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Quantifying N₂O emissions from intensive grassland production: the role of synthetic fertilizer type, application rate, timing, and nitrification inhibitors.

Short title: N₂O emissions from a fertilised grassland

M.J. BELL^{1*}, J.M. CLOY¹, C.F.E. TOPP¹, B.C. BALL¹, A. BAGNALL², R.M. REES¹ AND D.R. CHADWICK³

¹*SRUC, West Mains Road, Edinburgh, EH9 3JG, UK*

²*SRUC, Dairy Research Centre, Heston House, Dumfries, DG1 4TA, UK*

³*School of Environment, Natural Resources and Geography, Bangor University, Bangor LL57 2UW, UK*

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SUMMARY

Increasing recognition of the extent to which nitrous oxide (N₂O) contributes to climate change has resulted in greater demand to improve quantification of N₂O emissions, identify emission sources and suggest mitigation options. Agriculture is by far the largest source and grasslands, occupying *c.* 0.22 of European agricultural land, are a major land-use within this sector. The application of mineral fertilizers to optimize pasture yields is a major source of N₂O and with increasing pressure to increase agricultural productivity, options to quantify and reduce emissions whilst maintaining sufficient grassland for a given intensity of production are required. Identification of the source and extent of emissions will help to improve reporting in national inventories, with the most common approach using the IPCC emission factor (EF) default, where 0.01 of added nitrogen (N) fertilizer is assumed to be emitted directly as N₂O. The current experiment aimed to establish the suitability of applying this EF to fertilized Scottish grasslands and to identify variation in the EF depending on the

* To whom all correspondence should be addressed: madeleine.bell@sruc.ac.uk

application rate of ammonium nitrate (AN). Mitigation options to reduce N₂O emissions were also investigated, including the use of urea fertilizer in place of AN, addition of a nitrification inhibitor (NI) dicyandiamide (DCD) and application of AN in smaller, more frequent doses. Nitrous oxide emissions were measured from a cut grassland in south-west Scotland from March 2011 to March 2012. Grass yield was also measured to establish the impact of mitigation options on grass production, along with soil and environmental variables to improve understanding of the controls on N₂O emissions. A monotonic increase in annual cumulative N₂O emissions was observed with increasing AN application rate. Emission factors ranging from 1.06–1.34 % were measured for AN application rates between 80 kg N/ha and 320 kg N/ha, with a mean of 1.19 %. A lack of any significant difference between these EFs indicates that use of a uniform EF is suitable over these application rates. The mean EF of 1.19 % exceeds the IPCC default 1 %, suggesting that use of the default value may underestimate emissions of AN-fertilizer-induced N₂O loss from Scottish grasslands. The increase in emissions beyond an application rate of 320 kg N/ha produced an EF of 1.74 %, significantly different to that from lower application rates and much greater than the 1 % default. An EF of 0.89 % for urea fertilizer and 0.59 % for urea with DCD suggests that N₂O quantification using the IPCC default EF will overestimate emissions for grasslands where these fertilizers are applied. Large rainfall shortly after fertilizer application appears to be the main trigger for N₂O emissions, thus applicability of the 1 % EF could vary and depend on the weather conditions at the time of fertilizer application.

INTRODUCTION

The potential impact of nitrous oxide (N₂O) emissions on global climate is important because it is a greenhouse gas (GHG) 298 times more powerful than carbon dioxide (CO₂) and can destroy stratospheric ozone (Barneze *et al.* 2015). Agricultural expansion and intensification

have resulted in an increase in atmospheric N₂O concentrations from 290 parts per billion by volume (ppbv) to 324 ppbv between 1950 and 2011 (Smith & Massheder 2014). Agricultural soils contribute *c.* 0.40 of the 6.7 million tons N₂O-N/yr released into the atmosphere from global anthropogenic sources (Kim *et al.* 2014). Increased use of industrial fertilizers for agriculture is the main reason for the observed rise in atmospheric N₂O concentrations since pre-industrial times (Henault *et al.* 2012), with applications of nitrogen (N) in excess of crop requirements leading to losses of surplus N to the environment (Dai *et al.* 2013; Shcherbak *et al.* 2014). Although demand and fertilizer use in Europe is decreasing, it is increasing globally, with growth rates > 2.5 % in Africa and South Asia (Smith & Massheder 2014). Historically, fertilizer recommendation rates have ignored environmental impacts (Brown *et al.* 2005), however substantial research on their contribution to N₂O emissions is increasing recognition of the requirement to apply N fertilizer at a rate that will limit N₂O losses, whilst allowing optimum crop production.

Grassland occupies *c.* 0.22 of Europe's agricultural land (Hansen *et al.* 2014), and N₂O emissions from fertilized cut and grazed temperate grasslands make up > 0.10 of total N₂O emissions from global agricultural land (Jones *et al.* 2011). With increasing consideration being given to reducing GHG emissions and meeting government targets (Bell *et al.* 2014), the ability to quantify emissions and emission sources is gaining more importance. Despite the extent of the emissions from grassland, there is a notable lack of published research into the influence of fertilizer application rate and form on N₂O losses from grassland. Past studies have assessed emissions from fertilized Scottish grasslands cut for silage (Dobbie *et al.* 1999; Dobbie & Smith 2003a) and grazed (Rees *et al.* 2013), however more contemporary research would add support to that work, which was conducted 8–15 years ago. The value of the emission factor currently recommended for use in Tier 1 inventories (EF1) is a 'static' 1 % (IPCC 2006) when quantifying N₂O emissions from

fertilized grasslands and producing GHG emission inventories for the UK, but recent studies indicate non-linear relationships between N inputs and emissions (Grant *et al.* 2006; Zebarth *et al.* 2008; Cardenas *et al.* 2010; Kim *et al.* 2014). Those studies, along with others investigating the relationships between soil, environmental conditions and N₂O emissions (Dobbie *et al.* 1999; Skiba & Smith 2000; Dobbie & Smith 2003b; Lesschen *et al.* 2011) all contribute to the evidence base from which the appropriateness of the standard ‘static’ IPCC 1 % value can be judged. Higher emissions are also often observed from fertilized grasslands than croplands (Fowler *et al.* 1997; Skiba & Smith 2000) as a result of grasslands generally being located in wetter areas and on soils with high organic carbon contents, factors not accounted for in the current standard ‘static’ EF approach.

The main attraction and advantage of the IPCC’s EF1 approach to calculating N₂O emissions from fertilized soils is its ease of use (Skiba & Smith 2000; Kim *et al.* 2014) and the limited amount of input data required compared to alternative modelling approaches (Gao *et al.* 2011). Spatial and temporal variability in fluxes, however, and variation in the proportion of N emitted depending on N input rate make this approach questionable. The default EF of 1 % is based on evidence presented in Bouwman *et al.* (2002a, b) and Stehfest & Bouwman (2006), and assumes a linear relationship between N application and N₂O emissions, where 1 % of all N applied to the field in the form of synthetic fertilizer is subsequently released direct to the atmosphere as N₂O. There are calls for a move towards N-input-dependent EFs to replace the current value of 1 %, which has been claimed to be ‘too conservative’ for high synthetic N input rates (Shcherbak *et al.* 2014) and emissions will be overestimated for low synthetic N input systems (Kim *et al.* 2014). If more evidence can be gathered to illustrate an exponential relationship between N application and N₂O emissions then a case to make this EF value dependent on synthetic N input could be made.

In addition to concerns over the influence of N input rate on N₂O emissions, the chemical form of synthetic fertilizer N and the influence of soil water-filled pore space (WFPS) and rainfall at the time of fertilizer application raise further questions. Wide annual and seasonal variation in emissions can result from weather conditions, indicating that further measurements to supplement short-term studies or those undertaken in extreme weather (Burchill *et al.* 2014) are needed. Although many studies are in agreement with regard to environmental controls on N₂O emissions, there is still uncertainty and site variations in many of these relationships. Dobbie & Smith (2003b) report a strong relationship between increasing N₂O emissions and increasing WFPS, however, Burchill *et al.* (2014) observed low emissions following high rainfall and high soil WFPS as a result of saturation of heavy-textured soils.

With respect to the type of synthetic fertilizer, although Lesschen *et al.* (2011) found no variation in N₂O emissions when they analysed a dataset from Stehfest & Bouwman (2006), other studies have shown greater emissions from nitrate than ammonium-based fertilizers and urea (Dobbie & Smith 2003a; Smith *et al.* 2012; Smith & Massheder 2014). If N₂O emissions are dependent on the type of N fertilizer then this has implications not only for emission calculation and inventory production, but could also indicate potential N₂O emission mitigation options. Another mitigation option currently being considered is the use of nitrification inhibitors (NIs), mixed and applied with different N sources or sprayed directly on soils. Reported results vary depending on the type of experiment (field or laboratory), the time of year, soil and weather conditions (Barneze *et al.* 2015) and the type of fertilizer applied (McTaggart *et al.* 1997; Merino 2001), with more research specific to grassland soils in Scotland required.

The large impact of fertilizer N on N₂O emissions and control over the application of fertilizers provides much scope for altering agricultural management practices to reduce

emissions (Brown *et al.* 2005). For this change in management to take place a strong evidence base presenting the reductions in emissions achievable is required. It is predicted that wetter autumns and winters will become more frequent in the future, with wetter soil conditions increasing the extent of soil N₂O emissions (Kim *et al.* 2014). This threat of greater N₂O emissions combined with the ability to manage agricultural systems and therefore the extent of these emissions highlights the need for further research into current N₂O mitigation techniques.

The current study measured N₂O fluxes from a fertilized grassland cut for silage in Scotland (representative of a grassland cut for silage and subsequently grazed in rotation) for one year, with intensive daily measurements after fertilizer application. Use of a fully replicated experimental design captured spatial variations and identified relationships between N₂O and driving soil variables. The study measured emissions generated from the application of synthetic N fertilizer to soil, to allow direct comparison to the IPCC 1 % EF. Although measured emissions can be considered to represent those generated after N application to grasslands cut for silage and/or grazed, the absence of compaction and trampling from grazing animals in the current cut grassland experiment means that extrapolation of these results to grazed land must be made with caution. Emissions of N₂O from N deposited in the form of dung and urine from animals grazing grasslands fertilized with synthetic N are calculated separately, and would be added to those measured from synthetic fertilizer addition.

The current research is part of a UK-wide study, with the aim of improving the evidence base on which grassland EF values for application of inorganic N fertilizers are constructed. The aim is to help identify variations in and controls on N₂O emissions from grassland soils amended with different forms and rates of fertilizer N. This will enable better quantification of emissions on a UK and potentially global scale, and identify potential

drawbacks with the current EF approach. Investigation into the use of dicyandiamide (DCD) as a NI, more frequent (but smaller) applications of fertilizer, and the role of environmental conditions in emission generation will also help identify potential mitigation options to reduce N₂O emissions from this agricultural land-use.

MATERIALS AND METHODS

Field site and experimental design

The field experiment was located at Crichton Royal Farm, Dumfries (55° 02' N, 3° 35' W) in south-west Scotland, UK, on a long-term permanent grassland site. It is one of a network of sites in the UK where comparative studies on grass and arable land with the same treatments (Hinton *et al.* 2015) were undertaken, and others where emissions were measured from animal excreta (Bell *et al.* 2015). Each site was located on a different soil type and in areas with contrasting climates, with the sites chosen following a geographical assessment of UK agricultural land under a range of soil/rainfall zones, and a 'gap analysis' to identify zones that were lacking in current or planned experimental data. No animals were present in the field throughout the experiment period to allow N₂O emissions from fertilizer application to be measured and assessed without the possibility of inadvertently measuring emissions from the deposition of dung and urine patches, the subject of another experiment at this site in 2013 (Bell *et al.* 2015). There was no history of long-term organic manure applications and no manure applications or grazing 6 months prior to establishment of the experiment. The Crichton site is representative of a wet climate zone, with a 30-year (1971–2000) long-term average rainfall of 1140 mm, and mean annual temperature of 9.1°C. The soil is a free-draining sandy to sandy-loam, with organic matter (OM) content of 48–73 g/kg.

The experimental period ran from March 2011 to March 2012, where a control and nine fertilizer application treatments were tested (Table 1). The treatments were replicated in

a randomized block design, with three replicate blocks. Emissions of N₂O were measured from five static closed chambers per plot, providing a total of 15 N₂O measurements per treatment on each sampling occasion. In compliance with IPCC guidelines (IPCC, 2006) the N₂O measurements were made for a 12-month period to determine annual EFs. Plot sizes were large enough (16 × 2 m) to allow dedicated areas for N₂O chamber placement, soil sampling, grass cutting and yield measurements. The yield measurement areas were 15 m² per plot and they were not disturbed apart from during the fertilizer applications.

Fertilizer application rates and dates

The recommended annual fertilizer application rate for ammonium nitrate (AN) at this site (320 kg N/ha) was based on guidance given in Defra's Fertilizer manual (RB209; Defra 2010) for a three-cut grass silage system to achieve an indicative yield of 10–11 t DM/ha. This recommended rate was then adjusted to produce fertilizer treatment levels both above and below the recommended rate (Table 1) to allow assessment of varying fertilizer rates on EFs. All but one of the fertilizer treatments was applied in four doses at rates and timings based on commercial practice, but adjusted when necessary depending on seasonal conditions. To assess the difference in N₂O emissions between forms of fertilizer N, the same recommended fertilizer rate was also applied as urea, in the same doses and timings as AN. The NI DCD was applied along with AN and urea at their recommended rates to assess its potential to mitigate N₂O emissions from both forms of fertilizer. A further treatment was added to assess the impact of smaller but more frequent AN fertilizer applications, where each dose of AN was applied in smaller quantities; on six occasions rather than four (Table 1). For the inhibitor treatment, DCD was applied as a 2 % solution at a rate equivalent to 10 kg DCD/ha within 1 h of fertilizer application using a knap-sack sprayer. As DCD contains

650 g/kg N the amount of AN or urea applied to these plots was reduced to match the total amount of N applied (Table 1).

Nitrous oxide emission sampling and flux calculation

Nitrous oxide emissions were measured using the closed static chamber technique described in Chadwick *et al.* (2014), with all gas sampling events undertaken between 10:00 h and 12:00 h on each sampling occasion to minimize variation in N₂O flux resulting from diurnal variation. At the beginning of the experimental period, five circular chambers made of opaque polypropylene (400 mm diameter, 300 mm height and soil surface area coverage of c. 0.126 m²) were placed on each plot and inserted 5 cm into the soil. Daily gas samples were taken on ten occasions over the first 2 weeks after fertilizer application. Sampling frequency was reduced to 2 days per week for the following 3 weeks. A fortnightly sampling strategy was then implemented for the next 5 months (or until the next fertilizer application) and reduced to monthly sampling for the remaining 6 months. This sampling strategy was followed after each split fertilizer application, reverting to the start of the strategy after each subsequent application. In addition, one set of background N₂O measurements was taken in the week prior to application. Prior to chamber sampling five ambient air samples were collected, representative of N₂O concentration at time zero. Lids were placed onto chambers, sealed, and left in place for 40 min. At the end of the 40-min closure period a 50 ml sample of gas was extracted from each chamber using a syringe, through a valve with a three-way tap. Each gas sample was transferred to a pre-evacuated 20 ml glass vial so that it was under pressure. A further five ambient samples were taken at the end of the closure period and analysed along with the five samples from time zero to provide an average ambient air concentration. The order in which the blocks were sampled was randomized each day, to avoid any further bias from diurnal variation.

Following transportation back to the laboratory, prior to analysis, a needle was used to release excess pressure within the vials. Gas samples were analysed for N₂O concentration in the laboratory using an Agilent 7890A Gas Chromatograph (GC) fitted with an electron capture detector (Agilent Technologies, Berkshire, UK), with an N₂O detection limit of 0.025 ppmv. The GC response was calibrated using certified standard N₂O gas mixtures with N₂O concentrations of 0.35, 1.1, 5.1, and 10.7 ppmv. Nitrous oxide flux from each chamber was calculated by measuring the difference between chamber headspace concentration at the end of the 40-min closure period and that of the average concentration in ambient air samples. The large number of chambers used in the current study meant that the collection of N₂O samples at time zero for every chamber, followed by samples after 40 min closure was not practical. Ambient air samples were used as a surrogate for time zero samples, to allow the number of chambers to be maximized and the spatial variability in N₂O emissions to be captured. The assumption that ambient air is representative of time zero chamber headspace N₂O is based on analysis of data from eight experimental sites in the UK covering a range of soil types, environmental conditions and N fertilizer applications, in which there were no significant differences between time zero and ambient air samples in 0.56 of the cases assessed (Chadwick *et al.* 2014). A lack of any particular bias when a significant difference was observed meant that the use of ambient air to represent time zero N₂O was recommended (Chadwick *et al.* 2014). The assumption of linear gas accumulation in the chamber over the 40-min closure period is also based on this recent detailed assessment and evidence of linear accumulation provided in Chadwick *et al.* (2014) from experiments undertaken at this site and others across the UK. Details of other investigations that provide evidence of linear gas accumulation in similar static chamber experiments with a closure time of *c.* 40 min is provided in Dobbie *et al.* (1999).

The N₂O flux was calculated using N₂O concentration, chamber height, the ideal gas law, and the air temperature and chamber closure time. These details were entered into a standard spreadsheet used by all sites in the UK GHG Platform Project. The mean flux for the five chambers for each plot was calculated and then used to derive the mean flux and standard error (S.E.) for each treatment on any sampling occasion. Plot values rather than individual chamber values were used in all statistical analysis to avoid pseudo-replication. Cumulative fluxes were calculated by interpolating the area under the curve between sampling points and a mean cumulative flux and S.E. was calculated for each treatment using plot means. Emission factors were calculated by subtracting the cumulative emission from the control treatment in each block from the cumulative emission from individual treatments in the same block, as in the IPCC methodology, displayed in Eqn (1).

$$EF = \left(\frac{\text{Cumulative } N_2O \text{ flux (kg } N_2O\text{-N)} - \text{cumulative } N_2O \text{ flux from control (kg } N_2O\text{-N)}}{N \text{ applied (kgN)}} \right) \times 100 \quad (1)$$

Grass yield and nitrogen uptake measurements

A cut of grass was taken on three occasions over the experimental period, from the 15 m² yield measurement area on all of the treatments and control plots. The date of the cut was determined by the weather and when the grass reached the height at which it would be cut in normal practice in a grass-silage system. To allow conversion of the fresh yield to dry matter yield the sample of grass was dried to a constant weight at 65°C and weighed. Although not used to measure yield, grass within the individual chambers was cut when it reached the height of the chambers to enable lid closure and gas sampling to continue. This grass was cut to a height of 4 cm and the clippings were removed from the chamber.

Soil mineral nitrogen

Soil samples were collected on 15 occasions during the experimental period and coincided with N₂O emission measurements. A delay in soil sampling meant that no soil samples were collected for mineral N determination until after the second dose of fertilizer had been applied (fourth dose of the split fertilizer treatment), with no samples taken from the AN 240 and AN 320 treatments throughout the whole experimental period. The soils were collected weekly in the month following the remaining fertilizer applications, with frequency then reduced to one sample every 4–7 weeks for the remaining period. The measurements were made on one representative bulked sample from each plot, made up of five random samples from the 0–10 cm soil layer of the plot. These samples were analysed for NH₄⁺-N and NO₃⁻-N by colorimetric analysis (Singh *et al.* 2011), using a Skalar SAN⁺⁺ segmented flow analyser, after 2M potassium chloride (KCl) extraction of a sieved (< 4 mm) sample, with a soil: extractant ratio of 1:2.

Meteorological and additional soil data

A meteorological station was set up to record daily precipitation and minimum, maximum and mean air temperature. Five random soil samples from each block were collected from the 0–10 cm soil layer to enable the determination of gravimetric soil moisture content. These samples were bulked for each block, resulting in a total of three soil moisture measurements for every day of sample collection, with the measurements taken on each day of gas sampling. Additional soil samples from the same depth were also taken to measure bulk density on two occasions during the experimental period, enabling conversion of the gravimetric soil moisture values to volumetric soil moisture. A particle density value of 2.65 g/cm³ was assumed (Elliott *et al.* 1999) and used to convert volumetric soil moisture to %WFPS. Measurements of soil field capacity and permanent wilting point, pH (in water), extractable phosphorus (P), potassium (K), sulphur (S) and magnesium (Mg), total N, total

organic content (TOC) and particle size distribution were also made at the beginning of the experiment. This site characterization data was used to investigate the influence of these soil properties on N₂O emissions and to provide input data for future mechanistic modelling of N₂O fluxes.

Data analysis

All statistical analysis was undertaken using GENSTAT (GenStat 16th Edition. Release 16.1., VSN International Ltd., Oxford, UK). The measured data were separated into two groups, with each group analysed to allow assessment of the impact of AN rate, the addition of DCD, the application of fertilizer in smaller, more frequent doses and the difference resulting from urea *vs.* AN. Data was grouped and analysed as follows:

1. To assess the impact of AN rate: data analysed = Control, AN 80, AN 160, AN 240, AN 320, AN 400
2. To assess i) the impact of DCD addition, ii) the impact of smaller more frequent fertilizer applications, and iii) the difference between urea and AN: data analysed = AN 320, AN 320 + DCD, urea 320, urea 320 + DCD, AN 320 6 splits

Initial analysis of annual cumulative emissions, EF and grass yield was carried out using a mixed model and the restricted maximum likelihood (REML) algorithm. The random effect model was block. The effect of treatment was tested using the Wald statistic. For all analysis the data were transformed when necessary, to more closely satisfy the assumption that residuals and random effects were normally distributed. The type of transformation varied depending on which gave the better fit when normality of the residuals was analysed. When assessing the impact of AN rate the cumulative N₂O emissions were transformed using a Box-Cox transformation (Atkinson 1985), to determine the most suitable value of λ to use in the transformation (where $\lambda = 1$ indicates no transformation, 0 = log transformation and 0.5

= square root transformation). The equation used in the transformation is shown below Table 2. Residuals for the EF and yield data were normally distributed and did not require transformation. When assessing the impact of DCD, application of AN in smaller more frequent doses, and the form of N fertilizer, the cumulative N₂O emissions and EFs were both transformed using a Box-Cox transformation, with the equations used in the transformations displayed below Table 3. Residuals for the yield data were normally distributed and did not require transformation. Results were considered statistically significant at $P < 0.05$. The REML regression analysis was also undertaken on the AN rate data, to identify the relationship between AN application rate and N₂O emissions. Block was included as a random effect in this analysis.

RESULTS

The effect of ammonium nitrate application rate

Annual cumulative nitrous oxide emissions

There was a significant difference in annual cumulative N₂O emissions between AN fertilizer rates ($P < 0.001$, Table 2) and a general increase with AN application rate. Significantly lower ($P < 0.001$) mean emissions were measured from the control than from any of the AN treatments and from the lowest AN fertilizer application rate (AN 80) than from any of the higher rates. Although higher emissions were measured from the AN 240 than from the AN 160 treatment this difference was not statistically significant. Emissions from AN 320 were significantly higher ($P < 0.001$) than from AN 160, though not so from the AN 240 treatment. Emissions from AN 400 were significantly higher ($P < 0.001$) than emissions from any other AN application rate. REML regression analysis of Box-Cox transformed N₂O cumulative emissions for the AN rate treatments revealed a significant ($P < 0.001$) non-linear relationship between N application rate and N₂O emissions (Eqn 2).

$$\text{Box-Cox } N_2O \text{ (kg } N_2O\text{-N/ha)} = 0.35 (\pm 0.133) + 0.0049 (\pm 0.00032) \times N \text{ application rate (kg N/ha)} \quad (2)$$

Emission Factors

Annual EFs differed significantly between AN application rates ($P < 0.05$, Table 2), indicating that the use of one constant value for any AN fertilizer application rate is not suitable. However, a significant difference ($P < 0.05$) between EFs was only observed between the highest application of 400 kg N/ha and all rates < 320 kg N/ha, with no significant difference between the EFs measured for AN 80, AN 160, AN 240 and AN 320. The mean EFs for AN treatments AN 80, AN 160 and AN 240 were slightly higher than the IPCC default EF of 1 % (Table 2), but lower than the recently adjusted value of 1.25 % used in the national inventory until 2015 (IPCC 1997), suggesting that this adjustment was worthwhile. The measured EF for AN 320 (recommended fertilizer application rate for this soil and locality) was 1.34 %. The non-significant difference in EFs between fertilizer application rates up to 320 kg N/ha reflects the general increase in mean annual cumulative emissions with increasing N application rate. The EF of 1.74 % for AN 400 is much higher than the IPCC default of 1 %, and its significant difference ($P < 0.05$) from the EFs for lower AN application rates corresponds to the disproportionate increase in annual cumulative emissions once the AN application rate exceeds 320 kg N/ha.

Grass yield and yield intensity

Annual grass yield differed significantly between AN application rates ($P < 0.001$, Table 2). The grass yield from the control treatment was significantly lower ($P < 0.001$) than from any of the AN application rates. The yields from AN 80 and AN 160 were significantly lower ($P < 0.001$) than those from subsequently higher AN applications. There was no significant difference in the yields among 240, 320 and 400 AN application rates. When cumulative N_2O

emissions were expressed as a fraction of grass yield in a calculation of yield intensity, the lowest yield intensity of 0.27 kg N₂O-N/t DM was produced from AN 80. The yield intensities for AN application rates of 160 kg N/ha (0.33 kg N₂O-N/t DM) and 240 kg N/ha (0.40 kg N₂O-N/t DM) were very similar to the control of 0.35 kg N₂O-N/t DM. Greatest yield intensities were produced from AN 400 (0.74 kg N₂O-N/t DM) and from AN 320 (0.52 kg N₂O-N/t DM).

The impact of split fertilizer application, fertilizer type, and the addition of dicyandiamide

Annual cumulative nitrous oxide emissions, emission factors and grass yield

Annual cumulative N₂O emissions differed significantly ($P < 0.01$, Table 3) between fertilizer treatments applied at a rate of 320 kg N/ha. Emissions from urea 320 + DCD were significantly lower ($P < 0.01$) than from AN 320 + DCD, AN 320 and AN 320 six splits. There were however no significant differences in emissions between AN 320 and urea 320, between AN 320 and AN 320 + DCD, between urea 320 and urea 320 + DCD, or between AN 320 and AN 320 six splits. Statistical analysis of the EF data revealed the same significant and non-significant differences between treatments as for annual cumulative emissions (Table 3). Grass yield data (Table 3) revealed no significant difference between any of the 320 kg N/ha treatments.

Environmental variables and their relationship with daily nitrous oxide emissions

Two of the three largest daily rainfalls throughout the experiment were recorded in April and July 2011 (Fig. 1a), corresponding with the two largest measured N₂O emission peaks (Fig. 1b and f). On 5 April 2011, 23.8 mm of rainfall was recorded, with a large peak in N₂O emissions from the maximum fertilizer treatment measured only 3 days later, a total of 18 days after fertilizer was applied, and only 7 days before the second main application. Only a

small peak in N₂O emissions was measured following the second main fertilizer application on 15 April, with maximum emissions generated on 6 May. During this time period rainfall remained very low (Fig. 1a). The third highest N₂O emission peak was measured on 26 May 2011, 8 days after the third main fertilizer application and was also preceded by a substantial combined total of 40.1 mm of rainfall on 21 and 22 May 2011, 4–5 days before the emission peak (Fig. 1b and f). The largest N₂O emission peak was measured from the maximum fertilizer treatment level on 19 July, again 3 days after a large rainfall of 24.1 mm on 16 July, 15 days after the fourth main fertilizer application. Although the highest daily rainfall of 27 mm did not correspond to a large N₂O emission peak, this can be expected as it occurred several months after fertilizer application, when there was only a low supply of N available for transformation into N₂O (Fig. 1c, d, g, h).

The %WFPS varied throughout the experiment, with the highest levels > 80 % recorded in winter 2011. Low %WFPS values in the spring and summer of 2011 were measured after several consecutive dry days, with %WFPS increasing after more prolonged rainfall periods (Fig. 1a). The peaks in N₂O emissions did not have as strong a relationship with %WFPS as with rainfall, due to peak N₂O fluxes being generated from single large rainfall events even when the soil was dry e.g. the N₂O emission peak on 19 July (Fig. 1b and f). Although %WFPS was high (71 %) at the time of the large N₂O emission peak on 5 April 2011, it was only 58 % when the flux of 290 g N₂O-N/ha was measured on 19 July. This data suggests that rainfall had a stronger influence than %WFPS on N₂O emissions, as %WFPS is also dependent on air and soil temperature, and takes longer to respond to a rainfall event.

Total rainfall (1211 mm) and mean annual air temperature (10.18 °C) over the experiment were slightly higher than the 30-year (1971 to 2000) annual averages of 1140 mm and 9.1 °C respectively. The experiment was thus undertaken in slightly warmer and wetter conditions than recent/historical weather in this location. To ensure that weather in the

months of fertilizer application was not atypical of this location, rainfall and air temperature in these months were compared to the respective monthly 30-year values (Fig. 2). Although lower than the long-term average, precipitation in March and April 2011 was not representative of extreme conditions. Rainfall in July 2011 was 30 mm greater than the long-term monthly average, and application of fertilizer coinciding with a large rainfall event (Fig. 1) suggests that the high N₂O emission peaks in July may be higher than if rainfall was closer to average values. Temperature in April was > 3 °C higher than the 30-year average for April at this site (Fig. 2), but was very consistent with the long-term average for all other months when fertilizer was applied.

Soil mineral nitrogen and daily nitrous oxide emissions

Following fertilizer application on 18 May 2011 soil ammonium-N (NH₄⁺-N) and nitrate-N (NO₃⁻-N) both increased until 24 May for all fertilizer treatments (Fig. 1c, d, g, h), followed by a decrease from most treatments until 1 June 2011. Soil NH₄⁺-N levels continued to decline, whilst NO₃⁻-N levels peaked again on 16 June 2011 (Fig. 1c, d, g, h). Following the next fertilizer application on 4 July there was another increase in soil NH₄⁺-N and soil NO₃⁻-N for most treatments, reaching a peak on 7 July 2011. Soil NH₄⁺-N levels then declined rapidly, reaching background levels on 21 July 2011, with a further small peak on 1 August. A larger peak in soil NO₃⁻-N was also observed from most treatments on 1 August. A lack of soil mineral N measurements following the first four applications of fertilizer means that the role of fertilizer and soil N content at this time cannot be determined.

DISCUSSION

The maximum annual cumulative N₂O emission of 8.17 kg N₂O-N/ha observed in the current experiment from AN 400 is lower than the maximum emissions from fertilized Scottish

grasslands cut for silage in Dobbie & Smith (2003b) and Dobbie *et al.* (1999), where fluxes >16 kg N₂O-N/ha from applications of 300–320 kg AN/ha were reported. The overall range of emissions in the current study from the varying rates and forms of fertilizers (2.14–8.17 kg N₂O-N/ha) is within the same magnitude as the range reported in these other Scottish cut grassland studies, and fits into the range reported from UK grazed grasslands of 0.85 kg N₂O-N/ha to 51.3 kg N₂O-N/ha (Cardenas *et al.* 2010; Rees *et al.* 2013; Burchill *et al.* 2014). However, in these grazed grassland experiments, emissions from the deposition of animal excreta and compaction from trampling were not isolated from those generated from fertilizer N addition, thus a direct comparison between these and the results of the current study is not ideal and the high emissions reported in Rees *et al.* (2013) may include emissions generated from animal dung and urine. A lack of significance between emissions from fertilized grazed and fertilized un-grazed grasslands reported in Flechard *et al.* (2007) and the low European annual average of 1.77 kg N₂O-N/ha reported in Burchill *et al.* (2014) does, however, suggest large spatial variation in fertilizer-induced N₂O emissions across European grassland, highlighting the requirement for more regionally specific measurements and estimates. The higher than European average flux measured in the current study could be explained by a higher mean background flux of 1.31 kg N₂O-N/ha from the control compared to other areas of Europe where background fluxes range from 0.5–1.2 kg N₂O-N/ha (Flechard *et al.* 2007). When added to the fertilizer-induced flux this would produce a greater total flux from the current Scottish grassland study. In a similar manner, Burchill *et al.* (2014) attribute their high recorded emissions to high and variable background levels in Ireland of –1.6 to 4.66 kg N₂O-N/ha. This variation in emissions across Europe highlights spatial variation and the complex environmental factors involved in N₂O generation. Soil type, soil conditions, weather and climate all have a large influence on emissions that must not be overlooked or disregarded in emissions calculations. Although the results of the current study can be used to

estimate emissions from fertilized grasslands with a similar soil type and climate, the role of rainfall and the variation in emissions between years evident in Dobbie & Smith (2003a,b) mean that extrapolation of the results of a one-year study must be made with caution. It is realized that such variability in weather conditions could influence the magnitude of emissions in another year, however the trend between treatments is likely to remain.

Processes of denitrification are apparent in the current experiment and appear to be responsible for generation of the largest N₂O emission peaks. A decline in soil NO₃⁻-N between 24 May and 6 June corresponds with an N₂O emission peak on 26 May, and the large N₂O emission peak on 19 July follows a decline in soil NO₃⁻-N between 7 and 21 July, both suggesting that denitrification is generating emissions. A small peak in N₂O emissions on 8 August also relates to a decline in soil NO₃⁻-N between 5 August and 29 August. Decreases in soil NH₄⁺-N and corresponding increases in soil NO₃⁻-N on several dates during the current experiment imply that nitrification was also taking place and could be a possible explanation for some of the smaller observed N₂O emission peaks. The small peak in N₂O observed from most treatments between 27 June and 6 July coincides with an increase in soil NO₃⁻-N from these treatments between 20 June and 7 July. Although there was clearly a relationship between the application of N fertilizer, soil mineral N, and N₂O emissions in the current experiment, this is a complex relationship which appears to depend on interactions between soil and environmental factors, e.g. rainfall amounts when fertilizer induced soil N levels are conducive to N₂O emissions, and the time of year and stage of grass growth. A difference in the magnitude of N₂O emission peaks following declining soil NO₃⁻-N levels at different times of the year also suggests that other processes of N loss such as nitrate leaching must be considered.

The higher EFs calculated at this Scottish site for all AN treatments, when compared to the IPCC default of 1 %, suggest that N₂O fluxes may be underestimated when the 1 % EF

is applied in such locations. This one-year experiment was, however, carried out in a slightly warmer and wetter year than the long-term average suggests, and as such the default EF may be more suitable in colder and drier years, and where soil conditions are less conducive to N₂O generation. Lower reported EFs for AN applications of 300 kg N/ha of 1.13 % and 0.61 % from the same experimental farm in 2003 and 2004 (Smith *et al.* 2012) further supports this argument. The observed relationship between large rainfall events and N₂O emissions when fertilizer N was available in the soil suggests that emissions in this locality could differ in other years depending on rainfall, as was observed by Smith & Massheder (2014). Findings by Hansen *et al.* (2014), however, indicate that the assumption of greater emissions in wetter years should not be considered a certainty, especially in free-draining soils where denitrification is limited, where high rainfall could result in rapid leaching of N from the soil, and where soil temperature may play a more dominant role.

Although higher than the IPCC default, consistency in the EF calculated from the AN treatments in this experiment suggests that use of the current 'static' EF approach can be used to generate accurate emission estimates when AN fertilizer is applied up to a rate of 320 kg N/ha. Given the observed exponential relationship with increasing N application rate, which was also found in previous studies (McSwiney & Robertson 2005; Grant *et al.* 2006; Zebarth *et al.* 2008; Cardenas *et al.* 2010; Hoben *et al.* 2011; Kim *et al.* 2013; Shcherbak *et al.* 2014), this can only be relied on to provide accurate emissions for fertilizer applications at or lower than optimal rates for crop N uptake. A lack of any significant difference in grass yield between AN 320 and AN 400 treatments suggests that this increase in EF with increasing N application rate could be the result of N application in excess of plant demands (McSwiney & Robertson 2005) and supports the findings of Kim *et al.* (2014), of an abrupt increase in emissions beyond optimal N rates. Although the exponential increase in N₂O emissions found in the current experiment supports much recent research, it does not correspond with

the findings of a sister study by Hinton *et al.* (2015) where the same treatments (which included N additions beyond the recommended rate) were applied at a Scottish arable site but a linear increase in emissions was found. Combined with the different controls on N₂O emissions between arable and grassland sites identified by Dechow & Freibauer (2011), this emphasizes the requirement to consider different relationships when estimating and quantifying N₂O emissions and EFs from grassland and arable crops.

As the N₂O emissions generated from the application of fertilizer at the recommended rate at this location of 320 kg N/ha are not significantly greater than those generated from the application of 240 kg N/ha, a recommendation to apply fertilizer at a rate lower than used in current practice purely for N₂O emission mitigation cannot be supported at this site from the current 1-year study. Agronomic considerations and the impact on grass yield are, however, major factors that must be considered in such a decision, with these results suggesting that once AN fertilizer rate exceeds 240 kg/ha there is no longer a significant increase in yield, and that applications above this rate are of no benefit to grassland production. Application of AN fertilizer at the current recommended rate of 320 kg N/ha thus appears to be an inefficient use of N, suggesting that a reduction in fertilizer use could yield the same level of grass production. Although the difference between N₂O emissions for these two application rates is not significant this is due to the variability within treatments and further research is needed, supported by the lower yield intensity of 0.40 kg N₂O-N/t DM from AN 240 compared to 0.52 kg N₂O-N/t DM from AN 320. Findings of significantly greater N₂O emissions from AN320 would add support to a call for a reduction in N application rate.

The results assessing the impact of changing fertilizer form, dosage rate and the use of NIs suggests that urea 320 with added DCD could be used in place of AN 320 to reduce N₂O emissions, although the economics of such a change would need to be assessed. When assessing the mitigation option to change fertilizer type from AN to urea, although emissions

from urea 320 were lower than from AN 320 there was not a significant reduction with this change. The higher emissions from AN than urea support the finding of greater emissions from nitrate-based fertilizers than from urea presented by Smith *et al.* (2012); however caution must be taken, as losses of N by other pathways (especially ammonia volatilization from urea applications, if left unchecked) may offset the GHG emission reductions achieved through a reduction in N₂O. As such, measurements of NH₃ emissions should be an essential requirement of any further study. This adds to the uncertainty observed by Smith *et al.* (2012) and Smith & Massheder (2014), where the difference in EFs measured from urea and AN varied between sites, and thus the current experiment cannot be used to promote application of urea instead of AN as an N₂O emission mitigation measure. It does, however, indicate that further investigation over several years is required, and that, although there is uncertainty in the literature, there is potential for a reduction in emissions with this change in land-management. A comparison of emissions from AN 320 6 splits with AN 320 reveals that applying fertilizer in smaller more frequent doses to better match crop demand would actually increase emissions; however this difference was again found to be non-significant, and was most likely related to weather conditions at the time of the additional split applications. The grass yield measurements suggest that a decision to change fertilizer type, or to add DCD to fertilizer applied at these rates can be made based on the reductions in N₂O emissions achievable, without any compromise in yield production.

Although the EF measured in the current experiment from urea 320 (0.89 %) was not significantly lower than from AN 320 (1.28 %), this lower value suggests that use of the IPCC default of 1 % may overestimate emissions from urea fertilizer. More investigation is required into this mitigation option, to determine if a significant difference in EFs can be identified, and if lowering the EF value when quantifying emissions from land where urea is applied is justified. The EF calculated in the current experiment when DCD was applied

along with urea (0.59 %) was *c.* 40 % lower than the current IPCC default EF and is significantly lower than the EF measured from AN 320, indicating that quantification of emissions from land where this treatment is applied will be greatly overestimated if the current default 1 % is used. The EF calculated for the AN 320 with six splits treatment (1.60 %) was not significantly different to the EF for AN 320, however use of the 1 % default could lead to an underestimation of emissions from this land management.

Although investigation into use of the NI DCD identified no significant reductions in N₂O emissions at this grassland site, the lower EFs and annual N₂O emissions displayed in Table 3 do suggest that there is potential for reduced emissions with this mitigation measure. The mean annual emissions of 4.49 kg N₂O-N/ha from AN 320 + DCD are almost 1 kg lower than those from AN 320, and indicate a non-significant 17 % reduction with the use of DCD. A need for more investigation into the use of DCD is highlighted by variability in measured emissions within treatments and uncertainty in the literature, with some studies indicating 40–81 % reductions in emissions with application of NIs (McTaggart *et al.* 1997; Shoji *et al.* 2001), and others where the success rate was found to vary depending on the type of fertilizer to which DCD was added (Merino *et al.* 2001; McTaggart *et al.* 1997). Research into the effect of temperature on the half-life of DCD (Kelliher *et al.* 2008; Kim *et al.* 2012; Kelliher *et al.* 2014) and the mean temperature of > 10 °C in 4 of the 5 months in which DCD was applied in the current study suggests that the less effective performance of DCD could be the result of the environmental conditions in which it was applied. Reference to Fig. 1f indicates that DCD was the least effective at reducing N₂O emissions after application in March, and it is possible that less productive grass growth at this time of year could have caused the NH₄⁺ retained by DCD to be subsequently released as N₂O due to a lack of plant uptake. The current finding of no significant difference in the pasture yield of DCD and non DCD amended plots corresponds to the results of Li *et al.* (2014) and Cookson & Cornforth (2002);

however, increased yields presented by Moir *et al.* (2003) and decreased yields presented by Hinton *et al.* (2015) again emphasize the uncertainty surrounding the agricultural and environmental impacts of this potential mitigation measure, and the necessity for more research specific to individual land-use and fertilizer types.

CONCLUSION

The EFs presented in the current study ranging from 1.06–1.34 % when AN is applied at rates up to 320 kg N/ha indicate that use of the current IPCC EF default value of 1 % would slightly underestimate emissions from Scottish grasslands under similar environmental conditions, when treated with AN fertilizer at or below current recommended application rates. A disproportionate increase in emissions beyond this application rate, and an EF of 1.74 % for AN applied at a rate of 400 kg N/ha indicates that emissions will be underestimated to a much greater extent at higher N application rates. The non-linear increase in emissions, combined with no increase in grass yield indicates an increased loss of N as N₂O, once crop demands are exceeded, suggesting that EFs used to estimate N₂O emissions when fertilizer is applied to grassland at higher than the recommended rate should be N dependent, and that calculations using the default EF of 1 % would underestimate emissions. Assessment of the reduction in emissions achievable through use of mitigation options identified significantly lower N₂O emissions from grassland receiving urea and DCD than from application of AN fertilizer at the same rate. Although the use of DCD alongside AN or urea did not cause a significant reduction in emissions when compared to the respective fertilizers without DCD, lower EFs of 1 % for AN + DCD and 0.59 % for urea + DCD suggest the potential for reduced emissions with this mitigation measure. A lower EF of 0.89 % for urea applied at a rate of 320 kg N/ha compared to AN applied at the same rate (1.28 %) indicates not only the potential to reduce N₂O emissions through use of urea in place of AN,

but also implies that emission calculations using the default value of 1 % for grasslands treated with urea will be overestimated. This finding of a lower EF from urea than AN was however not statistically significant, but does highlight the need for further research. The results of this single-year study at one site in Scotland provide an important insight into the relationship between N₂O emissions, N fertilizer application, soil N availability and high rainfall levels, and emphasise the importance of combining the results of this research with further experiments under different weather and soil conditions.

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Table 1. Fertilizer application rates (kg N/ha) and date of application for each treatment. AN = ammonium nitrate; DCD = dicyandiamide.

Numbers in brackets indicate the amount of DCD applied, where the amount of N fertilizer has been reduced to allow for the N content of DCD

Treatment	Application rate & timing (kg N/ha)						Total applied (kg N/ha)
	21/03/11	04/04/11	15/04/11	22/04/11	18/05/11	04/07/11	
Control	0	0	0	0	0	0	0
AN 80	20	0	20	0	20	20	80
AN 160	30	0	40	0	50	40	160
AN 240	40	0	60	0	80	60	240
AN 320	70	0	70	0	100	80	320
AN 400	90	0	90	0	120	100	400
AN 320 + (DCD)	64 (+6)	0	64 (+6)	0	93 (+7)	73 (+7)	294 (+26)
Urea 320	70	0	70	0	100	80	320
Urea 320 + (DCD)	64 (+6)	0	64 (+6)	0	93 (+7)	73 (+7)	294 (+26)
AN 320 6 doses	40	30	40	30	100	80	320

Table 2. The impact of ammonium nitrate (AN) fertilizer application rate on annual cumulative N₂O emissions, emission factors (EFs), and grass dry matter (DM) yield. Transformed and back transformed values are presented when transformation was necessary to meet the requirements of statistical analysis, along with the measured values and their standard error (S.E.) where n = 3. Details of the transformation applied are contained in the Data Analysis section and below. Means that do not share a letter are significantly different

Treatment (kg N/ha)	cumulative N ₂ O-N emission (kg N ₂ O-N/ha)				EF (%)		Grass yield (DM t/ha)	
	Transformed*	Back transformed	Measured	S.E.	Measured	S.E.	Measured	S.E.
Control	0.28 ^a	1.31	1.34	0.17			3.85 ^a	0.35
AN 80	0.79 ^b	2.14	2.18	0.33	1.06 ^a	0.31	8.18 ^b	0.26
AN 160	1.21 ^c	3.14	3.15	0.17	1.14 ^a	0.15	9.72 ^c	0.35
AN 240	1.53 ^{cd}	4.16	4.28	0.71	1.23 ^a	0.31	10.81 ^d	0.25
AN 320	1.86 ^d	5.52	5.63	0.82	1.34 ^{ab}	0.29	11.03 ^d	0.56
AN 400	2.34 ^e	8.17	8.31	1.09	1.74 ^b	0.28	11.28 ^d	0.06
S.E.D.	0.168				0.187		0.343	
D.F. (n.d.f, d.d.f)	5, 10				4,8		4,8	

*Transformed using $((\text{kgN}_2\text{O}^{**}0.1)-1)/-0.1$

Table 3. The impact of split fertilizer application, fertilizer type (AN = ammonium nitrate), and the addition of DCD on annual cumulative N₂O emissions, emission factors (EFs) and grass dry matter (DM) yield. Transformed and back transformed values are presented when transformation was necessary to meet the requirements of statistical analysis, along with the measured values and their standard error (S.E.) where n = 3. Details of the transformation applied are contained in the Data Analysis section and below. Means that do not share a letter are significantly different

Treatment (kg N/ha)	cumulative N ₂ O-N emission (kg N ₂ O-N/ha)				EF (%)				Grass yield (DM t/ha)	
	Transformed*	Back transformed	Measured	S.E.	Transformed†	Back transformed	Measured	S.E.	Measured	S.E.
Urea 320 + DCD	0.94 ^a	3.25	3.26	0.13	-0.51 ^a	0.59	0.60	0.07	10.84	0.43
Urea 320	1.09 ^{ab}	4.19	4.24	0.36	-0.12 ^{ab}	0.89	0.91	0.14	10.74	0.62
AN 320 + DCD	1.13 ^b	4.49	4.53	0.37	-0.005 ^b	1.00	1.00	0.06	10.91	0.63
AN 320	1.23 ^{bc}	5.46	5.63	0.82	0.25 ^{bc}	1.28	1.34	0.29	11.03	0.56
AN 320 6 splits	1.31 ^c	6.42	6.50	0.62	0.48 ^c	1.60	1.61	0.17	11.16	0.06
S.E.D.	0.072				0.192					
D.F. (n.d.f, d.d.f)	4,8				4,8				4,8	

* Transformed using $((\text{kgN}_2\text{O}^{**}-0.4)-1)/-0.4$

† Transformed using $((\text{EF}^{**}0.1)-1)/0.1$

Fig. 1 (a). Variation in daily precipitation and water filled pore space (%WFPS) throughout the experimental period. (b). daily N₂O emissions for all AN fertilizer treatments. (c). Variation in soil NH₄⁺-N for AN fertilizer treatments. (d). Variation in soil NO₃⁻-N for AN fertilizer treatments. (e). Variation in daily precipitation and water filled pore space (%WFPS) throughout the experimental period. (f). Mean N₂O emissions from all 320 kg N/ha fertilizer treatments throughout the experimental period. (g). Variation in soil NH₄⁺-N for all 320 kg N/ha fertilizer treatments. (h). Variation in soil NO₃⁻-N for all 320 kg N/ha fertilizer treatments. Note: Data presented in Figs c, d, g, h from May 2011 onwards due to a delay in soil mineral N sampling. Error bars indicate the standard error of the mean (n = 3). Large arrows indicate application of fertilizers, small arrows indicate the additional application of fertilizer for the AN 320 6 split treatment.

Fig. 2 (a). A comparison of the monthly precipitation totals for the months of fertilizer application in 2011 to the historical 30 year average values (b). a comparison of the mean monthly air temperatures in the months of fertilizer application in 2011 to the historical 30 year average values.

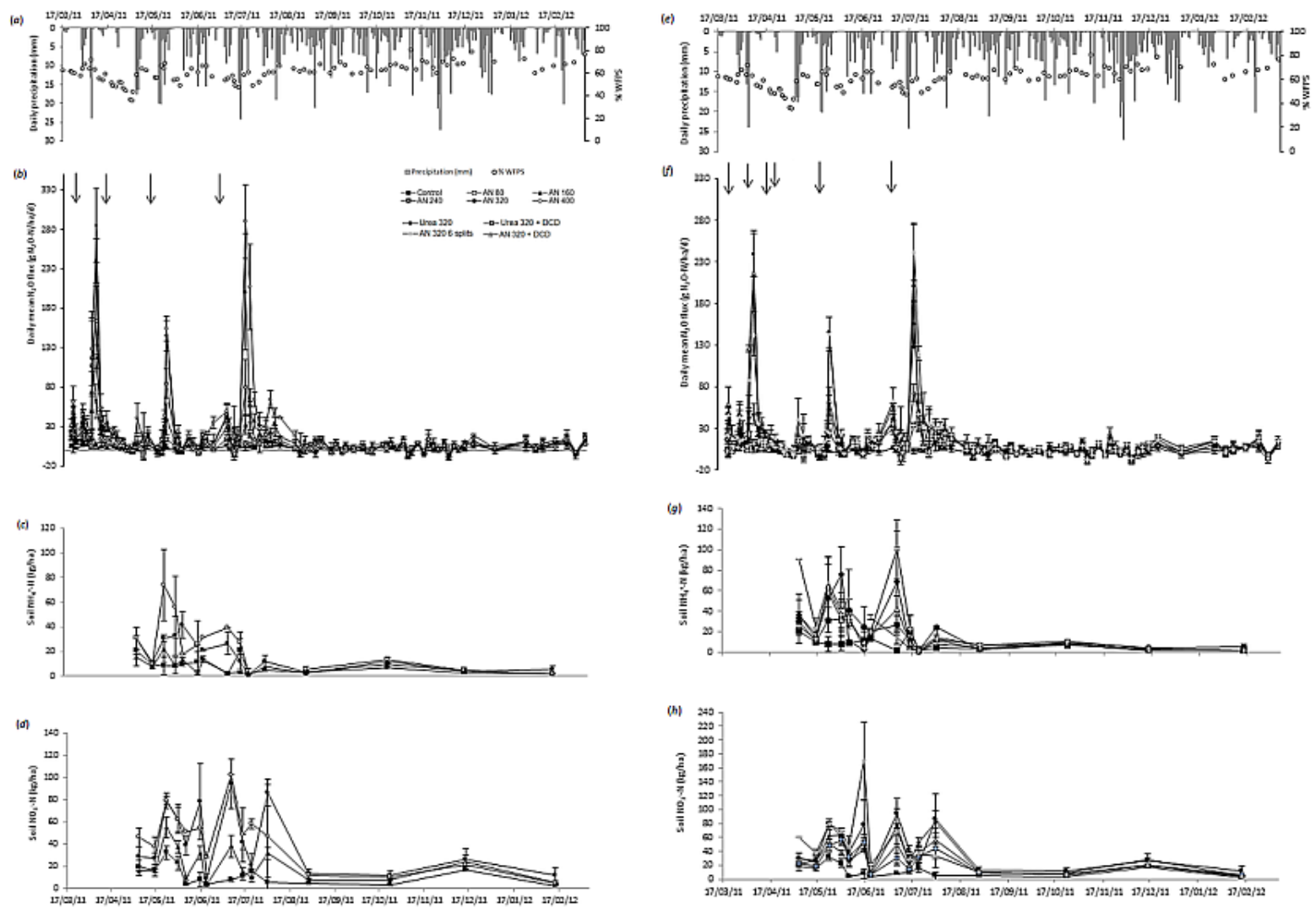


Fig. 1.

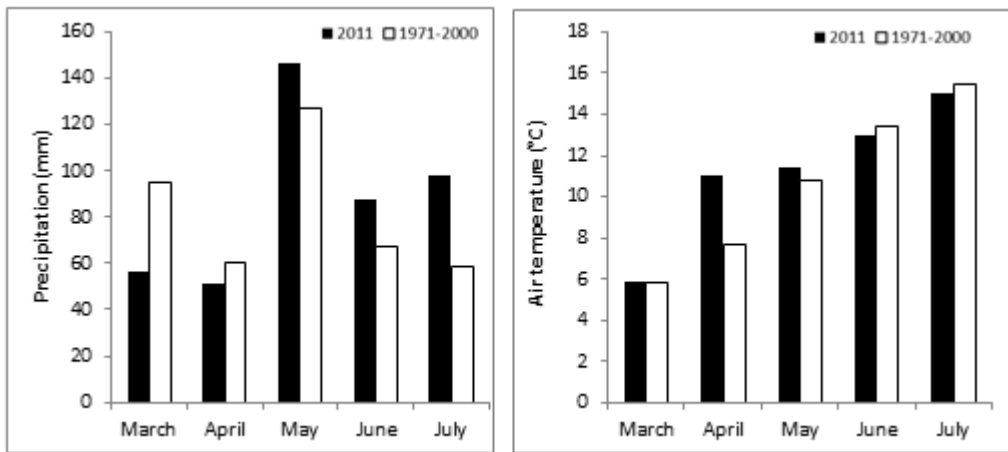


Fig. 2.

Fig. 1 legend

