewal in autumn results in higher soil mineral N content in autumn compared to spring renewal. The results of this experiment confirm this hypothesis: The soil mineral N content of the 0–90 layer of A_R_ST was 115 kg N ha^{-1} and 99 kg N ha^{-1} significantly higher than the control and S_R, respectively (Table 3). Renewal in spring resulted in a high N uptake of the new sward during growing season (Fig. 1), resulting in a similar soil mineral N of the S_R and control treatment in autumn. The N mineralization in autumn and the limited N demand of the grass at that moment induced a relatively high soil mineral N content (Fig. 2), which, considering the

lack of crop N uptake over the winter, suggests that autumn renewal increases the potential risk of NO_3 leaching. This is supported by the difference in soil mineral N content (Supplementary data Table N) in the 0–90 cm layer in the following spring compared to autumn of 131 kg N ha^{-1} (A_R_ST) and 42 kg N ha^{-1} (S_R). This difference in soil mineral N, however, only serves as an indicator for potential risk of NO_3 leaching, as there are more pathways for N losses, such as transformation to organic N and loss of N_2 through complete denitrification. Furthermore, the soil mineral N content in the 0–90 cm layer (Table 3) varies, averaged over all experiments, from 45 to 223 kg N ha^{-1} during autumn sampling. This is considered to be high; for example, a similar experiment was conducted by Velthof et al. (2010) where they measured soil mineral N contents in the 0–90 cm layer in November for grassland renewal in autumn and a control. They reported soil mineral N contents of 46–77 kg N ha^{-1} higher for autumn renewal than the control. This further shows that the N application rate was high as previously mentioned. An example of manure legislation in Flanders, Belgium, considers a soil mineral N residual of 90 kg N ha^{-1} as environmental harmful as it results in enhanced leaching (D'Haene and Hofman, 2022; Nevens et al., 2002). The difference in soil mineral N of the 0–90 cm layer in autumn per individual experiment are high (Supplementary Table O). The soil mineral N for VP_21 ranged from 19 to 159 kg N ha^{-1}, whereas the soil mineral N of BEN_20 ranged from 55 to 232 kg N ha^{-1}. The difference in soil mineral N per experiment is reflected in the yield (Supplementary data Figure C), as VP_21 show the highest yield (11.1–14.8 t DM ha^{-1}) resulting in low soil mineral N and BEN_20 show the lowest yield (5.5–7.5 t DM ha^{-1}) resulting in high soil mineral N content in autumn.

It was also hypothesized (H2) that mitigation strategies for autumn renewal would result in lower soil mineral N contents. The results of this experiment do not support this: although most of the mitigation strategies for autumn renewal led to a reduction of the average mineral N content in autumn compared to A_R_ST, they were not statistically different. For example, reduced or zero tillage results in less mixing of the sward residues in the soil and could therefore in principles lead to less aeration of the soil, and therefore reduced mineralization, but there is no statistical evidence that supports this hypothesis. Similarly, treatments with a reduced N application throughout the growing season (A_R_N55 and A_R_N105) and addition of a nitrification inhibitor (A_R_NI) also showed lower soil mineral N contents, but they were not statistically different. Regardless of the tendency of the mitigation strategies to reduce soil mineral N (Table 3) and NO_3 leaching, the soil mineral N contents in autumn are high compared to S_R and control, suggesting an increased potential risk of NO_3 leaching. Furthermore, highest soil mineral N content was observed for A_R_MD and was significantly higher than all other treatments; this is likely due to the intensive mechanical destruction of the sward (instead of chemical destruction with glyphosate) that led to more disruption of the sward and soil structure. This subsequently enhances N mineralization, suggesting a higher potential risk of NO_3 leaching than chemical destruction. This is not in line with a comparable experiment by Helfrich et al. (2020), who did not found a clear difference. The loss of soil mineral N between autumn and the following spring sampling (Supplementary data Table N) underlines the potential risk of leaching after autumn renewal: whereas the average delta between the following spring and autumn of soil mineral N is limited for the control and S_R treatment, the delta N is between 84 and 172 kg N ha^{-1} for all autumn renewal treatments.

It was also hypothesized (H3) that conversion to maize would increase the soil mineral N content in autumn compared to continuous grassland and spring renewal. In contrast to the temporary increase of the soil mineral N content of the 0–30 cm layer of the S_R treatment, the soil mineral N content of the maize treatments (S_M and S_M_1) were statistically higher than the control and S_R on most experimental sites in autumn (Fig. 1), implying an increased risk of a high soil mineral N content in autumn (Fig. 2). The soil mineral N in the 0–90 cm layer of the maize treatments was statistically higher (122–126 kg N ha^{-1}) than the control and S_R, which increases the potential risk of NO_3 leaching. The difference of the soil mineral N between the following spring and autumn sampling is 99–77 kg N ha^{-1}, indicating a high loss of N compared to the control and S_R through NO_3 leaching, but also other N pathways as previously mentioned. Similar studies indicate an apparent NO_3 leaching of 88 kg N ha^{-1} (Buchen et al., 2017) and 108–113 kg N ha^{-1} (Kayser et al., 2008).

4.3. Effect of grassland renovation on nitrate leaching

Groundwater sampling of NO_3 during the following spring confirms the potential elevated risk of NO_3 leaching in (Table 4). As soil mineral N content in autumn is an indicator for risk of NO_3 leaching to the groundwater (Velthof et al., 2010), a positive relationship has been found between NO_3 concentration in the following spring and soil mineral N in autumn (Supplementary data Figure B). A similar relationship has also been established by D'Haene and Hofman (2022).

According to the EU Nitrate directive, areas should be categorized as nitrate-vulnerable zones if the nitrate concentration in surface- and groundwaters exceeds 50 mg NO_3 L^{-1} (11.3 mg NO_3-N L^{-1}) or if there is a risk of this threshold being exceeded unless measures are taken (Delin and Stenberg, 2021; D'Haene and Hofman, 2022; European Commission, 2021; WHO, 2017). It was hypothesized (H1) that renewal in autumn increases NO_3 leaching compared to renewal in spring. The results of the present study confirms this hypothesis. Averaged over all experiments, the NO_3 concentration of S_R (3.1 mg N L^{-1}) and control (4.4 mg N L^{-1}) are similar and well below the 11.3 mg N-NO_3 L^{-1} threshold, suggesting that renewal in spring does not lead to increased risk of leaching (Table 4). On the other hand, autumn renewal (A_R_ZT) shows not only a significant higher NO_3 concentration (17.2 mg N L^{-1}) than S_R, it also exceeds the threshold as established by the EU Nitrate Directive. This shows clearly that, at least on dry sandy soils, autumn renewal should therefore be avoided for water quality reasons.

It was also hypothesized (H2) that mitigation strategies in autumn would result in less NO_3 leaching. Even though most of the mitigation treatments to reduce leaching do not show a significantly higher nitrate concentration than S_R, they all show a trend of increased leaching and they exceed the threshold (Table 4). This is probably caused by a high variation of the NO_3 concentration between individual samples and per field experiment. Our results suggest that none of the strategies were enough to constrain the NO_3 concentration below the threshold. In line with the soil mineral N content, the highest NO_3 concentration was observed for the A_R_MD treatment. Although this is not significant different compared with chemical destruction (A_R_ST), on three of the five experiments the NO_3 concentration of the A_R_MD treatment had the tendency to be higher than the chemical destruction. Recently, the use of glyphosate is approved by the EU commission for another decade after a discussion on whether the substance should be banned due to its environmental impact (Finger et al., 2023). A possible ban of glyphosate would have restricted the possibilities options for grassland management.

It was also hypothesized (H3) that conversion to maize increases the risk of NO_3 leaching compared to continuous grassland and spring renewal. Similar to the previous hypothesis, no significant differences were observed (Table 4) but the results show a tendency of increased NO_3 concentration of both S_M (12.8 mg N L^{-1}) and S_M_1 (12.5 mg N L^{-1}), thereby slightly exceeding the threshold of 11.3 mg N-NO_3 L^{-1}.

Absolute NO_3 concentrations were highly variable among the field experiments and years. This is probably due to the weather conditions (Supplementary data Table C): a relative high precipitation rate in Nov-Mar for VP_19 and Dec-Mar for BEN_20 corresponds with relative high NO_3 concentrations. Due to the high variability of the NO_3 concentrations (among the field experiments and within treatments per field experiment) observed in the following spring, the question arises what indicator is most suitable for NO_3 leaching. The soil mineral N as

measured in autumn (Table 3) or the soil mineral N difference between the following spring and autumn (Supplementary data Table N) could be a better predictor of NO_3 leaching than measuring NO_3 concentrations in the groundwater as they are much less variable.

4.4. Effect of grassland renovation on nitrous oxide emissions

It was hypothesized (H4) that grassland renewal would increase N_2O emission. Although annual cumulative emissions do not show a structural significant increase of total N_2O emission as a result of grassland renewal (Table 5), a more detailed analysis of daily fluxes directly after field operations related to renewal shows evidence that supports this hypothesis (Fig. 3). Grassland renewal has been reported to increase the N_2O flux after ploughing for 1–2 months (Buchen et al., 2017; Mori and Hojito, 2007; Velthof et al., 2010), something which is confirmed in the present study. In between the period of chemical destruction of the sward and fertilization the flux was significantly higher than in the control treatment. Similarly, fluxes of S_R were significantly higher for several weeks (between chemical destruction and fertilization) from the end of March onwards, suggesting that both chemically destroying and ploughing enhance N_2O emission. This is in line with observations from several studies on the effect of chemical destruction and ploughing on N_2O emission (e.g. Buchen et al., 2017; Reinsch et al., 2018b). Chemical destruction of the sward results in decomposition of the sward and subsequently ploughing causes a better mixing of the sward residues in the soil, as well as a better aeration of the soil (Velthof et al., 2010). Krol et al. (2016) show similar peak emissions directly after ploughing up to 1.1 mg N m^{-2} h^{-1}, which is comparable to the results of the present study. Significant fluxes were also found for S_R at the BEN_20 site and A_R_ST at WAG_21 site, but fertilization was directly applied after ploughing. Therefore, the observed fluxes can also be an effect of fertilization. Emissions were expected to be higher after renewal in autumn compared to spring renewal, because of the limited N uptake by the grass and relative wet conditions. Results show, however, that peak emissions after renewal in spring and autumn are similar (0.2 mg N m^{-2} h^{-1} at BEN_20 and 1.0–1.5 mg N m^{-2} h^{-1} at WAG_21). The cumulative emission (Table 5) cannot confirm or reject the hypothesis (H4). Several management strategies (i.e. chemical destruction of the sward, tillage and fertilization) are included in the cumulative emission and therefore it is impossible to test whether grassland renewal only results in enhanced N_2O emission. Furthermore, the cumulative emission differed by a factor 10 per experimental site and ranged from 2.4 to 4.3 kg N_2O-N ha^{-1} at BEN_20 and 15.4–46.0 N_2O-N ha^{-1} at WAG_21. Similar experiments showed that N_2O emissions can vary largely from 0 to 29.1 kg N ha^{-1} $year^{-1}$ in the first year after ploughing (Reinsch et al., 2018b). The large difference between the experimental sites is probably a results of low precipitation at BEN_20 and relatively high precipitation at WAG_21 (Supplementary data Table C). The cumulative emissions from the present study are within this range, but some treatments (A_R_RT, A_R_ZT, S_M and S_M_1) of WAG_21 are higher (Table 5). This is caused by two extreme peak events with a flux of 5.2–11.9 mg N $m^{-2}h^{-1}$ on the 16th and 23rd of June for S_M and S_M_1 (not shown). For A_R_RT and A_R_ZT, extreme peak events were observed on the 15 h of September with fluxes of 7.0–7.7 mg N $m^{-2}h^{-1}$. The difference in cumulative emissions per field experiment also explains the difference in YSNE per field experiment. Also, it must be mentioned that N_2O emissions were only measured at two of the five sites and may lead to uncertainties. N_2O is known for its high temporal and spatial variation and is affected by many factors (precipitation, temperature) and therefore, N_2O measurements at all sites would lead to accurate estimations of N_2O emissions (Butterbach-Bahl et al., 2013).

4.5. Synthesis

To counteract declines in yield and quality of the grassland that occur over time due to sward deterioration, farmers may decide to renew the grassland (Vellinga et al., 2004). In the case of renewal, most farmers lean towards reseeding in autumn instead of spring because of better establishment of the sward, lower weed infestation, and lower yield losses. However, although this study shows that there might indeed be yield benefits, these agronomical advantages are accompanied by a higher risk of environmental losses to air and water.

The present study shows that the timing (spring vs autumn) of grassland renewal has both advantages and disadvantages. As the yield was significantly lower after spring renewal, the results support the farmers' preference for autumn renewal over spring renewal due to relatively high grass production in spring (Velthof et al., 2010). However, it must be noted that this may be a short-term effect, as the yield following grassland renewal could improve (both in quantity and quality) on the long term. In contrast to spring renewal, autumn renewal leads to significant increases in soil mineral N which increases the potential risk in NO_3 leaching. The mitigation strategies included in this study do show a reduction in soil mineral N, but significant differences could not be observed. On top of that, groundwater sampling reveal that autumn renewal also leads to significant increases in NO_3 concentration in the groundwater which cannot be mitigated by any of the technical measures that were considered in this study, even though the results does cause a (non significant) reduction in NO_3 concentration. In fact, for six out of seven of the autumn renewal treatments the threshold of 50 mg NO_3 l^{-1} was exceeded. Renewal in spring did not lead to increased risk of leaching. In terms of management for environmental losses, autumn renewal should therefore be avoided. However, it must be noted that the measured NO_3 concentrations highly varies among the different experimental sites and years. On three (VP_20, VP_21 and WAG_21) of the five experimental sites the average NO_3 concentrations were mostly below the threshold of 11.3 mg N l^{-1} for all grassland treatments as well as maize treatments. This underlines the importance of repetition of similar field experiments. Furthermore, this study shows that grassland renewal results in temporary enhanced N_2O emission regardless of timing of renewal. It can be concluded that losses of N through leaching and N_2O emission can probably not be avoided completely, but this study can provide a basis for farmers as well as policy makers to determine when grassland renewal has beneficial agronomic or less harmful environmental effects.

The results of this study shows that treatments with reduced N application (A_R_N55 and A_R_N105) have the tendency to have lower soil mineral N and lower NO_3 concentrations, with indications that no loss of yield occurs on the short term. This suggests that the N application could be reduced. However, evaluation on the effect of a reduced N application on yield on the longer term is out of the scope of this paper.

Proper grassland management transcends minimizing nitrogen losses as there are more aspects to consider. Periodic renewal of grassland is considered to be important to maintain the yield and nutritional value of the grass, as well as to keep up other ecosystem services related to grass production. Additionally, permanent grassland (i.e. absence of grassland renewal) can act as a sink for carbon and may contribute to climate change mitigation and adaptation. Iepema et al. (2022) compared young grasslands (5–15 years since latest renewal) with old (>20 years since latest renewal) and shows that the carbon stock in the 0–10 cm layer was greater in old (62 Mg C ha^{-1}) compared with young grassland (51 Mg C ha^{-1}). It is well known that soil carbon is lost when grassland is transformed into arable land (Reinsch et al., 2018a), leading to an associated net release of CO_2 (Johnston et al., 2009). Grassland renewal can also significant reduce soil carbon stocks (Necpálová et al., 2014) and a long-term simulation of Reinsch et al. (2018a) indicates that renewal in spring carries a higher risk of soil carbon loss compared to renewal later in the year. Therefore, developing a basis for farmers and policy makers requires an integral approach that includes both environmental effects (carbon and nitrogen losses to air and groundwater) and agronomical effects.

CRediT authorship contribution statement

Ros Mart: Writing – review & editing, Supervision, Methodology. **van Schooten Herman:** Writing – review & editing, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Velthof Gerard:** Writing – review & editing, Supervision, Methodology, Funding acquisition. **van Groenigen Jan Willem:** Writing – review & editing, Supervision. **van Middelkoop Jantine:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **van 't Hull Jordy:** Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Gerard Velthof reports financial support was provided by Dutch Ministry of Agriculture. Herman van Schooten reports financial support was provided by Dutch Ministry of Agriculture. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.109549.

Data availability

Data will be made available on request.

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