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ORIGINAL ARTICLE

Nitrous oxide emissions from a fertile grassland in Western Norway following the application of inorganic and organic fertilizers

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Abstract In Norway, 65 % of the agricultural land is under grassland for feeding ruminants. The objective of the present study was to quantify N_2O emissions from grassland on a fertile sandy loam in Western Norway, and to estimate the response of seasonal N_2O emissions to added inorganic N, cattle slurry (CS) N and clover N. Ammonium nitrate (AN) and CS were applied manually at annual rates of 0, 100, 150, 200 and 250 kg AN-N ha^{-1}, 80 kg CS-N ha^{-1} or as a combination of 200 kg AN-N ha^{-1} and 80 kg CS-N ha^{-1}. Background N_2O emissions were five times higher in summer season 2009 than in 2010, but the relative amount of N_2O derived from AN was constant in both periods, amounting to 0.11 % of applied N. CS had no measurable impact on N_2O emissions in 2009, but 0.15 % of CS-N was emitted as N_2O during summer 2010. In the warm year of 2009, which included a drought period, 1–24 % of the N_2O emissions were attributed to the effect of clover depending on fertilization. Clover had no effect on N_2O fluxes in the cool and moist year 2010. Our results suggest that N_2O emissions in fertile Norwegian grasslands are to a great extent controlled by inter-annual variations in background emissions and variable contribution of biologically fixed N and CS-N.

Keywords N_2O · Background emission · Emission intensity · Ammonium nitrate · Cattle slurry · Clover

Introduction

Grasslands cover 22 % of agricultural land in Europe (Soussana et al. 2007) and differ widely in management, ranging from extensive cultivation with no

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reseeding and fertilization to intensive cultivation with regular fertilizer inputs, irrigation and periodic ploughing and reseeding. In Norway, 65 % of the agricultural land is under grassland production for feeding cows and sheep. Along the West coast of Norway, where grass is the dominant crop, precipitation is often high, summers are cool, and animal husbandry is dense. Animal manures (liquid or solid) are commonly returned to the fields, in addition to NPK fertilizer at total rates of up to 300 kg N ha^{-1} $year^{-1}$. Reported N_2O emissions in temperate grassland in Europe range from 0 to 51 kg N_2O-N ha^{-1} $year^{-1}$ (Clayton et al. 1997; Jones et al. 2005; Flechard et al. 2007; Kim et al. 2010; Rees et al. 2013). This large variation appears to be related to site-specific differences in soil inorganic N content and aeration (e.g. Clayton et al. 1997; Smith et al. 2012; Rees et al. 2013), which, in turn, are heavily influenced by soil type, local climate and farm management.

Emissions of N_2O increase with increasing N fertilization rates in agricultural soils (arable and grasslands; Bouwman 1996; Roelandt et al. 2005; Stehfest and Bouwman 2006). However, quantifying fertilizer-induced emissions (FIE) in mixed-species grasslands is complicated by a long growing period, absence of annual tillage, high internal N cycling with carry over between years, and the difficulty to quantify the contribution of biological nitrogen fixation. In particular, large variations in background emissions were reported in mixed swards containing legumes (Rees et al. 2013). The only study carried out on a grassland in Western Norway reported high N_2O emissions after fertilization, with highest rates in compacted soil (Sitaula et al. 2000). No whole-growing season study on N_2O emissions has been reported for Norwegian grasslands.

Nitrous oxide emissions from grasslands are often reported as N_2O-N loss per area or as percentage of N applied, without taking account of the magnitude and quality of the commodity produced. Because there is a need to minimize the emission per produced unit, N_2O emissions from forage production should also be expressed as a function of dry matter (DM) yield or foliar N-content (emission intensity, N_2O emitted as a portion of N-uptake by the above-ground crop). Schils et al. (2008) reported emission intensities of 0.29 % for a grass sward on a sandy soil that was fertilized with calcium ammonium nitrate (CAN) and cattle slurry (CS), and 0.17 % when CAN was the only fertilizer. This was substantially lower than the emission intensities of 0.8–1.5 % of total N accumulated in the above ground biomass calculated for arable crops (maize, wheat, potato, onion, rice) based on 19 studies summarized by Van Groenigen et al. (2010). Per date, it is not known whether grasslands have systematically lower emission intensities than arable crops, and how they are influenced by edaphic and management factors.

The objectives of the present study were (1) to quantify N_2O emissions in a fertile grassland in Western Norway, (2) to partition total emissions between background and emissions induced by N additions [ammonium nitrate (AN) and/or CS and biologically fixed clover N (BfcN)], and (3) to estimate the N_2O emission intensity of the grassland.

Materials and methods

Site description

The experiment was conducted in a managed grassland in its 4th and 5th year (in 2009 and 2010) situated on a south-facing slope (6 % inclination) at Tingvoll (62°54′42″N, 08°12′24″E, elevation 27 m asl), Western Norway. The long-term annual mean precipitation (1961–1990) is 1,160 mm and the mean daily air temperatures (1995–2009) is 1 °C in January and 15 °C in July. The atmospheric deposition of N in the region is <0.3 g N m^2 $year^{-1}$ (Aas et al. 2012). The soil is a well-structured sandy-loam overlaying a dense layer of marine clay, classified as a Haplic Stagnosol (Ruptic) (WRB 2006). Soil texture and relevant chemical and physical properties are given in Table 1.

The field has been under grassland for more than 20 years, harvested twice a year, ploughed and reseeded every 5–6 years. The ley consisted of grass, clover and dicotyledonous weeds (mainly dandelion, *Taraxacum officinale*). The dominant grass species were smooth meadow-grass (*Poa pratensis*), meadow fescue (*Festuca pratensis*) and timothy (*Phleum pratense*). Clovers were white (*Trifolium repens*) and red clover (*Trifolium pratense*), with large heterogeneity in coverage (Supplementary material, Table S1). There was no tractor traffic on the field during the experiment.

Treatments

The field experiment followed a completely randomized block design with seven N application rates

Table 1 Soil texture and physico-chemical properties at the study site in a fertile grassland in Tingvoll, Norway

Texture[a] and chemical properties (0–20 cm)	
Sand (g kg^{-1})	710
Clay (g kg^{-1})	70
Silt (g kg^{-1})	210
Soil pH[b]	5.8
Soil C (mg g^{-1})	44
Physical properties (7–11 cm ± SD, n = 6)	
Soil bulk density (g cm^{-3})	1.1 ± 0.1
Soil porosity (% vol)	59 ± 4
WFPS at field capacity (%)	52 ± 7
Air permeability (μm^2)	19 ± 8

[a] Soil texture was analysed according to Stokes law of the sedimentation of spherical particles as described 'by (Elonen 1971)

[b] Soil pH was measured on samples taken in spring and summer of 2009 and 2010 and in the autumn of 2009 (1:2 soil:distilled water)

replicated four times in 2 × 8 m plots. Fertilizer treatments are given as total kg N added as NH_4NO_3 (AN) in or/and CS per ha and year. AN alone was applied in treatments 100AN, 150AN, 200AN and 250AN, CS alone in the treatment 80CS (75 kg total N ha^{-1} in 2009 and 80 kg total N ha^{-1} in 2010), and AN and CS together in the treatment 280ANCS (200 kg N ha^{-1} as AN both years plus 75 kg total N ha^{-1} in CS in 2009 and 80 kg total N ha^{-1} in 2010). Fertilizers were broadcast manually on the surface of the plots. One treatment remained unfertilized as a zero-N control (0N).

Cattle slurry was applied once in spring (April 22nd 2009 and April 29th, 2010), diluted with water to 200 % of the original volume to facilitate spreading by can with a spreading plate (In total 5 dm^3 m^{-2}). The CS was obtained from a local farm and contained, on average, 6.8 % DM, 3.1 g kg^{-1} of N, 1.9 g kg^{-1} NH_4-N, 0.34 g kg^{-1} P, 4.0 g kg^{-1} of K, and had a C:N ratio of 23.5 at the time of application. The AN was spread by hand and the application was split, with 60 % applied in spring (April 22nd 2009 and May 10th 2010) and 40 % after first harvest (June 19th 2009 and June 28th 2010). The AN was applied as a mixed fertilizer containing 22-2-12 (NPK) in 2009 and 25-2-6 in 2010. The water provided by CS was not compensated for in the other treatments. However, the CS application did not significantly affect soil moisture content in 0–20 cm depth (Fisher *t* test, data not shown).

Ancillary variables

Hourly air temperature was monitored continuously in the field and rainfall was measured daily at a weather station on an adjacent field 600 m away from the trial (Table 2). Air temperature in the chambers used for gas flux sampling were monitored every minute using a data logger (USB-500, MCC, Norton, MA, USA) placed in one of the closed chambers.

Flux sampling and calculations

Soil N_2O flux was measured using static chambers adapted from Rochette and Bertrand (2008). In spring 2009, one aluminium frame (52 × 52 × 25 cm) per plot was inserted to a depth of 10–12 cm. The frames had a groove filled with water to ensure air-tight connection with 20-cm high vented aluminium chamber. An extra frame (55 × 55 × 33 cm) was added when the grasses were taller than 25 cm. A 16-mL air sample was taken from the chamber headspace at regular intervals (0, 15, 30, 45 min), using a 20-mL air-tight polypropylene syringe. The samples were injected into pre-evacuated 12-mL glass vials, (Model 10-CV-Crimp, Chromacol, Herts, UK) and analyzed by a gas chromatograph (GC) (Model 7890A, Agilent, Santa Clara, CA, US), using a 20-m wide-bore Poraplot Q (0.53 mm) column at 38 °C with backflushing and He as carrier gas. The electron captor detector (ECD) conditions were 375 °C with 17 mL min^{-1} $ArCH_4$ (90/10 %vol) as makeup gas.

Soil-surface N_2O fluxes were measured weekly during the snow free period from April 2009 to the end of August 2010. The first 2 weeks after fertilizer applications, flux measurements were carried out every fourth day. Soil surface N_2O fluxes (μg N_2O-N m^{-2} h^{-1}) were calculated according to Rochette and Hutchinson (2005):

$$F_{N_2O} = \frac{dC_{N_2O}}{dt} \times \frac{V_C}{A} \times \frac{M_m}{V_m} \quad (1)$$

where dC_{N_2O} (ppm N_2O h^{-1}) is the rate of change in gas concentration in the chamber headspace, V_c is the chamber volume (L), A is the surface area covered by the chamber (m^2), M_m is the molecular weight of N in

N_2O (28 g N mol^{-1}) and V_m is the molecular volume of the gas at the average temperature during chamber deployment (L mol^{-1}).

Calculations of cumulative N_2O losses, FIE and estimates of BfcN

Cumulative N_2O losses (g N_2O-N ha^{-1}) were calculated by linear interpolation for the following selected periods: "early spring" (before AN fertilization; 2010 only), "spring" (from AN fertilization until 1st harvest), "summer" (from 1st harvest to 2nd harvest), "fall" (from 2st harvest to 3rd harvest; 2009 only) and "late fall" (after 3rd harvest; 2009 only). The summer season was defined as the sum of spring and summer periods. Because of cooler weather in 2010, the plots were fertilized and harvested later than in 2009, resulting in slightly different observation periods between the years. The periods are shown in the footnote of Table 4.

Fertilizer-induced emissions were calculated for every period as the slope of the linear relationship between total N applied and cumulative N_2O-N emitted. The impact of N applied as NH_4NO_3 (FIE_{AN}), cattle slurry (FIE_{CS}) and non-harvested biologically fixed clover N (FIE_{BfcN}) was estimated by multiple linear regression of the overall N_2O emission response and the respective N amounts applied (Eq. 2). Background emission is given as the intercept of the regression.

$$Y = z + x_1AN + x_2CS + x_3BfcN \quad (2)$$

where Y = g N_2O-N ha^{-1} emitted in the given period, Z = g N_2O-N ha^{-1} background emission, $X_{1,2,3}$ = g N_2O-N ha^{-1} emitted per kg N applied as AN, CS or non-harvested, biologically fixed clover nitrogen (BfcN) in the previous period ha^{-1}. No correction was made for NH_3 volatilization.

Biologically fixed clover nitrogen was estimated for each period from measured clover DM and N content according to Høgh-Jensen et al. (2004) using Eq. 3:

$$BfcN = DM_{clover} \times N\% \times P_{fix} \times (1 + P_{root+stubble} + P_{transsoil} + P_{immobile}) \quad (3)$$

where DM_{clover} is the harvested clover DM in Mg DM ha^{-1}, N % the average N concentration in clover in percent of dry weight (n = 4), P_{fix} the proportion of N in the shoot DM of clover that is fixed from the atmosphere in percent, $P_{root+stubble}$ the fixed N_2 in the root and stubble, $P_{trans\ soil}$ the below-ground transfer of BfcN to grasses, and $P_{immobile}$ the BfcN immobilized in soil. The proportion of non-harvested N from BfcN was assumed to be 55 % of aboveground BfcN where $P_{root+stubble}$, $P_{trans\ soil}$ and $P_{immobile}$ were assumed to amount to 25, 5 and 25 %, respectively. DM_{clover} was measured in all treatments in both years, whereas data for N % in DM (3.8 % N for 1st harvest and 3.4 % N for later harvests) were only available for 2010. We assumed the same N concentration in DM_{clover} in 2009 and 2010 based on results from a related field trial at the same site, which found similar N contents in clover 2009 and 2010 (unpublished data). P_{fix} was assumed to be 80 % for treatments fertilized with more than 100 kg N (150AN, 200AN, 250AN and 280ANCS) and 95 % for the rest. The high level of assumed P_{fix} in plots receiving low N inputs was based on the low level of soil mineral nitrogen for these treatments (Fig. 1), and the work of Carlsson et. al (2009), who observed that in a mixed grass-clover sward, the grasses take up N more efficiently than clover, resulting in higher P_{fix} than in monoculture. In grass-clover mixtures, N-fertilization will decrease the proportion of clover before P_{fix} will be reduced, as observed by many authors (e.g. Nesheim and Øyen 1994). Our assumption that P_{fix} was not affected at the lowest fertilization levels, is supported by the findings of Oberson et al. (2013) who observed that N input of up to 150 kg N ha^{-1} did not affect P_{fix}. Of the available field trials that investigated P_{fix} in a grass clover sward, the field at Vågønes (Nesheim and Øyen 1994) was the one with climatic conditions closest to our site. They reported a P_{fix} of 95 % at the lowest fertilization levels.

Since values for P_{fix}, $P_{root+stubble}$, $P_{trans\ soil}$ and $P_{immobile}$ were based on literature values, our estimates of BfcN are inherently uncertain. We therefore estimated the effect of clover on N_2O emissions both based on percentage of clover at the end of the growth period (Supplementary material, Table S4), and based on the amount of non-harvested BfcN calculated for the previous growth period (Eq. 3, Table 5). The percentage of clover is given in Table S1 and non-harvested BfcN in Table S3. The data is split into periods in the regression analyses, so that the 2 years can be compared. We assumed that most of BfcN was released after the sward was cut, because N release is enhanced when the plant is cut, and only a small

amount of N is released from fresh residue (Carter and Ambus 2006). BfcN was not included in the regression analysis for early spring 2010, assuming that biological fixation was negligible in early spring before fertilization, and that no carry over of N from BfcN from the previous year occurred. For the spring periods (from 1st AN fertilization until 1st harvest), the non-harvested BfcN in the same period was used. Regression analyses based on clover % showed no effect of clover on N_2O emissions neither in early spring 2010 nor in the periods from spring until 1st harvest in 2009 and 2010 (Table S4).

Harvest method and analysis

Above-ground biomass was harvested manually three times in 2009 (10 June, 4 August, 18th September) and twice in 2010 (23 June, 18 August) from both a 5 m^2 sub-plot and from inside each permanent frame used for N_2O flux measurements. Sub-samples were dried at 60 °C to obtain DM yield. The botanical composition (grass, legumes and weeds) was determined for each plot by hand-sorting freshly harvested samples. For each harvest in 2010, the N content in grass samples from the permanent frame was determined for two replicates of each treatment using a ThermoFinnigan Flash EA 1112 CN analyzer (Carlo Erba, Milan, Italy).

Soil sampling and analysis

Soil samples (0–20 cm) were collected at every gas sampling date. In 2009, all soil samples from the same treatment were pooled before the analysis. In 2010, one sample per plot and gas sampling date was analysed. Gravimetric soil moisture content (u, g water g $soil^{-1}$) was determined by drying 60 g fresh soil at 105 °C for 24 h.

Water filled pore space was calculated as:

$$\text{WFPS}\ (\%) = (\text{u} \times \text{BD}) / (1 - (\text{BD}/\text{PD})) \qquad (4)$$

where BD is the bulk density (Mg m^{-3}), and PD is the particle density (Mg m^{-3}) calculated as 2.66–0.014 * ignition loss (Riley 1996).

Bulk density, soil porosity and air permeability, reported in Table 1, were determined in undisturbed 100 mL cylinders cores taken at 7–10 cm depth on 11 June 2009 shortly after 1st harvest (n = 6) and on 19 August 2010, shortly after second harvest (n = 12). Soil porosity was calculated from the ratio of bulk density (BD) and estimated particle density (PD). Air permeability was measured at −10 kPa tension (assumed field capacity), as described by (Green and Fordham 1975).

Soil mineral N (NH_4^+, NO_3^-) was extracted using 2 M KCl (1:2.5 field moist soil:KCl). Extracts were stored at −20 °C until analysis by automated colorimetry (Model QuickChem 8000 FIA+, Lachat Instruments Ltd, Loveland, CO, USA).

Data treatment and statistical analysis

After log-transformation ($y = \log_{10}(x + 10)$), measured N_2O fluxes were analysed by repeated-measures ANOVA using PROC GLM method of SAS (version 9.2, SAS statistical software, Cary, NC, USA) to test for differences between treatment and sampling dates. Multiple linear regression was used to estimate the effect of applied N as NH_4NO_3, cattle slurry or BfcN on cumulative N_2O emissions for each growth period (for details, see chapter 2.5). As the cumulative N_2O emissions and DM yields were normally distributed, no log-transformation was applied to these data. The relationship between N_2O flux and soil parameters (mineral-N (NH_4-N, NO_3-N), precipitation, air temperature and WFPS) was analysed by Pearson's correlation using PROC CORR method (SAS). Treatment means were compared using an LSD test at $\alpha = 0.05$. Differences in soil mineral N in 2010 were analyzed by ANOVA using PROC GLM method in SAS.

Results and discussion

Environmental conditions

Mean daily temperatures from April to September ranged from 4 to 23 °C in 2009 (Fig. 1d) and from −12 to 20 °C in 2010 (Fig. 2d), on average 2.9 °C warmer in the 2009 growing season than in 2010. Likewise, the average daily temperature in 2009 was higher than the long-term average (11 °C), measured at the Tingvoll weather station (Table 2). Spring came earlier than usual in 2009, whereas there was a late snowfall (2 cm) that covered the field from May 2–4 in 2010. In 2009, there was a water deficit (evaporation minus precipitation) during the summer because of high evaporation and limited precipitation (Fig. 1), whereas in the summer of

Table 2 Total precipitation and mean daily temperature for 2009 and 2010 at a nearby weather station, 600 m from the study site in Tingvoll, Western Norway

Month	2009		2010	
	Precipitation (mm)	Mean daily temp. (°C)	Precipitation (mm)	Mean daily temp. (°C)
April	19	7.8	78	3.9
May	74	10.6	72	6.4
June	83	12.5	153	9.4
July	113	15.1	88	14.0
August	188	14.6	130	14.6
September	206	11.1	85	10.0
October	125	4.3	111	7.4
November	40	4.2	33	−2.1
December	33	−0.3	3	−5.1
Sum/mean	882	8.9	753	6.5

2010, water was in excess. This resulted in greater water-filled pore space (WFPS) values in 2010 (50–70 %) as compared with those in 2009 (20–57 %) (Figs. 1d, 2d).

The winter preceding the 2009 growing season was mild and short with average monthly air temperatures during the period December to March of 0.5, 1.4, −1.0 and 3.6 °C. In contrast, the winter preceding the 2010 growing season was colder with average monthly air temperatures of −0.3, −4.4, −5.0 and 1.0 °C during the same period.

N_2O flux rates

In 2009, N_2O flux rates in the control plots (0N) varied from slightly negative values to 97 ± 15 (SE) μg N_2O-N m^{-2} h^{-1}. At one occasion (5th June 2009), the control treatment even had higher N_2O emissions than the fertilized treatments. However, this was not significant, and at all other measurement dates, N_2O emissions from the control plots are at the lower range of values or the lowest value.

Flux dynamics were characterized by short transient peaks during summer, while rates levelled off to values around 20 μg N_2O-N m^{-2} h^{-1} in the period Sept.–Oct. (Fig. 1a). The N_2O emission rates measured in the unfertilized grassland during summer 2009 are greater than those reported in many other studies (Clayton et al. 1997; Glatzel and Stahr 2001; Tilsner et al. 2003) but of the same magnitude as rates measured in intensively managed grassland by Chadwick et al. 2000 (up to 100 μg N_2O-N m^{-2} h^{-1}).

In 2010, N_2O emission rates in the control plots (0–14 ± 2 μg N m^{-2} h^{-1}) were one order of magnitude smaller than in 2009 and no pronounced N_2O emission peaks were observed (Fig. 2a). While soil mineral N concentrations in the control plots ranged from 10 to 20 mg N kg $soil^{-1}$ from April to June in 2009, they were near the detection limit throughout 2010 (Figs. 1b, c, 2b, c). Higher flux rates in 2009 than in 2010 could be explained by the mild winter and warm spring that likely resulted in early season N mineralization rates exceeding plant uptake and thus increased the availability of soil mineral N for N_2O producing processes (Fig. 1). The low emissions in spring 2010 are consistent with Roelandt et al. (2005) and Dechow and Freibauer (2011) who found in their meta-analysis of grasslands that N_2O emissions were greater after a mild than after a cold winter.

Ammonium nitrate applied in 2009 tended to increase N_2O flux, but the impact was small (Fig. 1; Table 4). Cattle slurry had no significant effect on N_2O emissions in 2009. Emissions from fertilized plots were seldom higher than from control plots in 2009, except shortly after second AN fertilization and in late July and early August, when heavy rainfall events increased WFPS from 20 to 45 % (Fig. 1). This rewetting likely increased the anoxic volume and redistributed inorganic N in the soil. We attribute the lack of N_2O response to N fertilization in 2009 to the good aeration of the soil, and to the high availability of soil mineral N (mainly NH_4-N) in all treatments (Fig. 1) after several years of intensive cattle slurry and inorganic N fertilization prior to 2009.

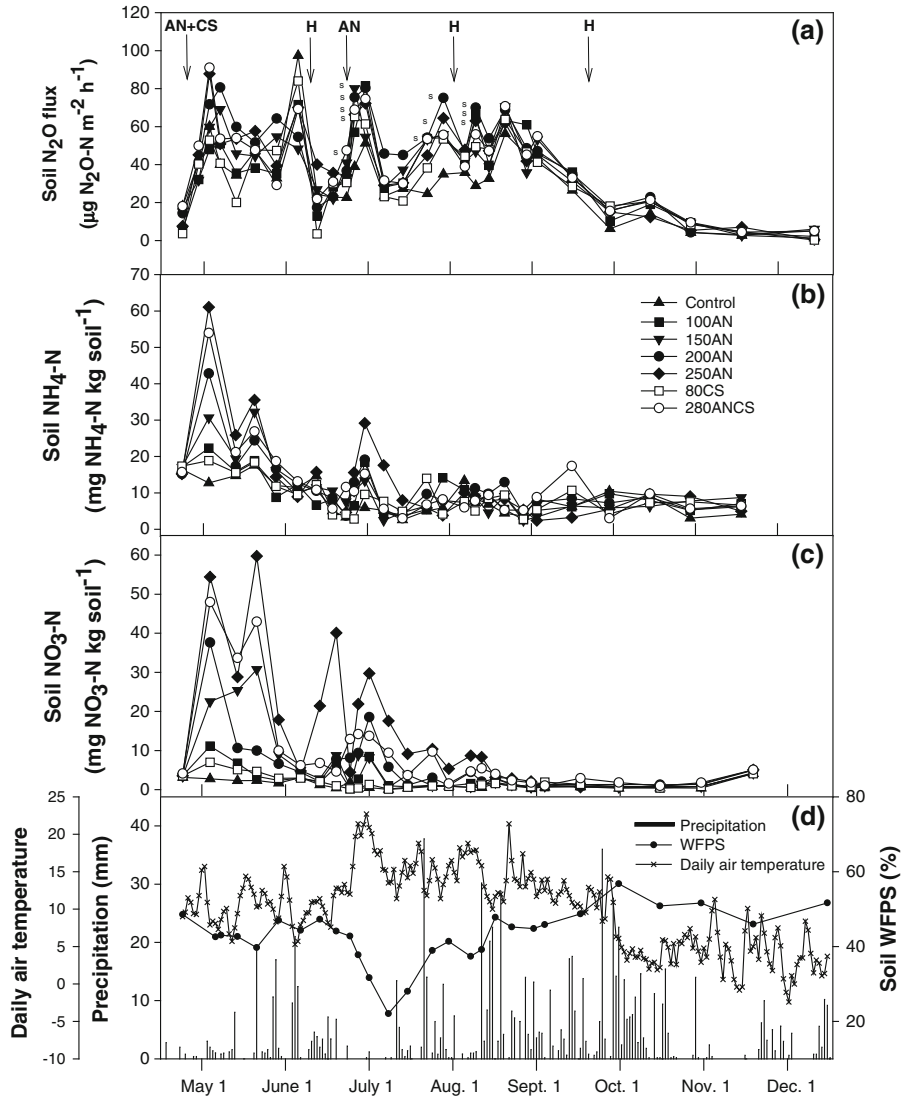

Fig. 1 Temporal dynamics of **a** N_2O fluxes, **b** soil NH_4-N concentration, **c** soil NO_3-N concentration, and **d** ancillary variables measured in fertilized grasslands in Western Norway in 2009. *Significant* differences in N_2O emission rate are marked with an **s** left of the symbol. Values are the average of 4 replicate plots per treatment (**a**) or from one composite sample per treatment on each date (**b**, **c**). Fertilizer treatments are total nitrogen added in NH_4NO_3 (AN) or cattle slurry (CS). *Black indicators* = AN = ammonium nitrate application, *white indicators* = CS = cattle slurry application, H = harvest

In 2010, emissions were highest in the treatment combining AN and CS (280ANCS) (Fig. 2). The emissions were significantly ($p < 0.05$) higher than in control plots except for the periods before fertilization in spring and before the second harvest. N_2O emissions in plots fertilized with cattle slurry (80CS, Fig. 2a) were significantly higher than in control plots only on two sampling dates right after CS application. In contrast, significantly higher N_2O emissions were observed with the highest AN fertilization rate (250 kg N ha^{-1}) up to 30 days after AN fertilization. Elevated emissions in other AN treatments were more sporadic and short-lived. It is evident that the combination of AN and freshly supplied CS enhanced the N_2O emission as emission rates in 280ANCS were significantly higher than in 250AN at three measurement dates following AN application in spring (8 days), despite the similar amount of mineral-N

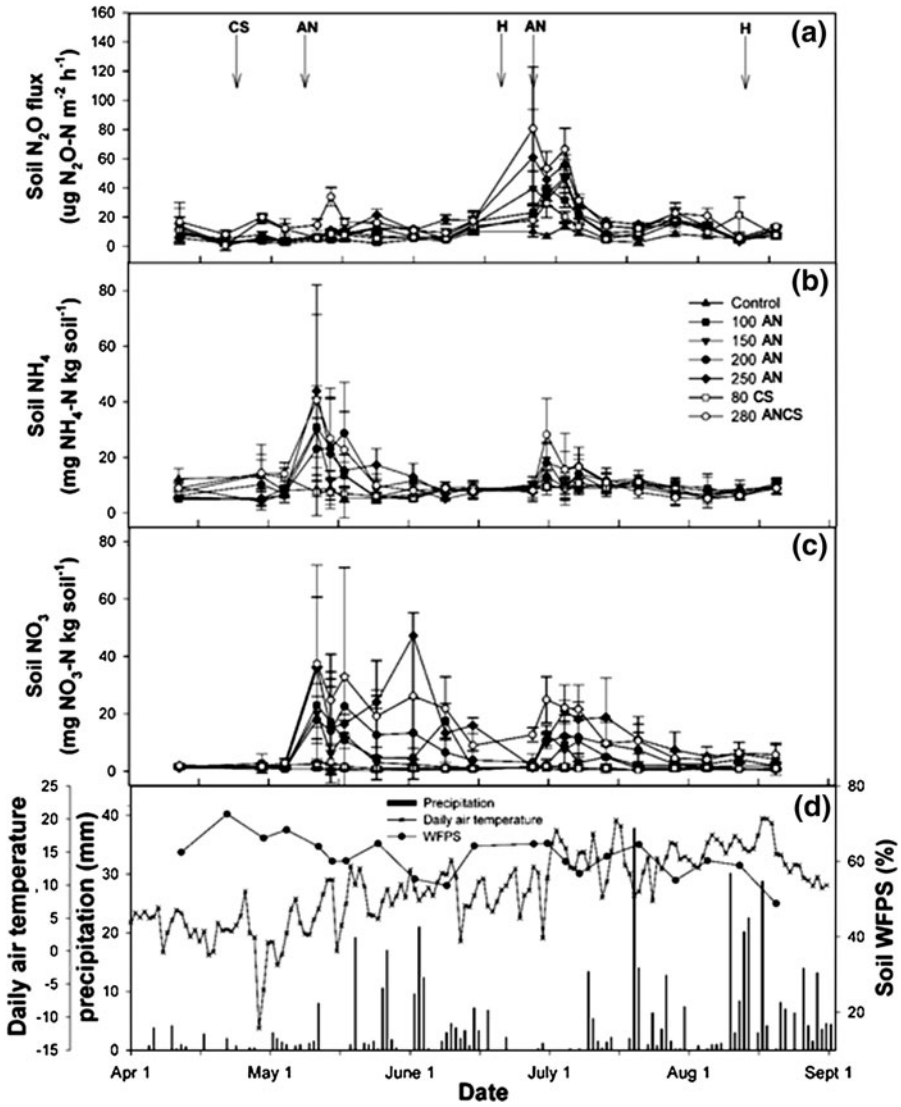

Fig. 2 Temporal dynamics of **a** N_2O fluxes, **b** soil NH_4-N concentration, **c** soil NO_3-N concentration, and **d** ancillary variables measured in fertilized grasslands in Western Norway in 2010. Abbreviations are described in Fig. 1. Values in **a**, **b** and **c** are averaged from 4 replicate plots per treatment. *Vertical lines* indicate SD

applied with the two treatments (249 kg N ha^{-1} with 280ANCS, and 250 with 250AN).

Highest N_2O emissions in 2010 were measured during a 2-week period in early July shortly after AN fertilization, reaching 80 ± 42 μg N_2O-N m^{-2} h^{-1} in the 280 N plots (Fig. 2). Higher N_2O emissions are usually measured at high soil WFPS (Davidson et al. 2000; Dobbie and Smith 2003). In our study, we found high N_2O fluxes during the drier year (2009) when WFPS never exceeded 56 %. In contrast to several previous studies (Hansen et al. 1993; Clayton et al. 1997; Smith et al. 1998; Jones et al. 2005; MacDonald et al. 2010), N_2O emission peaks were not generally related to rainfall events except in early June 2009, when the annual maximum N_2O flux occurred after 40 mm rainfall (Fig. 1a, d). On the other hand, peak N_2O fluxes in April and July 2009 coincided with a 5 °C increase in air temperature (Fig. 1d), resulting in a significant ($p < 0.0001$) but weak (r = 0.38) correlation between N_2O flux and air temperature in 2009 (Table 3). We found a negative correlation between N_2O emissions fluxes and WFPS in 2009, whereas no correlation was found in 2010 (Table 3). We hypothesize that the WFPS in our soil rarely reached the threshold value above which N_2O production from denitrification is triggered, and that nitrification was the major source of N_2O at our well-drained, sloping site. A similar negative relationship between N_2O flux

Table 3 Pearson's correlation coefficients describing the relationship between N_2O flux and soil and climatic variables in fertilized grasslands, Western Norway

Parameters	N_2O flux		
	2009	2010	Combined years
Soil			
NH_4-N	0.33***	0.25***	0.39***
NO_3-N	0.41***	0.11*	0.15***
NO_3-N + NH_4-N	0.38***	0.20***	0.29***
WFPS	−0.37***	-0.02^{ns}	−0.44***
Air temperature	0.38***	0.23***	0.31***
Cumulative precipitation	−0.20**	0.09*	-0.002^{ns}

Correlation coefficients were calculated for n = 266 in 2009 and n = 588 in 2010. WFPS = water-filled pore space, cumulative precipitation = total precipitation between each sampling date

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns = no significant effect

and WFPS was reported from a non-fertilized green manure ley in southeast Norway in 2009 (Nadeem et al. 2012), suggesting that temperature can be an important factor for N_2O emissions in boreal grasslands.

Cumulative N_2O losses and fertilizer-induced emissions (FIE)

Emissions from unfertilized plots

Cumulative N_2O emission from control plots during spring and summer were four to five times greater in 2009 than in 2010 (Table 4; Figs. 3, 4). Cumulative emissions from the control plots reached 1,230 g N ha^{-1} during the 2009 snow-free season and the emissions were greater during the summer season (spring + summer) in 2009 (700 ± 90 g N ha^{-1}) than in 2010 (140 ± 13 g N ha^{-1}). This resulted in high "background emissions" in 2009 which we attributed to mineralization of organic N built up during previous years of intensive fertilization. Average clover % in the control plots was lower than in fertilized plots in 2009 (Table S1), as was the portion of N_2O emission related to recently fixed N from clover (BfcN) (Fig. 3). Great inter-annual variation in N_2O emissions from unfertilized grasslands have been reported from other studies in humid climates (Chadwick et al. 2000; Ball et al. 2004; Schils et al. 2008). In a study of ten sites across Europe, Flechard et al. (2007) found that the yearly variation in N_2O emission from unfertilized grasslands was positively correlated with soil temperature, but not with WFPS. This is consistent with our findings. However, our site was a well-drained sandy loam, and different responses could be expected on poorly drained soils.

AN fertilisation

AN is applied twice a year on grasslands in the region, when the grass sward is 5–10 cm high in spring and after first harvest. Except for spring 2009, AN fertilisation resulted in a slight increase in cumulative N_2O emission irrespective of season (Fig. 3). The AN-induced emissions were remarkably similar between the study years, amounting to 0.11 % of applied N when cumulated for the spring and summer period. The low variability in AN-induced emissions between the years, in spite of the high variability in background emission, is consistent with the findings of Schils et al. (2008) who reported AN-induced emissions of 0.17 and 0.13 % in two following years. However, other fertilization trials with AN reported large inter-annual variation in emission response. For example, Clayton et al. (1997) found that 0.4 % of applied N was emitted as N_2O in 1993 and 1.2 % in 1994, while Smith et al. (2012) reported AN-induced emissions of 0.15 % in 2003 and 1.42 % in 2004 in one site, and emissions of 1.13 % in 2003 and 0.61 % in 2004 at another site. The site-specific responses probably reflect differences in the soil aeration due to changing weather conditions from year to year.

Including N_2O emissions from early and late fall 2009, the FIE_{AN} for our observation period was 0.21 % (Table 5), which is well below the default EF1 value of 1 % for N-input proposed for national greenhouse gas inventories using the IPCC Tier-I methodology (IPCC 2006). A comparison of N_2O emissions in European grasslands showed that annual emission factors for fertilized grasslands can vary considerably (0.01–3.65 %; Flechard et al. 2007). Contradictory to what we expected in this humid climate, our FIE_{AN} was at the lower end of this range. The reason for the low FIE_{AN} and the low variability between years in our study, despite the large variability in background emissions, could be the good

Table 4 Average daily N_2O emission in each season and cumulative N_2O emissions for 2009 and 2010 in fertilized grasslands, Western Norway

Fertilizer treatment kg N ha^{-1} $year^{-1}$	Daily N_2O emissions (g N_2O-N $ha^{-1}day^{-1}$)							g N_2O-N ha^{-1}	
	2009 sampling periods				2010 sampling periods			Total	
	Spring[a]	Summer[b]	Early Fall[c]	Late Fall[d]	Early Spring[e]	Spring[a]	Summer[b]	2009	2010
	49 days	55 days	54 days	87 days	28 days	44 days	53 days	245 days	125 days
0	6.2	7.2	7.7	1.3	3.4	1.4	1.5	1,226	234
80CS	6.1	8.5	8.9	2.5	2.8	2.0	3.4	1,465	347
100AN	6.0	10.1	9.9	2.1	2.8	2.4	3.3	1,560	358
150AN	6.2	10.5	9.1	2.5	1.8	2.6	3.9	1,590	373
200AN	6.8	12.3	10.2	2.4	0.3	2.5	3.9	1,773	329
250AN	6.3	11.0	9.3	2.0	1.9	4.1	4.9	1,590	491
280ANCS	6.7	10.8	9.8	2.6	2.5	4.5	5.6	1,678	564
$LSD_{5\ \%}$	2.1	2.5	1.8	1.3	2.0	1.2	1.3	328	165

Fertilizer treatments indicate the total nitrogen added as NH_4NO_3 (AN) and/or cattle slurry (CS) ha^{-1} $year^{-1}$. The number of days (d) in each sampling period is given under each season, and differs between the years

[a] From fertilization to 1st harvest (22nd April–10th June, 2009; 10th May–23rd June, 2010)

[b] From 1st harvest to 2nd harvest (10th June–4th Aug, 2009; 28rd June–20th Aug, 2010)

[c] From 2nd harvest to 3rd harvest (4th Aug–18th Sept., 2009)

[d] After 3rd harvest (18th Sept–14th Dec., 2009)

[e] Before first fertilization with AN (12th April–10th May, 2010)

aeration of the top soil at our study site (Table 1) which has likely resulted in little anoxic volume for denitrification throughout the year. The absence of top soil compaction by heavy machinery during the experimental period was likely an additional factor limiting denitrification. Loose and well-aerated soil provides favourable conditions for root growth, thus increasing plant uptake of applied AN probably also during the colder and wetter year 2010. The dominant role of plant N uptake for the N status of our soil is also illustrated by the fact that more above-ground N was harvested than applied as AN at most AN rates (Table S2), similar to what was the case in the grasslands studied by Schils et al. (2008). We can however not exclude loss of AN fertilizer by NO_3 leaching and run-off from this porous soil on sloping ground.

Our FIE_{AN} estimates covers the period during which the soil was not covered by snow. The absence of AN treatment effects on N_2O emissions in late fall and early spring suggests that omitting measurements from snow-covered soil was a negligible source of error in quantifying the AN-induced emissions in this trial.

Cattle slurry

Spring application of cattle slurry increased cumulative N_2O emissions in spring and summer 2010, but not in 2009 (Tables 4, 5; Fig. 3). Animal slurries are a significant source of labile C and N substrates for nitrification and denitrification and increases in N_2O have been observed following their application to agricultural soils (Rochette et al. 2000; Tilsner et al. 2003). Conversely, other studies found no effect of cattle slurry on N_2O emissions from grassland (Glatzel and Stahr 2001 and Jones et al. 2005), indicating that other factors may offset the stimulation of N_2O production by slurry C and N. A large fraction of the slurry NH_4-N can be volatilized as ammonia shortly after spraying (Beauchamp et al. 1982; Rochette et al. 2008) and the slurry-$NH4^+$ transferred to soil is likely taken up efficiently and rapidly by grasses in extensively fertilized grassland (Glatzel and Stahr 2001). We suspect that NH_3 losses were greater in 2009 (13 °C surface soil temperature, 3.1 m s^{-1} wind speed, 0.6 mm precipitation) than in 2010 (9.5 °C, 1.7 m s^{-1}, 2.2 mm) when the soil was covered by

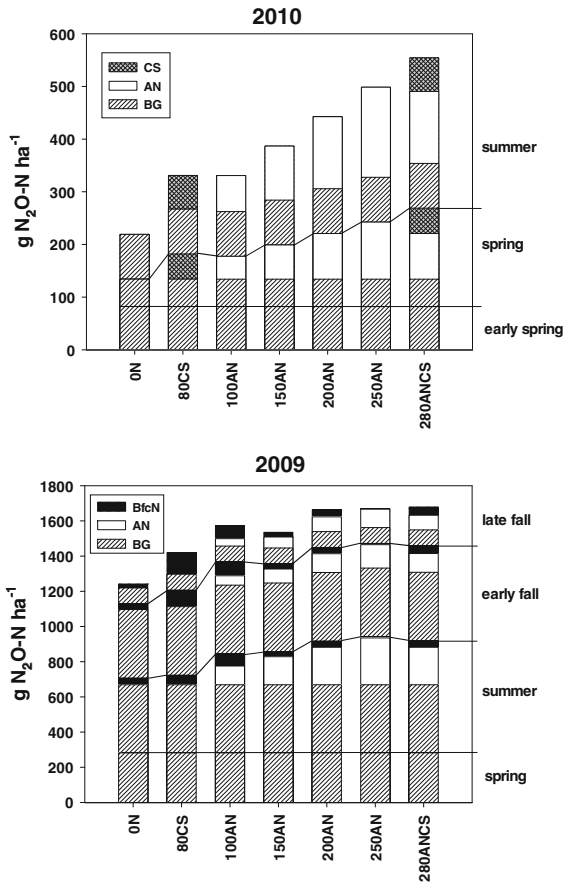

Fig. 3 Impact of N source and monitoring period on N_2O emission during the years 2009 and 2010 based on regression equations given in Table 5. The N-sources are background (BG), NH_4NO_3 (AN), cattle slurry (CS) and non-harvested BfcN. The length and dates of the monitoring periods are given in Table 4. Non-significant sources of N_2O emissions were not included in the figures. Note the difference in the scale of the y-axis in 2009 and 2010

snow for 48 h after CS application. Moreover, slurry-NH_4^+ transferred to soil was likely taken up rapidly by the grasses in the warm spring and summer 2009.

Clover

While biological nitrogen fixation per se does not increase N_2O emissions (Rochette and Janzen 2005), biologically fixed N can contribute to N_2O-producing processes after N-rich legume residues are mineralized (IPCC 2006). We found a statistically significant effect ($p < 0.05$) of clover on cumulative N_2O-emissions in 2009, but not in 2010, either calculated as BfcN (Table 5; Fig. 3) or as percent clover in herbage DM (Supplementary material, Table S4). The small variations in the intercept, as well as in the significance of clover, AN or CS on the N_2O-emissions, whether we used BfcN or clover in the regression analysis, indicate that the effect of clover was consistent and independent of the chosen calculation method. The effect of clover was greatest in late autumn 2009 when N_2O emission attributed to BfcN was greater than background emission in plots with high clover content (Fig. 3). The portion of N_2O attributed to non-harvested BfcN varied between 1 and 24 %, depending on N fertilization regime, and was probably related to pronounced drying-rewetting in July–August 2009 (Fig. 1), which may have resulted in release of N from nodulated clover roots. In 2010, when no effect of clover on N_2O-emission was found, the summer was colder, there was no drought, and the percentage of clover in the stand had decreased (Table S1). Our results from 2009 contrast Rochette and Janzen (2005) who found small or negligible effects of clover N on N_2O emissions. We surmise that the N mineralized after the harvest is rapidly taken up by grasses in mixed forage stands during the summer season, as reported by Carlsson et al. (2009). Even in pure alfalfa stands, Rochette et al. (2004) did not observe any effect of forage cuts on N_2O emissions. Our finding from 2009 suggests that clover can be a significant source of N_2O in certain years.

Yield-scaled N_2O emissions

According to expected yields for Norwegian grassland (Bioforsk 2012), our study site represents a highly fertile grassland. In both years, AN and CS fertilizers increased DM yields, but not above fertilization rates of 150 kg AN-N ha^{-1} $year^{-1}$ (Table 6; Fig. 4b), which is well below Norwegian fertilization recommendations (200–300 kg N ha^{-1} $year^{-1}$, Bioforsk 2012) for these forage yield levels.

Dry matter yields were higher in 2009 than in 2010, reflecting better growth conditions, probably due to higher mineralization rates during the warmer 2009 growing season. In addition, there were more weeds growing in 2010. We evaluated the ratio of N_2O-N emitted to DM yield to assess N_2O emission intensity relative to herbage DM production (g N_2O-N Mg DM^{-1}). To be able to compare 2009 and 2010, only the summer season is shown in Fig. 4c. When fall and late

Table 5 Impact of N source on N_2O emission estimated by linear regression (see Eq. 2 in the text) where Z is the background N_2O emission in each sampling period, X_1 = g N_2O-N ha^{-1} emitted per kg N applied as AN ha^{-1} $period^{-1}$, X_2 = g N_2O-N ha^{-1} emitted per kg N applied as CS ha^{-1} $period^{-1}$, X_3 = g N_2O-N ha^{-1} emitted per kg non-harvested BfcN in the previous period

Periods	Z:Background g N_2O-N ha^{-1}	X_1:AN g N_2O-N kg N^{-1}	X_2:CS g N_2O-N kg N^{-1}	X_3:BfcN g N_2O-N kg N^{-1}	R^2
Year 2009					
Spring	$282 \pm 66^{***}$	$0.2 \pm 0.5^{ns, a}$	$0.1 \pm 0.8^{ns, h}$	$0.9 \pm 2.9^{ns, i}$	0.01
Summer	$387 \pm 44^{***}$	$2.6 \pm 0.5^{***, b}$	$0.1 \pm 0.5^{ns, g}$	$3.9 \pm 1.9^{T, i}$	0.50
Early fall	$390 \pm 30^{***}$	$1.4 \pm 0.4^{***, b}$	$0.0 \pm 0.4^{ns, g}$	$3.6 \pm 0.4^{**, j}$	0.43
Late fall	$90 \pm 22^{***}$	$1.1 \pm 0.3^{**, b}$	$0.1 \pm 0.3^{ns, g}$	$21.8 \pm 4.3^{***, k}$	0.58
Total period	$1{,}128 \pm 74^{***}$	$2.1 \pm 0.4^{***, c}$	$0.6 \pm 0.9^{ns, g}$	$6.1 \pm 1.3^{***, l}$	0.64
Year 2010					
Early spring	$82 \pm 18^{***}$	$-0.5 \pm 0.3^{ns, b}$	$0.1 \pm 0.3^{ns, h}$		0.12
Spring	$52 \pm 17^{**}$	$0.7 \pm 0.1^{***, d}$	$0.6 \pm 0.2^{**, h}$	$-0.2 \pm 1.4^{ns, i}$	0.61
Summer	$85 \pm 24^{**}$	$1.8 \pm 0.4^{***, e}$	$0.8 \pm 0.3^{*, h}$	$2.7 \pm 2.8^{ns, j}$	0.60
Total period	$218 \pm 55^{***}$	$0.9 \pm 0.3^{**, f}$	$1.6 \pm 0.7^{*, h}$	$1.0 \pm 2.8^{ns, m}$	0.41

X_1, X_2 and X_3 are average gradient coefficients ± SE (n = 4) for AN, CS and BfcN, respectively. Probabilities that coefficients were affected significantly by fertilization are indicated with asterisks or ns = not significant. Fertilizer induced emissions: N_2O-N in percent of N added (FIE_{AN}, FIE_{CS} and FIE_{BfcN}) are the gradient coefficients for AN, CS and BfcN respectively divided by 10. The length and time of the periods are given in Table 4

[a] AN applied in spring 2009, [b] AN applied shortly after first harvest 2009, [c] Sum of the two AN applications 2009, [d] AN applied spring 2010, [e] AN applied shortly after first harvest 2010, [f] Sum of the two AN applications 2010, [g] Cattle slurry applied spring 2009, [h] Cattle slurry applied spring 2010, [i] Estimated non harvested BfcN from spring growth of ley until 1st cut of ley, [j] Estimated non harvested BfcN from regrowth after 1st cut of ley until 2nd cut, [k] Estimated non harvested BfcN from regrowth after 2nd cut of ley until 3rd cut, [l] Estimated non harvested BfcN sum of the three first growth periods, assume no BfcN in late fall, [m] Estimated non harvested BfcN sum of the two first growth periods, assume no BfcN in early spring, Probabilities: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, [T] $p < 0.1$,[ns] $p > 0.1$

Table 6 Dry matter (DM) yield in 2009 and 2010 harvested within 1 m^2 frames from fertilized grasslands in Western Norway

Treatment	DM yield (Mg DM ha^{-1})						
	2009				2010		
	First harvest	Second harvest	Third harvest	Total (±SE)	First harvest	Second harvest	Total (±SE)
0	3.6	2.0	0.5	6.2 ± 0.1	2.1	1.6	3.7 ± 0.8
80CS	5.2	2.8	0.9	8.8 ± 1.0	4.1	2.5	6.6 ± 0.5
100AN	5.1	3.4	0.9	9.4 ± 0.3	3.5	3.0	6.5 ± 0.5
150AN	6.2	3.4	0.9	10.5 ± 0.9	4.1	3.4	7.5 ± 0.0
200AN	6.1	3.8	1.1	11.0 ± 1.0	4.1	3.4	7.4 ± 0.3
250AN	6.6	4.5	1.2	12.3 ± 0.4	4.3	3.9	8.1 ± 0.3
280ANCS	6.9	4.4	1.2	12.5 ± 0.6	5.2	4.5	9.6 ± 0.6
$LSD_{5\ \%}$	1.3	0.9	0.4	2.0	0.8	1.0	1.4

Fertilizer treatments indicate the total nitrogen added as NH_4NO_3 (AN) and/or cattle slurry (CS) ha^{-1} $year^{-1}$

DM yields from 5 m^2 harvest area outside the frames were of the same magnitude, but only two harvests in 2009

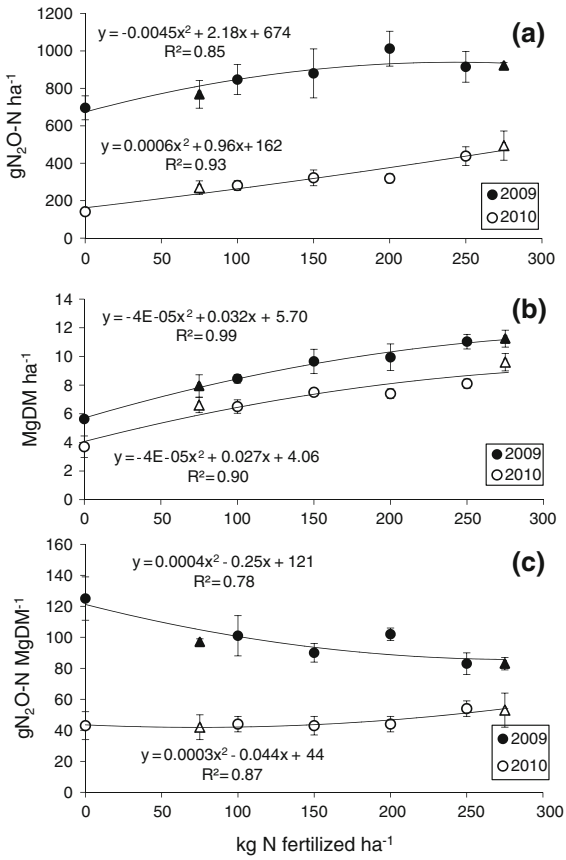

Fig. 4 **a** Cumulative N_2O-N emissions (g N_2O-N ha^{-1}) during summer season (from first AN fertilization until second harvest) in 2009 (104 days) and 2010 (97 days), **b** yield in harvested herbage dry matter (Mg DM ha^{-1}, sum of 1st and 2nd cut), and **c** emission intensity (g N_2O-N per Mg DM harvested) as a function of fertilizer N input. *Circular indicators* are fertilization with ammonium nitrate (AN), and *triangular indicators* are fertilization with cattle slurry

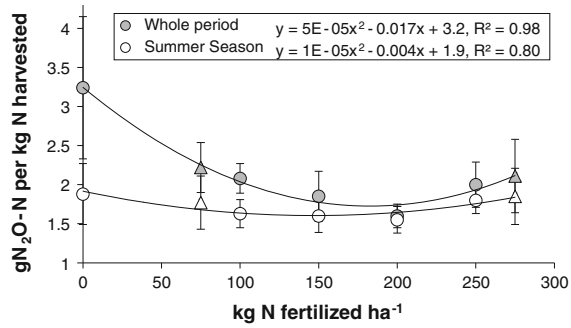

Fig. 5 Nitrogen emission intensity (g N_2O-N emitted per kg N harvested) during the 2010 monitoring period (from early spring until second harvest—125 days) and the 2010 summer season (from first AN fertilization until second harvest—97 days). *Circular indicators* are fertilization with AN, and *triangular indicators* are fertilization with cattle slurry

fall were included in 2009, g N_2O-N emitted per Mg DM was larger, but the shape of the curve was almost the same (g N_2O-N Mg $DM^{-1} = 0.0004x^2 - 0.35x + 215$, x = kg N fertilizer ha^{-1}, $R^2 = 0.84$) (Figure not shown). Cumulative N_2O emissions in percentage of the N-uptake by the above-ground crop were small (0.2–0.3 % of harvested N, Fig. 5) and were of the same magnitude as observed by Schils et al. (2008) in grassland on sandy soil. Because of scant data from grasslands, we cannot determine if the lower emission intensity from grasslands, compared to arable soils (Van Groenigen et al. 2010) is due to (1) a generally higher N use efficiency in grasslands, or (2) because the grasslands studied so far were on well aerated soils (this study and Schils et al. 2008). The quadratic shape of the observed emission intensity curve (Figs. 4, 5) is in accordance with Van Groenigen et al. (2010), who found that yield-scaled N_2O emissions decrease with N-application rate until the relationship between N uptake and N application rate levels off, and N_2O emission increases progressively with N application rate.

Conclusion

Nitrous oxide emissions measured throughout two growing seasons in a fertile, grass/legume forage sward in Western Norway showed high inter-annual variation with up to 10 times higher emission rates from unfertilized soil during a warm and dry year as compared with a cold and wet year. Fitting N_2O emissions aggregated for seasons to various fertilizer N sources (AN, CS, BfcN), showed i) that the inter-annual variation in flux magnitude was mainly due to pronounced differences in background emission, and ii) that the emissions induced by inorganic fertilizer during summer were surprisingly constant between the years (0.11 %), regardless of weather conditions. Including an estimate for the effect of clover N in regression analyses showed that clover was a significant source for N_2O in a warm-dry year, likely because of enhanced N release after drought. The effect of CS was not consistent across the years, and possibly linked to differences in ammonia losses. The observed emission intensity in this grassland was

much lower than observed in arable soils elsewhere. The good aeration and high fertility of this soil could be an important reason for this. Our data suggest high and variable background emissions in well-drained, fertile grasslands in Western Norway, while the response of N_2O emission to fertilization seems to be moderate. Together with the circumstantial evidence for clover induced emissions, this calls for caution when scaling up N_2O emissions from fertilizer-response alone.

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