Dear Editor and editorial team,

Please find below the combined files of the reply to both reviewers comments and the previous version of the manuscript with tracked changes.

Sincerely,

Dr Nicholas Cowan

Dear reviewer 1,

We would like to thank you for your extensive comments and suggestions on our original manuscript which you have clearly invested some time. We have made edits in our original manuscript based on these comments and suggestions, and we hope that our corrections and replies are satisfactory.

In response to reviewer 1

First, the motivation is unclear. The authors argue that urease inhibitors might not only decrease NH3 emissions but also increase N2O emissions compared to untreated urea. It is not explained by what mechanism this would occur.

Text added to intro:

Although there are positive studies which promote the pollution reducing capabilities of these chemicals (Misselbrook et al., 2014), some questions remain over the overall effectiveness of the inhibitors which face claims that reduction of one form of Nr pollution may increase another. This is most commonly observed for nitrification inhibitors in which the slowing on the conversion of NH_4^+ to NO_3^- in soils results in a decrease in N_2O at the expense of an increase in NH_3 volatilisation (Lam et al., 2016; Zaman et al., 2009). In theory, the use of a urease inhibitor should reduce both the emission of NH_3 by reducing the rate at which urea is converted to NH_4^+ in soils, thus limiting available nitrogen in all forms. This may however, limit the rate at which crops also receive Nr and reduce yields.

The two main N_2O producing processes of nitrification and denitrification use NH4+ and NO3- as substrates and consequently also depend on urea hydrolysis. Urease inhibitors should therefore rather decrease than increase N2O emissions. The reference cited by the authors (Lam et al., 2017) does also not mention a potential increase in N2O release by urease inhibitors, but in contrast discusses a potential increase in NH3 release by nitrification inhibitors that are designed to reduce N_2O emissions.

The reviewer is correct in that theoretically the urease inhibitors should reduce N_2O emissions by limiting the rate of available nitrogen in the soil released from the fertiliser, which was the reason for the field testing in this study. But there are many unknowns when it comes to the application of these inhibitors, especially as N_2O emissions are so unpredictable and yields may be affected. We cite the Lam et al., 2017 paper as a relevant example of the potential negative aspects of the addition of a type of inhibitor which is often mentioned in discussions surrounding the uncertain impacts of chemical additives to traditional fertilisers, albeit a fundamentally different one used in this study. We have added text to the introduction and discussion sections to address the reviewers concerns.

In addition, I am wondering about long-term effects of the urease inhibitor on NH_3 fluxes. NH_3 was measured over 14 days after fertilization (in contrast to a 30-day period for N_2O – why?). No increase in plant N yield was observed by the inhibitor treated urea compared to untreated urea and on average 55% of the applied fertilizer was accounted for in the experiments. Is it possible that a large fraction of the inhibited urea remained in the soil and will eventually release NH3 once the (short-term) inhibitor stops working? The short-term nature of the experiment is briefly mentioned in the conclusions, but the resulting limitations should be discussed more thoroughly.

The two week measurement period of NH_3 was based on the methodology and funding we had available. Extending this period was not possible, nor do we believe it was necessary. The measurements show that NH_3 emission reduced exponentially to near zero (or uptake) by the time two weeks had passed in all cases. Due to the limits of detection of NH_3 measurement methodology, proceeding longer than 2 weeks would not have provided any data of use. As the harvests were only approximately 1 month apart, if there had been residual nitrogen in the soil we would have observed an increase in yield, nitrogen content of the soil or emissions from the treated urea plots in the following harvest. On all occasions the soil nitrogen content was similar to the surrounding plots, indicating that there was no significant quantities of residual N.

Text added to method section:

Due to logistical constraints, we were limited in the number of measurements we could make using the FIDES method. Based on the extensive experience of the researchers in the field of NH_3 flux measurements, and numerous studies of NH_3 emissions (e.g. Gericke et al., 2011; Sanz-Cobena et al., 2011; Suter et al., 2013) we decided to measure for a period of two weeks, which would allow us to capture the vast majority of any cumulative emissions associated with the fertiliser event, which typically last only several days.

I further find the introduction and discussion of previous studies on the effect of urease inhibitors on plant yields, NH3 and N2O emissions rather shallow (e.g., introduction in lines 83-88, rare mentioning of previous work in the discussion). A range of papers have been published on the topic, e.g. recently Graham et al. (2018, Soil Science Society of America Journal), as well as multiple papers by Zaman et al.

Graham manuscript is behind a paywall, but further references and text has been added to introduction and discussion sections.

Added to introduction:

This is most commonly observed for nitrification inhibitors in which the slowing on the conversion of NH_4^+ to NO_3^- in soils results in a decrease in N_2O at the expense of an increase in NH_3 volatilisation (Lam et al., 2016; Zaman et al., 2009). In theory, the use of a urease inhibitor should reduce both the emission of NH_3 by reducing the rate at which urease is converted to NH_4^+ in soils, thus limiting available nitrogen in all forms. This may however, limit the rate at which crops also receive Nr and reduce yields.

Added to discussion:

Previous studies highlight a potential for pollution swapping with nitrification inhibitor treated urea (typically dicyandiamide, a.k.a. DCD), suggesting that a reduction in NH_3 emissions results in a higher N_2O production (Lam et al. 2016). that by reducing the rate of conversion of NH_4+

to NO_3- in soils that NH_3 emissions are increased (Lam et al., 2016; Zaman et al., 2009). Elevated N_2O and NH_3 emissions have been observed on occasion after the use of nitrification inhibitors (Scheer et al., 2017; Zaman et al., 2009); however reductions in both have also been observed (Di et al., 2006; Misselbrook et al., 2014) . This should not be the case for urease inhibitors as it slows the release of Nr from the applied fertiliser, thus reducing the potential of N_2O and NH_3 emissions. Previous studies have shown that the use of urease inhibitors can significantly reduce N_2O emissions (Singh et al., 2013; Zaman et al., 2009).

Line 34-35: Are the mentioned reductions by 90% and 47% or did the reduced values correspond to 90% and 47% of the comparison value?

Text reworded to make it clearer:

"The urea coated with a urease inhibitor did not significantly increase yields; however, ammonia emissions were only 10 % of the magnitude measured for the uncoated urea, and N_2O emissions were only 47 % of the magnitude of those measured for ammonium nitrate fertiliser."

Line 42: Please add examples of agriculturally important Nr forms.

Text changed:

Due to a large and rapidly expanding global population, modern-day agriculture requires regular inputs of industrially produced reactive nitrogen fertilisers (Nr) (i.e. nitrogen compounds that plant life can consume through root systems such as ammonium nitrate (AN) and urea) in order to keep up with increasing food demand (Lassaletta et al., 2014).

Lines 48-51: I suggest to change the sequence of this sentence, first introducing different pathways of N loss to link to the sentence before, and then mention the resulting environmental damage.

Text changed:

Typically, more than half of applied Nr is lost to the environment through various biological pathways and chemical processes (Lassaletta et al., 2014; Raun and Johnson 1999), such as nitrate (NO_3^-) run-off into streams and waterways (Lu and Tian 2017) as well as gaseous losses in the form of ammonia (NH_3) (Bouwman et al., 1997), nitrous oxide (N_2O) (Reay et al., 2012), and nitrogen oxides (NO_x) (Bertram et al. 2005). This relatively low nitrogen use efficiency (NUE) results in significant environmental damage at a global scale.

Line 62: Do you mean an increase in rates of fertilizer application? Please also add a reference to support this projection.

Added reference FAO, 2017

Line 90-93: Please rephrase this sentence. It is unnecessarily complicated and contains the word "required" three times.

Text changed:

Further work using specific products in different environments is needed to supply the evidence that will provide the agricultural community with the confidence to make the changes required to meet future NUE demands globally.

Lines 98-100: I am not sure I understand. Do you mean that the study area is representative for agriculturally used grasslands in the UK? Please clarify.

I have changed the text slightly to make it clear that the experiment aims to represent conditions in the UK:

This study aims to specifically investigate the effect of the Agrotain® urease inhibitor (Koch, KS, USA) on a typical grassland silage crop in Scotland, comparing it with the two most commonly used synthetic nitrogen fertilisers: Ammonium nitrate (Nitram®) and urea. Grasslands account for approximately 60 % of agricultural land use in the UK (approximately 74,000 km2) to which an estimated 120 kt of ammonium nitrate and 26 kt of urea are applied annually (BSFP, 2017). The results presented in this study are intended to represent to some extent this large coverage of agricultural land in the UK to which urease inhibitors may be applied in the future.

Lines 115-118: Please add some details to the description of the two fields. Are exposure, slopes, soil types etc. similar? Is grazing/mowing history similar? Anything else that might affect the results? I also suggest to move the different pH values presented in the paragraph below here.

Text changed to:

The soil in both fields is classified as a clay loam for the top 30 cm in fields, with a pH (in H_2O) of 6.5 and 6.1 for the Engineers and Upper Joiner fields, respectively. They are classed as an imperfectly drained Macmerry soil of the Rowanhill association (eutric cambisol, FAO classification). All fertiliser applications were of 70 kg N ha^{-1} (Table 1) which was consistent with the typical management regime of the fields. Both fields are used as grazing pastures for mainly sheep at high stocking densities of approximately 20 ewes per hectare. The sheep were vacated before and throughout the duration of the experiment and instead the grass was grown for silage.

Lines 138-140: How were these parameters measured?

See above

Line 142-144: Considering the high variability between replicates and often similar values of control and treatment plots, how did you treat uncertainties in this calculation and in others?

Text added:

Uncertainties in in these values are represented by 95 % confidence interval of the mean, calculated by multiplying the standard deviation by 1.96. The least squares method is used to combine uncertainties when subtraction or addition is used.

Line 261: Measurements of soil moisture and soil temperature are mentioned here but not presented in the manuscript. Soil moisture data in particular would be a good complement to the manuscript, for instance for interpreting the observed N2O fluxes.

The reviewer is correct in that soil moisture measurements are typically used to help describe N_2O fluxes, but we found no statistical correlation with N_2O flux and soil moisture in this experiment in any of the fertiliser applications. Due to the already long length of the

manuscript we decided to cut this out as is provided no additional explanation for variations in observed fluxes. We have now removed the text describing the measurements.

Line 268: Considering the discussed large variability of measured parameters, please present uncertainty estimates (e.g., standard deviation) together with the averages throughout the Results section.

Corrected

Line 273: How can meteorological conditions be ruled out as the reason behind the observed differences between the two fields in the two years? If site properties lie behind the differences, what specific properties could that be (e.g., are there differences in N availability to start with)? Also, in what sense was the 2016 yield "exceptional" – compared to 2017, or compared to the usually observed yield in such systems?

Text changed to clarify:

Although rainfall and temperature was similar during both years of measurement, crop yields for all treatments were substantially larger in the 2016 field plots (5.5 t ha^{-1}) than the 2017 field plots (1.48 t ha^{-1}) (Table 2), indicating that the Engineer's field was the more productive of the two experimental areas regardless of fertiliser application or meteorological conditions.

Line 288: Are the values after subtraction of the background?

Text added:

(after subtraction of the control)

Lines 292-293: I find it hard to see to see a consistent pattern of overall later peaks in the urea than the Nitram treatments.

Elevated fluxes can be seen after 10 days for the urea and treated urea in Figure 2.

Text changed to clarify:

Fluxes also increased after the urea and inhibitor coated urea applications, although the timing of the peaks in these emissions were more variable than those observed from the Nitram plots.

Line 309: Are these values after the subtraction of the background? Also, why was the measurement period for NH3 shorter than for N2O?

Text changed to:

In four of the five events, Nitram was the highest N_2O emitting fertilizer of the treatments after 30 days (minus background from control plots) with a mean EF between…

As discussed previously, the FIDES method was only carried out for 2 weeks for logistical and methodological reasons (see text added in 2.4).

Lines 308-309 and 314-315: These sentences have considerable overlap; please restructure.

Text changed to:

Differences in NH_3 from individual plots was typically larger than an order of magnitude of the mean value of the grouped treatments.

Lines 331-333: It looks more like an additional peak in NH4+ concentrations; there is also a peak in the beginning of similar magnitude than for the other events.

I believe that the additional peak in NH_4^+ concentrations shows a delay in urea hydrolysis. The peak in the other events is there as it is not delayed, as stated in the original text.

Line 334: What do you mean with variation on a log-normal scale?

Log-normal distributions are a common statistical phenomenon that we do not feel is necessary to describe in the text. We have re-worded the text to clarify.

Text changed to:

Concentrations of NO_3^- followed a log-normal distribution in a similar fashion to the NH_4^+ concentrations.

Line 339: Differences between sites and years cannot be distinguished in this setup; please rephrase accordingly.

Text removed

Line 353-355: Were the 2017 plots not grazed by sheep before? Please add potential differences in grazing history and intensity to the site description in the Material & Methods section.

Text added to method section:

While sheep were vacated from the 2016 field a month prior to the experiment, the 2017 plots had not been grazed for more than six months before the experiment.

Lines 357-359: With the scaling of the y-axes of Figure 4, it is almost impossible to compare initial ammonium and nitrate concentrations between fertilization events. It would help to present control concentrations here again to support the claimed differences in initial Nr concentrations between events.

I can understand the reviewer's frustration with this figure, but due to the log-normal distribution of the data, it is exceptionally difficult to present the data in a clear manner in any format. I disagree that it is not possible to compare the starting N in the treatments as it is clear from the Control column (LHS) that nitrogen in the soil is consistently at about 10 - 40 mg N kg^{-1} for the different events. We have tried to present this data in a variety of ways, and believe that changing its current format would cause more problems than it solves.

Line 375: The Lam et al. 2017 reference does not indicate that a reduction in NH3 emissions might result in higher N2O production – the paper is about nitrification inhibitors and a potential increase in NH3 emissions that follows reduced N2O emissions.

The reviewer is correct in this statement. We have altered the text to include more relevant comparisons with literature.

Text changed to:

Previous studies highlight a potential for pollution swapping with nitrification inhibitor treated urea (typically dicyandiamide, a.k.a. DCD), suggesting that by reducing the rate of conversion of NH_4^+ to NO_3^- in soils that NH_3 emissions are increased (Lam et al., 2016; Zaman et al., 2009). Elevated N_2O and NH_3 emissions have been observed on occasion after the use of nitrification

inhibitors (Scheer et al., 2017; Zaman et al., 2009); however reductions in both have also been observed (Di et al., 2006; Misselbrook et al., 2014) . This should not be the case for urease inhibitors as it slows the release of Nr from the applied fertiliser, thus reducing the potential of N_2O and NH_3 emissions. Previous studies have shown that the use of urease inhibitors can significantly reduce N_2O emissions (Singh et al., 2013; Zaman et al., 2009). In this study, emissions from the inhibitor treated urea were slightly larger overall compared to the urea; however, the treatments behaved similarly throughout the experiment and the differences observed in this study were not statistically significant (p-value = 0.42).

Line 392: Does the accounted for Nr include measured ammonium and nitrate, additional N in the harvested plants, as well as NH3 and N2O emissions? Please specify. Considering that no increase in plant N yield was observed by urease inhibitor compared to untreated urea and the short duration of the NH3 flux measurements, is it possible that a large fraction of the inhibitor-treated urea still remains in the soil at the end of the experiment and will eventually release NH3 once the (short-term) inhibitor stops working?

Text changed to:

After the N content of the crop, the N content of the soil and emissions of N_2O and NH_3 are taken into account, the majority (> 55 %) of applied Nr in the experiments remains unaccounted for by the time of harvest.

As discussed previously, the NH_3 emissions have declined to near zero after 2 weeks and it is unlikely that emission is statistically significant compared to deposition.

See text added to section 2.4.

Tables 2, 3, 4: Considering the high variability within treatments, please add information on whether differences between treatments (in particular also between background and fertilizer NH_3 and N_2O fluxes) were statistically significant.

As the standard deviations are presented in the table, and the number of data points for each replication is relatively low (n = 4) and often the data follows a log-normal distribution, I do not believe that individual significance tests offer much in the way of describing the data beyond what is already presented. The significance values for the important comparisons are presented in the text.

Figure 1: It might help to indicate the experimental periods (fertilizer addition and run time of flux measurements).

Text added to figure caption:

Fertiliser was applied at t = 0 days, and the measurements lasted up to 30 days for each event.

Line 25: The first "and" is superfluous.

Corrected

Line 32: The abbreviation "Nr" has not been introduced yet.

Corrected

Lines 33-34: "The urea coated with a urease inhibitor did not significantly increase yields" – compared to what treatment?

Corrected

Line 71: Add "to" before "convert".

Corrected

Line 73: Add "N" after "less".

Corrected

Line 77: Change "trialed" to "tested".

Corrected

Line 83: Typo, this should be "losses". Are the losses quantified in these studies in the form of NH3, N2O or both?

Corrected

Line 87-88: I suggest ". . . reduction of one form of Nr pollution may contribute to increase another".

Corrected

Line 147: Please change to "for both 2016 and 2017 experiments" or similar; the sentence gives the impression of measurements throughout the entire growing season of multiple years.

Corrected

Line 149: "a sealed lid"

Corrected

Line 238: "several"

Corrected

Line 239: "holds" and "centre"

Corrected

Line 253: "to provide"

Corrected

Line 335: Do you mean gaseous NH3 or NH4+ in the soil solution?

Corrected

Line 345: Change "was" to "were".

Corrected

Lines 348-349: Please add uncertainties.

Corrected

Line 350: Typo, should be "trials".

Corrected

Line 353: Why "although"?

Changed

Line 358: "show"

Corrected

Line 365: Change to "under the conditions".

Corrected

Line 368: "the treatment effect"

Corrected

Line 382: FIDES is the mathematical approach, not the measurement method.

Changed

Line 386: Change to ". . . while Nitram treatments do not . . .".

Changed

Line 399: Add "of" before "applied".

Corrected

Line 402: "under the right conditions". Also, which conditions would be "right"?

Changed text to:

…and microbial emissions of NO and N_2 can account for Nr losses of an order of magnitude higher than N_2O when water filled pore space (WFPS) is particularly low (< 40 %) or high (> 80 %) (Davidson 1993; Weier 1993).

Line 410: Change "emitters" to singular.

Corrected

Line 413: Please specify that this decrease is by 90%, not to a level of 90%.

Corrected

Figure 2: The headers are not consistent with the other figures (Ammonium Nitrate instead of Nitram).

Corrected

Dear reviewer 2,

We would like to thank you for your comments and suggestions on our original manuscript. We have made edits in our original manuscript based on these comments and suggestions, and we hope that our corrections and replies are satisfactory.

I believe the manuscript could benefit from a different statistical analysis and not just considering the average across both sites and all harvest.

I agree that an argument could be made for the approach suggested by the reviewer, but there is an equally strong argument that in comparing the fertilisers that all events should be considered together as this is more realistic of the impacts at a wider scale. Before our experiment began, we decided that the results from the different fields would be combined, and to not do so would be to alter the experimental assumptions based on the results. It is true that there is a large difference in yields observed between the fields, but these results are documented in detail in the text and tables provided. To separate the fields in the statistical analysis would only reduce the replicate size and provide two very different and not very useful values for the fertiliser types. A difficulty in this area of research is that in order to make a very good comparison between the fertilisers, we would need several years of repeated experiments and many replicates more than we could financially support to come to solid conclusions. We present our data in a way that future studies can extract the data and collate it with other datasets to increase its long term value.

The introduction focuses largely on the general, big problems of fertilizer application and the part, which should guide the reader, actually comes too short: What is expected from the different fertilizer types? What are the underlying mechanism? I understand that the urea fertilizer coated with urease inhibitor is meant to reduce NH3 emissions but I do not understand "the pollution swapping", meaning that it should increase net N2O fluxes (L86-88 and L373-375).

Text added to introduction:

This is most commonly observed for nitrification inhibitors in which the slowing on the conversion of NH_4^+ to NO_3^- in soils results in a decrease in N_2O at the expense of an increase in NH_3 volatilisation (Lam et al., 2016; Zaman et al., 2009). In theory, the use of a urease inhibitor should reduce both the emission of NH_3 by reducing the rate at which urea is converted to NH_4^+ in soils, thus limiting available nitrogen in all forms. This may however, limit the rate at which crops also receive Nr and reduce yields.

The other crop quality measures, crude protein, ME, MAD and D value are barely mentioned. These variables also need to be explained: What do they mean in this context? Why did you choose them? I also suggest to explain the expected effect of fertilizer type on crop quality measures in the introduction.

These are the common metrics by which agronomists will measure the quality of the grass for animal feed. These were measured to identify if there were any large differences as a result of the fertiliser, which there was not. We add the following text to clarify:

These indicators of digestibility and energy content are commonly used to indicate the quality of the silage grass for animal feed and our study suggests that there was no significant differences between the feedstock grown using the different fertilisers.

NH3 emissions were only monitored over two weeks after each fertilization event, which I would assume could match the period when the urease inhibitor is effective. But what happens when the inhibitor becomes ineffective? Basically my question is: how long is such inhibitor effective in the soil and how does this compare to the duration of NH3 emission observation? How would the NH3 emission look like if it would have been monitored over a longer period than two weeks?

This is a question asked by reviewer 1 as well (see reply to reviewer 1). NH_3 measurements are extremely difficult to capture from plot experiments due to the "sticky" properties of the gas. The application of the FIDES method is a novel way in which NH_3 emissions can be measured from plot scale experiments, of which this study is one of the first to report. Low emissions of NH_3 cannot be detected by any method in plot scale experiments; however, from previous experience (and the cited sources added to the methodology section), we expect more than 95 % of NH_3 emissions to occur within the first week, which we see in our study as cumulative flux stabilises over the 2nd week (or becomes negative due to low rates of deposition). If the inhibitor and the urea remained in the soil we would have seen an effect after subsequent treatments to the same plot, which we do not, even after 3 events. Based on our results, and previous studies, we do not expect measurements of NH_3 after the 2 week window to be large enough to be significant, therefore we did not expend resources to measure them.

Fig. 3: Different order of fertilizers compared to the other tables and figures is confusing.

The order is the same, with the addition of the control plots.

Fig. 4: Why is the median and not the mean plotted? Are there no error bars plotted or are they smaller than the symbols? One data point for ammonium in the urea treatment for the second fertilization event in 2016 looks like an outlier to me. If not then consider to use a break in the y-axis because the other data points are not readable due to the scaling.

This figure was also criticised by reviewer 1, and I offer the same reply here. Due to the log-normal distribution of the data and the limited number of measurements, it is exceptionally difficult to present the data (or meaningful error bars) in a clear manner in any format. When handling data that varies on a log-normal (exponential) scale, it is not statistically defensible to remove an "outlier" as it is a real value, nor do we want to "break the axis to present it. We have tried to present this data in a variety of ways, and believe that changing its current format would cause more problems than it solves. Although criticised, the figure is transparent and able to display much of the measured data in a way that is understandable to readers. In this form we can show trends in available nitrogen in the soil without specifying quantitative values for which there is no model to provide us with a way to handle the data or uncertainties.

I suggest to use repeated measures ANOVA to analyse the effect of fertilizer type on extractable ammonium and nitrate concentrations.

I disagree that this analysis is required in the text. Due to the log-normal distribution of data and small sample size, the assumptions of an ANOVA test are not met or easily applied in a meaningful way.

L25: Delete "and" after ammonia (NH3).

Corrected

L32: Delete "the" in "the urea".

Changed text to:

Overall, ammonium nitrate treatment was found to increase yields significantly (p-value < 0.05) when compared to the urea fertilisers used in this study

L33: Delete "The" in "The urea coated"

Corrected

L180: Comma missing before dC/dt.

Corrected

L238: Delete one "over" and consider re-writing this sentences.

Changed

L273: How can you conclude here that meteorological conditions were affecting yields differently in both years?

Reworded based on previous comment:

Although rainfall and temperature was similar during both years of measurement, crop yields for all treatments were substantially larger in the 2016 field plots (5.5 t ha^{-1}) than the 2017 field plots (1.48 t ha^{-1}) (Table 2).

L284: Data on ME, MAD and D value are not shown in Table 2 or anywhere else.

These were measured as part of a different work package within the project and although not really relevant to this study, we include reference to a lack of statistical significance in the differences. We have removed an earlier incorrect reference to table 2, but the description remains in the results section with some added description of its importance as a metric for quality of animal feed as it does not detract from the study either.

L327: How long were sheep grazing at the Upper Joiner Field in 2017? Were they also excluded from the plots one month before start of the experiment?

Text has been added to explain in better detail based on previous comment:

While sheep were vacated from the 2016 field a month prior to the experiment, the 2017 plots had not been grazed for more than six months before the experiment.

L334: On a log-normal scale? I do not understand this in this context.

Reworded based on previous comment to describe log-normal distribution of data, a common occurrence in scientific data handling.

L338 & 341: Nitrate does not decay. Describe it as a decrease in concentration over time.

Corrected

L355-357: That's speculation. Your experiment does not allow to conclude this.

Text changed to:

We speculate that the prior grazing of the sheep is also likely to have resulted in the residues of animal waste in the 2016 plots, which would explain the higher than expected yields and Nr in the soil measurements in these plots (Cowan et al., 2015).

L367-370 & L378-379: Here again: Description of statistical analysis is missing and no results of the statistical analysis are given.

This is true, but also of little relevance to the overall study. T-tests and p values will not add anything of value to this discussion regarding these variables.

L393-394: Re-write this sentence: ". . .fallen considerably in magnitude come harvest"?

Typically, Nr in the form of NH_4^+ and NO_3^- in the top 10 cm of soil has returned to concentrations on par with the control plots by harvest.

Nitrogen use efficiency and N_2O and NH_3 losses attributed to three fertiliser types applied to an intensively managed silage crop

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Key words: Ammonium nitrate, urea, grassland, urease inhibitor, FIDES, Bayesian statistics

Abstract

Three different nitrogen (N) fertilizer types, ammonium nitrate, urea and urea coated with a urease inhibitor (Agrotain®), were applied at standard rates (70 kg N ha^{-1}) to experimental plots in a typical and intensively managed grassland area at Easter Bush Farm Estate (Scotland). The nitrogen use efficiency of the fertilisers was investigated as well as nitrogen losses in the form of nitrous oxide fluxes (N_2O) and ammonia (NH_3) and during fertilisation events in the 2016 and 2017 growing seasons. Nitrous oxide was measured by the standard static chamber technique and analysed using Bayesian statistics. Ammonia was measured using passive samplers combined with the FIDES inverse dispersion model. On average, fertilisation with ammonium nitrate supported largest yields and had the highest nitrogen use efficiency, but as large spatial and seasonal variation persisted across the plots, yield differences between the three fertilizer types and zero N control were not consistent. Overall, ammonium nitrate treatment was found to increase yields significantly (p-value < 0.05) when compared to the urea fertilisers used in this study. Ammonium nitrate was the largest emitter of N_2O (0.76 % of applied N) and the urea was the largest emitter of NH_3 (16.5 % of applied N). The urea Urea coated with a urease inhibitor did not significantly increase yields when compared to uncoated urea; however, ammonia emissions were substantially smaller (90 %) only 10 % of the magnitude when compared tomeasured for the uncoated urea, and N_2O emissions were also smaller only (47 %) of the magnitude of those measured for when compared with ammonium nitrate fertiliser. This study suggests that urea coated with a urease inhibitor is environmentally the best choice in regards to nitrogen pollution, but because of its larger cost and lack of agronomic benefits, it is not economically attractive when compared to ammonium nitrate.

1. Introduction

Due to a large and rapidly expanding global population, modern-day agriculture requires regular inputs of industrially produced reactive nitrogen fertilisers (Nr) (i.e. nitrogen compounds that plant life can consume through root systems such as ammonium nitrate (AN) and urea) in order to keep up with increasing food demand (Lassaletta et al., 2014). This wide-scale intensive application of Nr has resulted in significant anthropogenic alterations of virtually every process in the natural global nitrogen cycle (Fowler et al. 2013; Vitousek et al., 1997). Typically, more than half of applied Nr is lost to the environment through various biological pathways and chemical processes (Lassaletta et al., 2014; Raun and Johnson 1999)~~.~~, such as nitrate (NO_3^-) run-off into streams and waterways (Lu and Tian 2017) as well as gaseous losses in the form of ammonia (NH_3) (Bouwman et al., 1997), nitrous oxide (N_2O) (Reay et al., 2012), and nitrogen oxides (NO_x) (Bertram et al. 2005). This relatively low nitrogen use efficiency (NUE) results in significant environmental damage at a global scale. ~~caused by Nr lost into the environment, such as nitrate (NO_3^-) run-off into streams and waterways (Lu and Tian 2017) as well as gaseous losses in the form of ammonia (NH_3) (Bouwman et al., 1997), nitrous oxide (N_2O) (Reay et al., 2012), and nitrogen oxides (NO_x) (Bertram et al. 2005).~~

After fertiliser application, the resulting volatilization of NH_3, especially from urea, will often contaminate the surrounding environment with deposition of Nr, in some cases causing significant damage to fragile biodiversities by increasing nitrogen loading (Phoenix et al. 2006). Fluxes of NH_3 also contribute to an increase of particulate matter ($PM_{2.5}$) in the atmosphere which has negative implications for human health (Paulot and Jacob 2014). Agricultural sources contribute an estimated 60 % of global anthropogenic N_2O emissions (Syakila and Kroeze 2011), primarily due to increasing the quantity of Nr in soils and aquatic systems in which N_2O is released as a byproduct of the microbial processes of nitrification and denitrification (Davidson et al. 2000). N_2O is a potent greenhouse gas as well as the most significant contributor to global stratospheric ozone depletion (Ravishankara et al., 2009) which doubly increases the incentive to mitigate these emissions.

Current projections predict that global rates of Nr fertiliser will continue to rise over the next century in order to cope with a growing population and an increase in meat production (FAO, 2017), and therefore, it has become increasingly urgent to address the issue of nitrogen pollution from agriculture sources. However, food supply is a sensitive issue both politically and economically, with limited options available to governments or environmental regulators that may attempt to mitigate the damage caused by agricultural nitrogen pollution. One favorable option which potentially benefits all parties is to attempt to increase the NUE of Nr applied to crops, therefore maintaining high yields while reducing Nr lost to the environment in its various damaging forms. Typically, when fertiliser is applied, the water soluble nitrogen compounds permeate into the rhizosphere allowing plant roots to absorb the nitrogen and the microbial community to convert Nr through the processes of nitrification and denitrification into gaseous compounds (N_2O, NO_x & N_2) which may then be lost to the atmosphere (Davidson et al., 2000). In theory, by slowing the release of the Nr, plants can outcompete the microbial populations and less N escapes into air and ground waters as leachate. This can result in increased NUE,

decreased environmental impact, improved crop yields and reduced fertiliser costs for farmers making these efforts an attractive prospect for combatting global nitrogen pollution.

Several methods have been ~~trialed~~ tested to slow down the release of Nr from synthetic fertilisers. In its simplest form, this can be achieved by increasing the particle size of the applied fertilizer pellets (Azeem et al., 2014; Shamsudin et al., 2014). More complicated methods of Nr inhibition come in the form of microbial inhibitors which directly target and slow a specific biological pathway (Abalos et al., 2014; Modolo et al., 2015). Synthetic fertilisers (typically urea) coated with chemical inhibitors that target urease hydrolysis and microbial nitrification are already commercially available.

Microbial inhibitors have been shown to reduce Nr losses for both N_2O and NH_3 under laboratory conditions and in field trails, but with varying success (Sanz-Cobena et al., 2016; Ni et al., 2014; Singh et al., 2013; Rose et al., 2017; Ruser and Schulz 2015). Although there are positive studies which promote the pollution reducing capabilities of these chemicals (Misselbrook et al., 2014), some questions remain over the overall effectiveness of the inhibitors which face claims that reduction of one form of Nr pollution may ~~contribute to~~increase another. This is most commonly observed for nitrification inhibitors in which the slowing on the conversion of NH_4^+ to NO_3^- in soils results in a decrease in N_2O at the expense of an increase in NH_3 volatilisation (Lam et al., 2016; Zaman et al., 2009). In theory, the use of a urease inhibitor should reduce both the emission of NH_3 by reducing the rate at which urea is converted to NH_4^+ in soils, thus limiting available nitrogen in all forms. This may however, limit the rate at which crops also receive Nr and reduce yields. The use of inhibitors in farming remains uncommon, mostly due to a reluctance to change to an uncertain practice, compounded by the drawback that treated fertilisers are typically more expensive than traditionally used products. Further work using specific products in different environments is ~~required~~ needed to supply ~~the~~ evidence ~~required to~~ that will provide the agricultural community with the confidence to make the changes required to meet future NUE demands globally.

This study aims to specifically investigate the effect of the Agrotain® urease inhibitor (Koch, KS, USA) on a typical grassland silage crop in Scotland, comparing it with the two most commonly used synthetic nitrogen fertilisers: Ammonium nitrate (Nitram®) and urea. Grasslands account for approximately 60 % of agricultural land use in the UK (approximately 74,000 km^2) to which an estimated 120 kt of ammonium nitrate and 26 kt of urea are applied annually (BSFP, 2017). The results presented in this study are intended to represent to some extent this large coverage of agricultural land in the UK to which urease inhibitors may be applied in the future.

In this study we aim to:

- Compare the nitrogen use efficiency of equivalent applications of pellet fertilisers in the form of ammonium nitrate (Nitram), urea and urea with a urease inhibitor (the percentage of applied nitrogen fertiliser that is converted into plant matter as a result of increased crop growth).
- Investigate differences in crop quality and yield as a result of the fertilisers applied.
- Quantify gaseous losses of nitrogen from the fertiliser types in the form of NH_3 and N_2O.

2. Materials and methods

2.1. Experimental Design

Fieldwork was carried out between May 2016 and September 2017. During this time, five applications of three different nitrogen fertiliser types were added to a grid of experimental plots (including a control) in intensively managed silage grassland fields (*Lolium perenne L.*) at Easter Bush Farm (Midlothian, UK, 55°51'57.4"N 3°12'29.3"W). The three fertiliser types used in the experiment were ammonium nitrate pellets (Nitram, $NH_4^+NO_3^-$), urea pellets, and urea pellets with a coating of powdered urease inhibitor (N-(n-butyl) thiophosphoric acid Triamide; Agrotain®). In 2016, fertiliser was applied twice to experimental plots known as the Engineers Field (Cowan et al., 2016). In 2017, fertiliser was applied three times to experimental plots in an adjacent similarly managed field (known as the Upper Joiner field). ~~All fertiliser applications were of 70 kg N ha^{-1} (Table 1) which was consistent with the typical management regime of the fields. Both fields are used as grazing pastures for mainly sheep at high stocking densities of approximately 20 ewes per hectare. The sheep were vacated before and throughout the duration of the experiment and instead the grass was grown for silage.~~

The soil in both fields is classified as a clay loam for the top 30 cm in fields, with a pH (in H_2O) of 6.5 and 6.1 for the Engineers and Upper Joiner fields, respectively. They are classed as an imperfectly drained Macmerry soil of the Rowanhill association (eutric cambisol, FAO classification). All fertiliser applications were of 70 kg N ha^{-1} (Table 1) which was consistent with the typical management regime of the fields. Both fields are used as grazing pastures for mainly sheep at high stocking densities of approximately 20 ewes per hectare. The sheep were vacated before and throughout the duration of the experiment and instead the grass was grown for silage. While sheep were vacated from the 2016 field a month prior to the experiment, the 2017 plots had not been grazed for more than six months before the experiment.

For each of the five fertiliser events there were a total of sixteen plots; four treatments (including the control) replicated four times. The layout of the experimental plots varied in the two different fields. In 2016 the sixteen (Engineer's Field~~, pH = 6.5~~) plots were separated into strips of 2 m by 8 m (with a 0.5 m spacing between them). The treatments were assigned a random plot position in order to capture the spatial variability across the experimental area during measurements. In contrast, in 2017 the (Upper Joiner Field~~, pH = 6.1~~) plots were arranged in a square grid, each measuring 20 m by 20 m with no spacing between them. The treatments were also assigned at random across the grid in 2017 to capture spatial variability. For each fertiliser event the grass was allowed to grow for as long as the farm manager recommended for a full harvest (weather dependent), then all plots were harvested on the same day (see Table 1).

2.2. Crop Yield and Quality Measurements

Each of the plots was harvested and above-ground biomass was dried at 60 °C for 24 hours and both wet and dry weights were recorded. For the smaller 2016 plots, a 1 m^2 section of each plot was harvested manually using sheers (i.e. 1 sample per plot). For the larger 2017 plots, a small harvester with onboard weighing capabilities (Haldrup F-55) was able to harvest an area of 30 m^2 from which yield data were obtained. After wet yield was recorded, subsamples were taken from each of the individual plots for further analysis (at SRUC Analytical Services, Midlothian, UK). The dry matter content, metabolizable energy (ME), crude protein, modified acid detergent (MAD), decimal reduction time (D value), total carbon and total nitrogen contents were all analysed from the subsamples.

The nitrogen use efficiency (NUE) reported in this study refers to the crop uptake efficiency of the total nitrogen fertiliser applied. This was calculated by subtracting the mean total nitrogen content of the harvested grass from the control plots from the mean of the treatment plots for each individual event. The NUE for each treatment was then calculated by dividing this difference by the input of N fertiliser for a known area, thus providing the overall impact of the fertiliser on crop growth. Uncertainties in in these values are represented by 95 % confidence interval of the mean, calculated by multiplying the standard deviation by 1.96. The least squares method is used to combine uncertainties when subtraction or addition is used.

2.3. N_2O Flux Measurements

Measurements of N_2O fluxes were taken ~~during all of the growing seasons~~for both 2016 and 2017 experiments using the static chamber approach. The chambers consisted of a cylindrical polyvinyl chloride (PVC) plastic pipe of 38 cm inner diameter (ID) and 22 cm height fitted with a sealed lid and a flange at the base. The chambers were placed onto a plastic flanged collar that had been inserted several centimeters into the soil (on average 5 cm) to form a seal in the soil. A layer of draught sealant material held in place by four strong gripping clips formed an airtight seal between the chamber and the collar for the duration of the flux measurement. Chambers were closed for 60 min, during which time four gas samples were collected via a syringe and a three-way tap fitted to the lid, at t = 0, 20, 40 and 60 minutes. Gas samples were stored in 20 ml glass vials which were flushed with 100 ml of air from the syringe using a double needle. Samples were analysed using gas chromatography (7890B GC system fitted with an electron capture detector, Agilent Technologies, UK), with a limit of detection of 7 ppb (Drewer et al., 2017). Measurements were carried out daily for two weeks after fertilisation, then every second day for a further two to four weeks. Measurements were made only on working days (Monday to Friday) between 09:00 and 15:00 GMT.

Fluxes were calculated as:

$$F = \frac{dC}{dt} . \frac{\rho V}{A} \qquad \text{(Eq. 1)}$$

where F is the gas flux from the soil (nmol m^{-2} s^{-1}), d*C*/d*t* is the rate of change in the concentration in time in nmol mol^{-1} s^{-1} estimated by linear regression, ρ is the density of air in mol m^{-3}, V is the volume of the chamber in cubic meters and A is the ground area enclosed by the chamber in square meters.

Cumulative fluxes over the experimental periods (30 days) were calculated using a Bayesian approach, taking into account the log-normal distribution of spatial samples and the lognormal peak-and-decay pattern in time (Levy et al., 2017). Based on the assumption that at a given time, N_2O fluxes, F, are typically log-normally distributed in space, the probability density is given by:

$$f(F) = 1/(\sqrt{(2\pi)}\sigma_{\log}F)\exp(-((log(F) - \mu_{\log})^2/(2\sigma_{\log}^2))) \quad \text{(Eq. 2)}$$

where $\mu_{\log}$ and $\sigma_{\log}$ are the location and scale parameters, equivalent to the mean and standard deviation of the log-transformed variate.

Following a fertilisation event, the time course of N_2O flux is expected to rise to a peak, then decay exponentially, and this basic pattern is reproduced by all process-based models (i.e. Li et al., 1992; Del Grosso et al., 2006) and is also well described by the log-normal equation:

$$\mu_t = 1/(\sqrt{(2\pi)}kt)\exp(-((\log(t) - \Delta)^2/(2k^2))) \cdot N_{in}\Omega \quad \text{(Eq. 3)}$$

where μ_t is the spatial mean of the N_2O flux at time t, Δ and k are analogues for the location and scale parameters, and with the additional term N_{in} is the fertiliser nitrogen input and Ω is the fraction of this which is emitted as N_2O as *t* tends toward infinity. Δ can be interpreted as the natural logarithm of the delay between fertiliser application and peak flux; k is a decay rate term. So, at time t following fertilisation, the mean flux is given by:

$$\mu_{\log,t} = \log(\mu_t) - 0.5\sigma_{\log}^2 \quad \text{(Eq. 4)}$$

The parameters μ, $\mu_{\log}$ and $\sigma_{\log}$ were estimated using the Markov Chain Monte Carlo (MCMC) method with Gibbs sampling (Gelman, 2013). This was implemented using the freely available JAGS software (Plummer, 2016). The prior distribution for Ω was based on the data collated by Stehfest and Bouwman (2006). The prior distributions for Δ and k were based on the dynamics of the DNDC model (Li et al., 1992, as described in Levy et al., 2017). To obtain the cumulative flux at time t, we use the standard log-normal cumulative distribution function:

$$F_{cum,t} = \Phi\left(\frac{\ln t - \Delta}{k}\right) N_{in}\Omega \quad \text{(Eq. 5)}$$

where Φ is the cumulative distribution function of the standard normal distribution.

To account for background fluxes (fluxes of N_2O expected in the absence of any applied nitrogen), a cumulative background flux was estimated using the mean of the fluxes measured from the control plots during each event. This cumulative background estimate was then subtracted from the cumulative fluxes estimated for each treatment. The reported EFs in this study take background fluxes into account when reporting final values.

2.4. NH_3 Flux Measurements

During the 2016 measurements we were unable to obtain wind tunnels to measure NH_3 flux as originally planned. Therefore, in 2017 fluxes of NH_3 were derived using the FIDES inverse dispersion model as described in detail in Loubet et al. (2010 & 2017). This approach requires relatively large plots (20 m^2), and according to the farmers requirements needed to be set up in the Upper Joiner field, diagonally opposite from the Engineers field. The basis of the model is the solution of the advection-diffusion equation by (Philip 1959), assuming power law profiles for the wind speed (U(z)) and the vertical diffusity ($K_z(z)$). The model assumes that the atmospheric NH_3 concentration (χ in µg NH_3 m^{-3}) at a given point (x, y, z) is the sum of the background concentration (χ_{bgd} in µg NH_3 m^{-3}) unaffected by the sources, and the influence of the sources (Equation 6). The latter is equal to all the source strengths per unit surface area (S in µg NH_3 m^{-2} s^{-1}) at locations (x_s, y_s, z_s) multiplied by the dispersion function ($D(x_s, y_s, z_s|x, y, z)$ in s m^{-1}), which expresses the contribution of each source to each receptor point at which the concentration is considered. The meaning of $D(x_s, y_s, z_s|x, y, z)$ can be viewed simply as the concentration at location (x, y, z) for a source of unit strength at location (x_s, y_s, z_s). (Loubet et al. 2010, 2017)

$$\chi_{model}(x, y, z) = \chi_{bgd} + \int_{all\ x_s\ and\ y_s} S(x_s, y_s, z_s) D(x_s, y_s, z_s|x, y, z) \quad \text{(Eq. 6)}$$

In order to calculate S, D was computed by the model, and both χ and χ_{bgd} were measured. To calculate D, the description of Philip (1959) was followed as shown in Equation 7 – 10. Here, the values of a, b, p and n are derived from a linear regression between ln(U), ln(K_z) and ln(z), over the height range $2 \times z_0$ to 20 m, using U(z) and $K_z(z)$ estimated based on the Monin-Obukhov similarity theory (e.g. Kaimal & Finnigan, 1994), where z_0 denotes the roughness length. In Equation 9, $X = (x - x_s)\sin(WD) - (y - y_s)\cos(WD)$, and $Y = (x - x_s)\cos(WD) - (y - y_s)\sin(WD)$, where WD is the wind direction; $\alpha = 2 + p - n$, $v = (1 - n)/\alpha$, and I_{-v} is the modified Bessel function of the first kind of order −v. Finally, in Eq. 10 C_y and m are parameters taken from Sutton (1932).

$$U(z) = az^p \quad \text{(Eq. 7)}$$

$$K_z(z) = bz^n \quad \text{(Eq. 8)}$$

$$D(X, Y, z) = \frac{1}{\sigma_y\sqrt{2\pi}} exp\left(-\frac{Y^2}{2\sigma_y^2}\right) \times \frac{z z_s^{(1-n)/2}}{baX} \times exp\left(-\frac{a(z^\alpha + z_s^\alpha)}{ba^2X}\right) \times I_{-v}\left(\frac{2a(zz_s)^{\alpha/2}}{ba^2X}\right) \quad \text{(Eq. 9)}$$

$$\sigma_y = \frac{1}{\sqrt{2}} C_y x^{(2-m)/2} \quad \text{(Eq. 10)}$$

Wind data were recorded by two sonic anemometers (IRGASON, Campbell Scientific, UT, USA) which were positioned at the north east and south west sides of the plots, 30 m from the borders of the plots in alignment with the two wind predominant wind directions. The anemometers measured 3D wind components at 10 Hz. Following Loubet et al. (2001), the source height was tuned to z_s = 1.01 z_0 + d, where d is the displacement height, in order to insure best comparison with Lagrangian Stochastic models and experiments (see also Loubet at al. 2010). The dispersion model embedded in FIDES is essentially similar to the Foken and Meixner (2001) footprint model, except for the retrieval of the a, b, p, n parameters which are here inferred by fitting the wind speed and diffusivity profiles over a height range 0.2-20 m while in Foken and Meixner (2001) it was computed by forcing the profiles at a reference height. The FIDES model was shown to behave similarly to a Lagrangian Stochastic model in Loubet et al. (2017).

For the concentration measurements, Alpha passive air samplers (Tang et al., 2001) were used. These samplers are small hollow plastic tubes (27 mm ID) with a PTFE membrane which allows air to pass through. Inside there is a layer of filter paper coated with citric acid which traps atmospheric NH_3 and holds it in place within the sampler. This method enabled us to measure cumulative NH_3 concentrations at a fixed point, integrated over ~~over~~ a certain period of time (t) of sever~~v~~al hours or days ~~can be determined~~. To observe χ_{meas}, duplicate samplers were positioned at the centre of the 16 treatment plots (20 by 20 m) at heights of 30 and 50 cm. In order to measure χ_{bgd}, samplers were installed in triplicate at the four edges of the experimental grid, 30 m away from the plots. Samplers were placed immediately before fertilisation and removed/replaced 0.25, 1, 2, 3, 7 and 14 days after fertilisation. Samplers were stored at 4 °C after collection before extraction by deionised water and analysis using Ammonia Flow Injection Analysis (AMFIA, CEH Edinburgh, UK). Due to logistical constraints, we were limited in the number of measurements we could make using the FIDES method. Based on the extensive experience of the researchers in the field of NH_3 flux measurements, and numerous studies of NH_3 emissions (e.g. Gericke et al., 2011; Sanz-Cobena et al., 2011; Suter et al., 2013) we decided to measure for a period of two weeks, which would allow us to capture the vast majority of any cumulative emissions associated with the fertiliser event, which typically last only several days.

2.5. Soil Measurements

Soil cores were sampled from a distance of approximately 2 m from the static chambers (within the appropriate experimental plot) each time N_2O flux measurements were made. Cores were 3 cm in diameter and 10 cm in depth. Samples were frozen immediately after collection and stored at -18 °C until further processing up to three months later. Potassium Chloride (KCl) solution (50 ml, 1 mol L^{-1}) was used to extract Nr (in the form of NH_4^+ and NO_3^-) from the samples (15 g, wet soil). Having added

the 1 M KCl solution to the samples, they were subsequently mixed on an orbital shaker for 60 mins before the solution was filtered using 2.5 µm filter paper (Fisherbrand, US) and stored at -18 °C for analysis up to three months later. A further 10 g of mixed soil was dried to provide the dry soil ratio of each soil sample.

Concentrations of NH_4^+ and NO_3^- in the soil extracts were measured using a SEAL AQ2 discrete analyser (SEAL Analytical, US) fitted with a cadmium coil. The widely used phenol-hypochlorite (for NH_4^+) and sulfanilamide (NO_2^- & NO_3^- after cadmium coil reduction) methods were used to provide the relevant colorimetry reactions. Concentrations of NH_4^+ and NO_3^- in soil was then calculated based on the mass of dry soil in the initial KCl extraction.

2.6. Meteorological data

~~Measurements of soil temperature and volumetric soil moisture were made using handheld probes (31/162/0, Brannan, UK & Hydrosense II, Campbell Scientific, UT, US) next to each flux chambers and when chamber measurements were carried out.~~ Long term meteorological and soil measurements were recorded at the permanent Easter Bush measurement station, which was situated at the edge of the Engineer's Field. This station provided measurements of air temperature (1.8 m), soil temperature (0.3 m depth) and rainfall (tipping bucket) at 30 min intervals throughout the measurement campaigns (Fig. 1).

3. Results

3.1. Crop Yield, NUE and Quality

Although rainfall and temperature was similar during both years of measurement, ~~Crop~~ crop yields for all treatments were substantially larger in the 2016 field plots (5.5 t ha^{-1}) than the 2017 field plots (1.48 t ha^{-1}) (Table 2~~)). The yields from the control plots were exceptionally high in 2016~~, indicating that the Engineer's field was the more productive of the two experimental areas regardless of fertiliser application or meteorological conditions. There was reasonably large variation in yield measurements from the harvests in both fields, and in some cases (October 2016) the effect of the addition of fertiliser (i.e. dry control yields subtracted from dry yields of fertilised plots) appeared to have a negative effect on yield (although these values fall well within the large uncertainty range around zero). The most efficient fertiliser overall was Nitram, increasing yields (after subtraction of the control) on average ~~-~~by 1.05 ± 0.61 t ha^{-1} with a mean NUE of 35.5 %. Urea and inhibitor coated urea increased yields by an average of 0.66 ± 0.62 and 0.69 ± 0.73 t ha^{-1}, respectively. Nitram treatment was found to increase yields significantly (p-value < 0.05) when compared to the urea fertilisers. The treated urea had a slightly higher average NUE than the untreated urea (24.6 and 20.7 %, respectively), but this difference was not statistically significant (p-value = 0.91).

Crude protein (and therefore nitrogen) content of the fertilised plots (154 g kg^{-1}) was typically higher than that of the control plots (102 g kg^{-1}) for all fertiliser treatments; however, there were no outstanding differences between the treatment types. Differences in metabolizable Energy (Grass ME), modified acid detergent (MAD) and decimal reduction time (D value) between the fertiliser treatments were also small, and varied more between the two field sites than the fertiliser types ~~(see Table 2)~~. These indicators of digestibility and energy content are commonly used to indicate the quality of the silage grass for animal feed and our study suggests that there was no significant differences between the feedstock grown using the different fertilisers.

3.2. N_2O Fluxes

N_2O fluxes from the chambers ranged from -0.39 to 24.47 nmol m^{-2} s^{-1} and showed a log-normal spatial distribution. The majority of flux measurements were close to zero with 81 % below 1 nmol m^{-2} s^{-1} in magnitude (Fig. 2). Observed fluxes increased in magnitude from the plots treated with Nitram immediately after fertilisation, typically peaking within a week of the Nr application. Fluxes also increased after the urea and inhibitor coated urea applications, although the timing of the peaks in these emissions ~~typically appeared several days after~~ were more variable than those observed from the Nitram plots.

Cumulative flux estimations of N_2O from the individual fertilisation events have a typical large relative uncertainty, due to the difficulty in extrapolating measurement data both spatially and temporally from small data sets. In this study we have chosen to calculate cumulative fluxes using the Bayesian model outlined in equations 2 to 5 rather than the trapezoidal method (linear interpolation between mean values) in order to better represent this uncertainty (Levy et al., 2017). Regardless of the large associated uncertainties in cumulative flux estimates, our measurements show that the Nitram fertiliser results in significantly larger N_2O emissions when compared to the urea and inhibitor coated urea applications of the same quantity of Nr (p-value < 0.05) (Table 3). In four of the five events, Nitram was the highest N_2O emitting fertilizer of the treatments after 30 days (minus background from control plots) with a mean EF between replicates of 0.76 ± 0.63 % (Table 3). Emissions from the urea and the inhibitor treated urea were comparable in magnitude, 0.29 ± 0.27 % and 0.36 ± 0.15 % of the applied Nr, respectively.

3.3. NH_3 Fluxes

Ammonia fluxes were only measured during the 3 fertilisation events in 2017. The majority of the NH_3 emissions occurred between 0 and 5 days after fertiliser was applied, and emissions beyond 7 days after fertiliser application were largely negligible. Emissions of NH_3 from the plots varied widely with cumulative flux values from individual plots ranging from -1.8 to 13.1 kg N ha^{-1} at the end of the 14 day measurement period (Fig. 3 & Table 4). Emissions from the plots treated with urea fertiliser were

consistently higher than those of the other treatments after fertiliser applications. Mean cumulative emissions for each of the fertiliser types after all three fertilisation events (n= 12) were -0.74, -0.95, 10.83 and 0.42 kg N ha^{-1} for the control, Nitram, urea and inhibitor treated urea, respectively.

~~Cumulative fluxes of NH_3 measured from the individual plots varied widely, with differences~~ Differences in NH_3 from individual plots was typically larger than an order of magnitude of the mean value of the grouped treatments. As the control plots represent a near zero influence situation, the mean flux observed from the control plots for each event were subtracted from the fluxes associated from the treatment measurements. Based on this, emissions from the urea treated plots (mean of 16.5 ± 5.0 % of applied N) were considerably higher than each of the other treatments (-0.3 ± 1.8 % and 1.66 ± 2.0 % for Nitram and the inhibitor coated urea, respectively). Fluxes measured from the Nitram plots were not significantly different to those from the control plots (p-value = 0.42), but emissions from the inhibitor coated urea were (p-value < 0.1).

3.4. Soil Chemistry

As shown in Fig. 4, concentrations of NH_4^+ varied by several orders of magnitude, with individual measurements ranging from 1.3 to 1525 mg of nitrogen per kg of soil sampled (mg kg^{-1}). Concentrations of NH_4^+ were consistently low in the experimental plots before fertiliser application; with the exception of the first fertiliser event in 2016 where elevated Nr was observed in the control plots, possibly due to residues from sheep grazing in the field close to one month before the experiment began. Concentrations of NH_4^+ typically rose in magnitude for several days after fertiliser application before returning to pre-fertiliser magnitudes by the end of the measurement period. Concentrations of NH_4^+ in soils treated with urea and inhibitor coated urea were typically higher than those that received Nitram fertiliser. During the third fertiliser event (13/03/17) there was a clear delay in the rate at which urea was hydrolysed into NH_4^+ in the soil (Fig. 4). This phenomenon was not observed during the other events.

Concentrations of NO_3^- ~~in soils varied on a log-normal scale~~ followed a log-normal distribution in a similar fashion to the NH_4^+ concentrations. Nr in the form of NO_3^- was typically lower than that of NH_4^+~~NH_3~~ with measured values ranging from 0.05 to 165 mg kg^{-1}. As with NH_4^+, NO_3^- concentrations in the experimental plots were near zero before fertiliser application, with the exception of the first event. After Nitram application, NO_3^- concentrations typically rose then ~~decayed~~ decreased in concentration with time. ~~The urea and inhibitor coated urea behaved differently at the two measurement sites. For the 2016 measurements the urea fertilisers behaved in a similar fashion to the Nitram, but during the 2017 measurements there was typically a delayed rise then decay after application (see Fig. 4).~~

4. Discussion

The yield and nitrogen uptake of the silage crop varied widely across the plots and seasons during the experiment. The quantity of the applied fertiliser that was consumed by the crops ranged from a maximum of 66 % to a negative value of -16 % compared with the adjacent control plots. As there ~~was~~ were only small differences between the total N content of the crop for the three different fertiliser types, the percentage of applied N that was present in the harvest from the plots scales closely with the overall dry yield. In this respect, the Nitram treated plots have the highest NUE of the three treatments with a mean NUE of 35 ± 19 % when compared to urea (21 ± 15 %) and the inhibitor treated urea (24 ± 20 %).

The perceived negative effect of fertiliser application during the 2016 trials ~~trails~~ may have been influenced by a considerably large amount of clover that had begun to grow in the plots by late spring. The nitrogen fixing properties of the clover may have had some impact on the results of the experiment, although not atypical of grazed grasslands (Marriott, 1988). We speculate that ~~T~~the prior grazing of the sheep is also likely to have resulted in the residues of animal waste in the 2016 plots, which would explain the higher than expected yields and Nr in the soil measurements in these plots (Cowan et al., 2015). Although unintentional, the presence of these two factors sheds some light into the importance of N-fixation and animal waste in grazed fields which often receive similar applications of N fertiliser as arable crops. The 2016 plots in our study show~~s~~ that when there is a large amount of Nr already present in the soils, the application of further Nr can have negligible effect on yield, while still contributing to N pollution. This highlights the future potential of precision farming methods which could take into account the spatial variability of Nr already present in the field and attempt to improve NUE by better managing where fertiliser is required, and where it is not (Auernhammer, 2001; Kindred et al., 2017).

The 2017 plots did not appear to be influenced by clover growth or residues of animal waste after visual inspection, and subsequently the observed NUE was more comparable to values considered typical under ~~in~~ the conditions (Raun and Johnson 1999). Overall, the Nitram application resulted in the highest average yield, but there was little difference in yield observed between the urea and inhibitor coated urea in this study. The crude protein content of the silage harvests varied largely between events, but the treatment effect was small and inconsistent. Differences in metabolizable Energy (Grass ME), modified acid detergent (MAD) and decimal reduction time (D value) between the fertiliser treatments were also small, with little variation observed between the events and the treatment types.

Emissions of N_2O were higher from the plots treated with Nitram fertiliser than from the other treatments. This observation is consistent with previous research which has identified Nitram as a higher emitter than urea fertiliser (DEFRA, 2006; Harty et al., 2016). Previous studies highlight a potential for pollution swapping with nitrification inhibitor treated urea (typically dicyandiamide, a.k.a.

DCD), suggesting ~~that a reduction in NH_3 emissions results in a higher N_2O production (Lam et al. 2016).~~ that by reducing the rate of conversion of NH_4^+ to NO_3^- in soils that NH_3 emissions are increased (Lam et al., 2016; Zaman et al., 2009). Elevated N_2O and NH_3 emissions have been observed on occasion after the use of nitrification inhibitors (Scheer et al., 2017; Zaman et al., 2009); however reductions in both have also been observed (Di et al., 2006; Misselbrook et al., 2014) . This should not be the case for urease inhibitors as it slows the release of Nr from the applied fertiliser, thus reducing the potential of N_2O and NH_3 emissions. ~~Although~~ Previous studies have shown that the use of urease inhibitors can significantly reduce N_2O emissions (Singh et al., 2013; Zaman et al., 2009). In this study, emissions from the inhibitor treated urea were slightly larger overall compared to the urea~~,~~; however, the treatments behaved similarly throughout the experiment and the differences observed in this study were not statistically significant (p-value = 0.42). The emissions of N_2O were not found to correlate well with any of the measured environmental variables such as rainfall or temperature, ~~although this~~which is not uncommon. The wide variety of complex interacting conditions that influence microbial processes often prevent predictive modelling and correlation with environmental variables (Butterbach-Bahl et al., 2013).

Emissions of NH_3 ~~observed~~ calculated using the FIDES method were consistently largest from the plots treated with urea fertiliser (mean EF of 16.5 % of applied Nr). The emissions from the Nitram plots were not significantly different from the control plots, suggesting that emissions were negligible from this treatment. These observations agree with previous studies in that urea treatments are expected to lose a large fraction of Nr as NH_3 emissions (Sommer et al., 2004) while Nitram ~~is~~ treatments do not (DEFRA, 2005). The urease inhibitor appears to have significantly reduced NH_3 losses from the inhibitor coated urea plots, reducing emissions of NH_3 by approximately 90 % when compared to the untreated urea. This effect has been observed in other similar studies when applying a urease inhibitor to urea fertiliser (Li et al., 2015: Rawluk et al., 2001). The large reduction in NH_3 volatilisation and lack of yield response does raise the question of the fate of the Nr in the urease treated urea plots.

After the N content of the crop, the N content of the soil and emissions of N_2O and NH_3 are taken into account, ~~The~~ the majority (> 55 %) of applied Nr in the experiments remains unaccounted for by the time of harvest. Typically, Nr in the form of NH_4^+ and NO_3^- in the top 10 cm of soil has ~~fallen considerably in magnitude come harvest, returning~~ returned to concentrations ~~near zero~~ on par with the control plots by harvest. When compared to the control plots, the remaining extractable Nr in the top 10 cm of the fertiliser treated plots at time of harvest accounted for less than 1 % of the applied nitrogen in all cases in this study. Other known pathways for large losses of Nr from agricultural soils include the leaching of NO_3^- into deeper soils and water systems, uptake of Nr into root systems, and microbial nitrification and denitrification which produces nitric oxide (NO) and gaseous nitrogen (N_2). Leaching can account for 2 - 33 % of applied Nr (Riley et al. 2001; Sebilo et al. 2013; Skinner et al. 1997), root systems may consume Nr in the same order of magnitude as the harvested shoots (Watson, 1987)

and microbial emissions of NO and N_2 can account for Nr losses of an order of magnitude higher than N_2O ~~in the right conditions~~ when water filled pore space (WFPS) is particularly low (< 40 %) or high (> 80 %) (Davidson 1993; Weier 1993). All of these potential processes may account for a significant fraction of the unaccounted Nr applied to the plots in this experiment and measurements should be included in future studies when logistically possible.

5. Conclusions

Large variations in crop yield measurements show that none of the fertiliser types used in this study consistently outperforms the others in terms of NUE. However, of the three fertilisers used, Nitram performed better on average than the urea compounds in this experiment with an average NUE of 35% when compared to urea (21 %) and the inhibitor treated urea (24 %). This study supports previous research which suggests that Nitram is the largest emitter~~s~~ of N_2O (0.76 % of applied Nr) and that urea fertiliser is the largest emitter of NH_3 (16.5 % of applied Nr) when the mineral fertilisers are compared. The use of the urease inhibitor resulted in a considerably large reduction in NH_3 losses from the urea fertiliser (decrease of 90 %) without significantly increasing emissions of N_2O; however, yields were statistically the same. The results of this study suggest that urease inhibitors, such as Agrotain®, can play an important role in mitigating Nr-related air pollution. However the agronomic benefits to the farmer appear to be negligible. With the higher costs of urea coated with urease inhibitors, there is no incentive for farmers to switch to these more environmentally friendly compounds. Our experiments are short term only. There certainly is a need for more long-term studies covering different climate zones, crop types and soil properties to investigate the economic and environmental benefits of switching from the preferred ammonium nitrate fertilisers in the UK to urea treated with urease inhibitors, or even double inhibition using nitrification and urease inhibitors.

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7. Author Contribution

N. Cowan managed the fieldwork, carried out data analysis and wrote the manuscript. P. Levy contributed to the Bayesian statistics and was involved in the writing of the manuscript. A. Moring, B. Loubet and P. Voylokov worked on the data analysis and the FIDES method. I. Simmons, C. Bache, A.

Stephens, J. Marinheiro, J. Brichet, L. Song, A. Pickard, C. McNeill, R. McDonald and J. Maire were involved in the fieldwork and laboratory analysis stages of the research. M. Sutton provided guidance on the measurement aspects of ammonia and helped develop the data analysis. U. Skiba is the primary investigator of the Cinag project at CEH Edinburgh, managing the project overall, contributing to all aspects of the research and the writing of the manuscript.

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673 **Table 1** Management of experimental plots over five fertilization events at Easter Bush Farm, 2016 &
674 2017. 70 Kg-N ha-1 was applied each time.

Field	Event	N Application	Harvest	No. of Plots	Plot Size	Days of Crop Growth
Engineers	1	13/06/2016	15/07/2016	16	16 m^2	32
Engineers	2	27/07/2016	03/10/2016	16	16 m^2	68
Upper Joiner	1	13/03/2017	25/05/2017	16	80 m^2	73
Upper Joiner	2	12/06/2017	19/07/2017	16	80 m^2	37
Upper Joiner	3	07/08/2017	15/09/2017	16	80 m^2	39

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677 **Table 2** Crop quality measurements of sub-samples taken from harvests of all experimental treatment
678 plots. Mean values and standard deviation of samples are provided (n = 4 replicates). Effect of N
679 addition is reported as the additional dry matter (DM) harvested compared to the control plots. The
680 total N content of the dry matter and NUE for each event are presented.

Event	Treatment	Dry Yield (t ha^{-1})	Effect of N Addition (t ha^{-1} DM)	Crude Protein (g kg^{-1})	N content (g kg^{-1})	NUE (%)
2016						
1	Control	6.7 ± 0.8		72.2 ± 6.2	11.6 ± 1	
1	Nitram	8.5 ± 0.5	1.8 ± 0.9	95.2 ± 15.3	15.2 ± 2.5	39.1
1	Urea	8 ± 1.2	1.3 ± 1.4	93.8 ± 21.5	15 ± 3.4	27.9
1	Urea & Inhibitor	7.9 ± 1	1.2 ± 1.3	111.8 ± 12.8	17.9 ± 2.1	30.7
2	Control	3.4 ± 1.1		120.8 ± 8.1	19.3 ± 1.3	
2	Nitram	3.8 ± 0.2	0.4 ± 1.1	122 ± 12.1	19.5 ± 1.9	11.1
2	Urea	2.9 ± 0.4	-0.5 ± 1.2	116.2 ± 28.3	18.6 ± 4.5	-13.3
2	Urea & Inhibitor	2.8 ± 0.8	-0.6 ± 1.3	117.8 ± 14.8	18.8 ± 2.4	-16.1
2017						
1	Control	0.6 ± 0.2		78.9 ± 3.8	12.6 ± 0.6	
1	Nitram	2.4 ± 0.8	1.8 ± 0.8	160.5 ± 37.4	25.7 ± 6	66.1
1	Urea	1.6 ± 0.2	1 ± 0.3	102.2 ± 5.4	16.4 ± 0.9	23.4
1	Urea & Inhibitor	2.1 ± 0.4	1.6 ± 0.4	130.9 ± 40.2	20.9 ± 6.4	47.8
2	Control	1.1 ± 0.3		94.8 ± 9	15.2 ± 1.4	
2	Nitram	2 ± 0.2	0.9 ± 0.3	191.8 ± 35.5	30.7 ± 5.7	27.6
2	Urea	2.1 ± 0.3	1 ± 0.4	165 ± 23.8	26.4 ± 3.8	26.4
2	Urea & Inhibitor	1.8 ± 0.3	0.8 ± 0.4	173.8 ± 9	27.8 ± 1.4	22.2
3	Control	0.7 ± 0.3		141 ± 13	22.6 ± 2.1	
3	Nitram	1 ± 0.4	0.4 ± 0.5	236.8 ± 31.9	37.9 ± 5.1	15.2
3	Urea	1.2 ± 0.6	0.5 ± 0.7	241.8 ± 17.9	38.7 ± 2.9	19.4
3	Urea & Inhibitor	1.1 ± 0.2	0.5 ± 0.4	251.8 ± 14.9	40.3 ± 2.4	20.2

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683 **Table 3** Cumulative N_2O fluxes estimated using the Bayesian interpolation method over a 30 day period
684 after fertilizer applications (70 kg N ha^{-1}) at two intensively managed grassland sites. Values presented
685 represent 4 plots (n = 4) per event at each field site. Emission factors (EF) account for the effect of N
686 application after the measured background flux has been deducted from cumulative totals.

Event	Fertiliser Type	Background Flux (kg N ha^{-1})	Cumulative Flux (kg N ha^{-1})	95 % C.I. min	95 % C.I. max	Flux Minus Background (kg N ha^{-1})	EF (%)
2016							
1	Nitram	0.25	1.59	1.02	2.86	1.34	1.92
1	Urea	0.25	0.52	0.37	0.78	0.27	0.38
1	Urea & Inhibitor	0.25	0.54	0.37	0.90	0.28	0.41
2	Nitram	0.19	0.45	0.32	0.68	0.25	0.36
2	Urea	0.19	0.30	0.24	0.40	0.11	0.15
2	Urea & Inhibitor	0.19	0.29	0.23	0.40	0.10	0.14
2017							
1	Nitram	0.92	1.39	0.97	2.26	0.48	0.68
1	Urea	0.92	0.99	0.72	1.48	0.07	0.10
1	Urea & Inhibitor	0.92	1.33	0.87	2.46	0.41	0.58
2	Nitram	0.51	0.50	0.39	0.67	-0.01	-0.01
2	Urea	0.51	1.06	0.64	2.10	0.55	0.79
2	Urea & Inhibitor	0.51	0.67	0.50	0.97	0.17	0.24
3	Nitram	0.93	1.53	1.08	2.34	0.60	0.85
3	Urea	0.93	0.97	0.77	1.27	0.04	0.05
3	Urea & Inhibitor	0.93	1.22	0.89	1.83	0.29	0.41

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689 **Table 4** Cumulative fluxes of NH_3 estimated the FIDES method over a 14 day period after fertilizer
690 applications (70 kg N ha^{-1}) at the Upper Joiner grassland. Values presented represent 4 plots (n = 4) per
691 event at each field site. Emission factors account for the effect of N application after the measured
692 background flux has been deducted from cumulative totals. The 95 % C.I. is calculated using the least
693 squares method to combine the standard error between the replicates for each treatment.

Event	Fertiliser Type	Cumulative Flux (kg N ha^{-1})	Std. Error in Cumulative Flux (kg N ha^{-1})	Flux Minus Background (kg N ha^{-1})	95 % C.I. (kg N ha^{-1})	EF (%)
1	Control	0.36	1.19			
1	Nitram	-0.83	1.28	-1.19	1.75	-1.70
1	Urea	11.37	1.76	11.01	2.13	15.73
1	Urea & Inhibitor	0.65	1.36	0.29	1.81	0.41
2	Control	-0.75	0.46			
2	Nitram	-1.19	1.05	-0.44	1.14	-0.63
2	Urea	8.04	0.99	8.79	1.09	12.56
2	Urea & Inhibitor	-0.16	0.88	0.60	0.99	0.86
3	Control	-1.81	1.77			
3	Nitram	-0.82	3.17	0.99	3.63	1.42
3	Urea	13.09	3.34	14.90	3.78	21.29
3	Urea & Inhibitor	0.78	1.81	2.60	2.54	3.71

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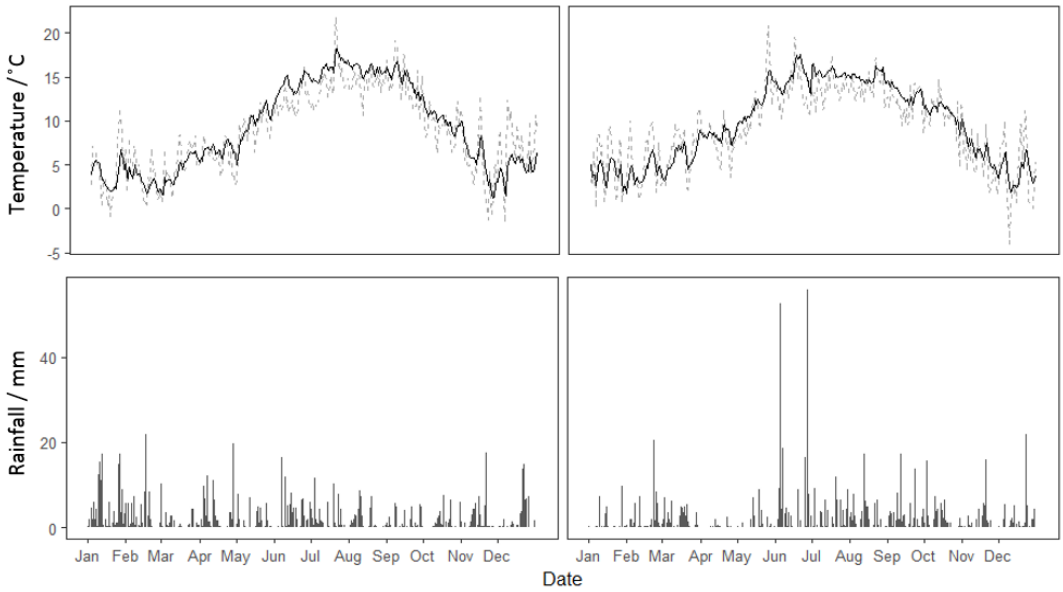

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697 **Figure 1** Meteorological data recorded at Easter Bush Farm over 2016 (left) and 2017 (right). Daily mean
698 soil temperature (black) and air temperature (grey) and daily cumulative rainfall are presented.

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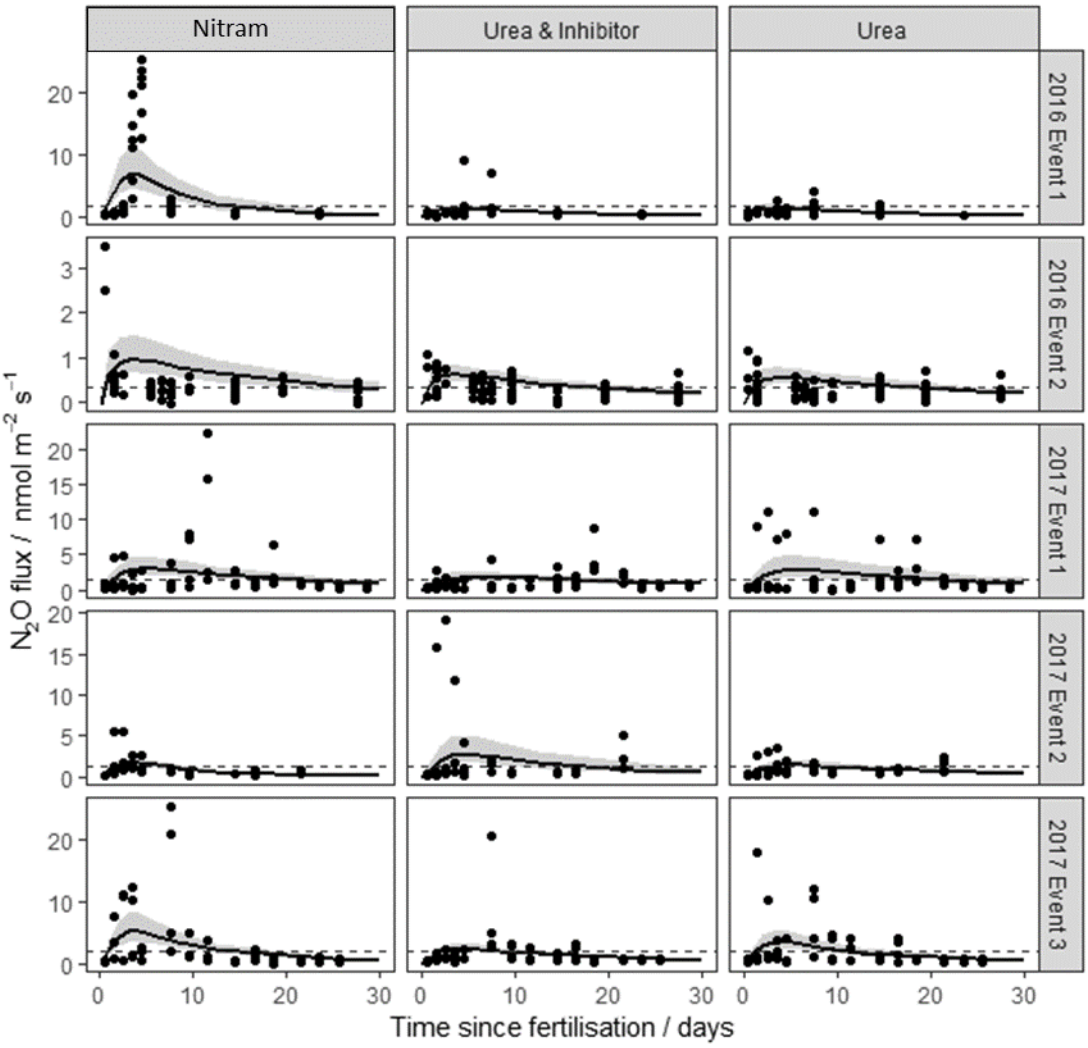


Figure 2 N_2O fluxes following fertilisation of the Engineer's field in 2016 and Upper Joiner field in 2017. Fertiliser was applied at t = 0 days, and the measurements lasted up to 30 days for each event. The log-normal model was used to estimate cumulative N_2O fluxes. The 95 % credible intervals of the posterior predictions are shown as the shaded area. Mean background fluxes from control plots are included for each event (dashed line).

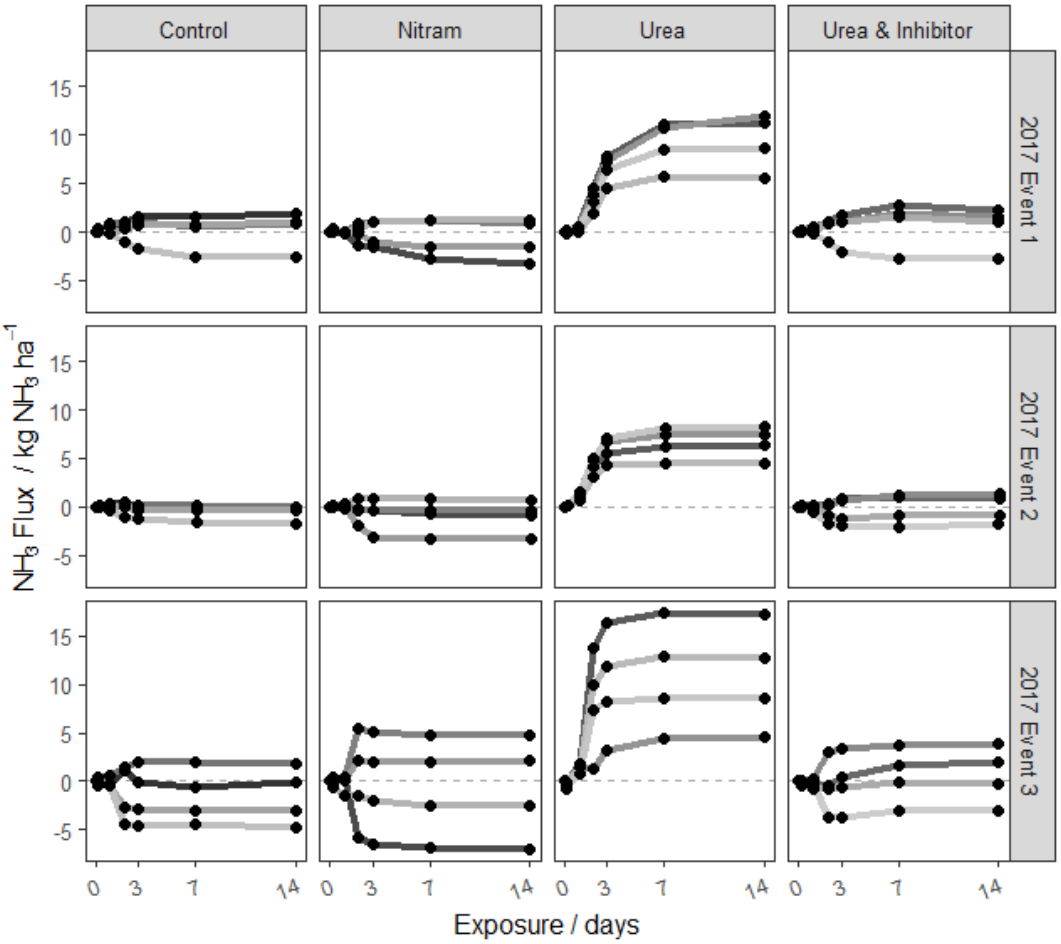
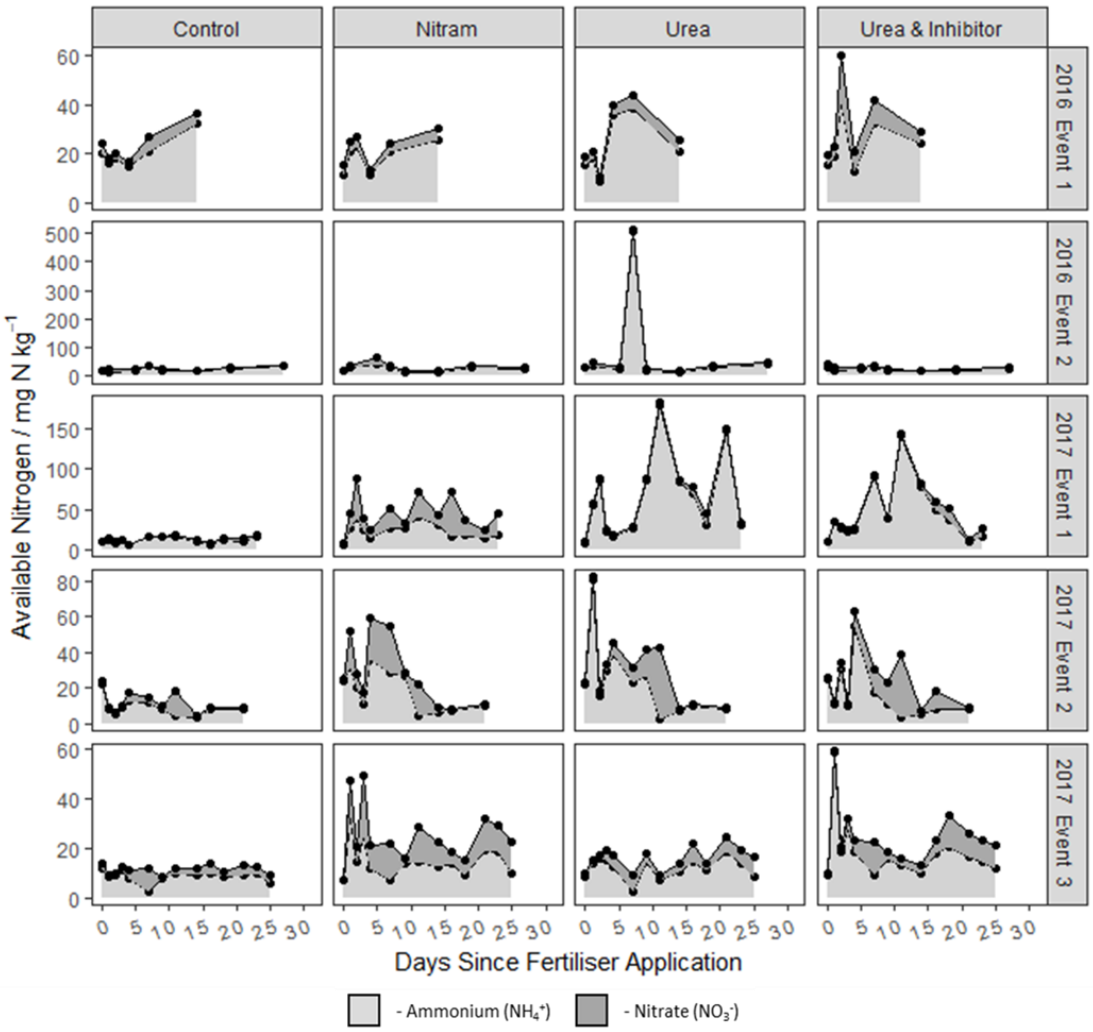


Figure 3 Cumulative fluxes from each of the experimental plots during three fertilisation events measured using the FIDES method (2017). Each shaded line represents one of the four plots replicated for each treatment.



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715 **Figure 4** Median available nitrogen concentrations measured in tandem with N_2O chamber

716 measurements after fertilisation events.

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