An Investigation Into the Effect of pH Level and Fertilizer Form on Nitrous Oxide Production from Agricultural Soils

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Abstract

Effective mitigation of climate change requires substantial reduction of greenhouse gas emissions across all sectors. As a result of the increasing use of nitrogen fertilizers, agricultural land has become the primary source of global N_2O emissions. Emissions of N_2O are set to rise as the pressures of global food insecurity require ever increasing agricultural production. The aim of this study is to investigate how inorganic fertilizer form and pH level effect N_2O emissions over four sampling days after application. This project hopes to shed light on the mechanisms of N_2O production and how these processes are altered by changing soil parameters. Cores were collected from *Westmill Organics* farm in Oxfordshire and a laboratory experiment was carried out using inorganic fertilizers. The experiment compared ammonium nitrate (AN), urea + nitrification inhibitor (U(NI)) and unfertilized control cores at two pH levels: ambient (7.8) and lowered (5.7). The results indicate that the NI was effective in reducing N_2O emissions by approximately 90% compared to the AN ($p<0.05$). The results also demonstrate the enzyme inhibitory effects of lowering the pH as both the AN and control cores exhibited significantly lower N_2O emissions from acidified cores compared with ambient. The same cannot be said for the U(NI) as no significant difference was observed between pH levels with this fertilizer. No significant change over time was observed in any of the fertilizer groups apart from AN which increased slightly over the four day sampling period. The findings of this project suggest that pH level interacts significantly with inorganic fertilizers with respect to N_2O emissions. Results suggest that decisions of fertilizer and inhibitor type should be made on a case-by-case basis. Broad scale strategies to mitigate N_2O production from agriculture may not be appropriate due to the wide variety of soil parameters which impact N_2O emission.

Key terms;

N_2O, Nitrification, Denitrification, Ammonium Nitrate, Urea, nitrification inhibitor

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1 Introduction

Nitrous oxide (N_2O) has a greenhouse warming potential which is 300 times greater than CO_2 (Misselbrook et al, 2014) and is estimated to contribute approximately 6% of the total global anthropogenic radiative forcing (Laville et al, 2011). The primary source of N_2O is agricultural land with approximately 70% of the atmospheric loading of N_2O emanating from agricultural soils (Baggs, 2011). There has been a 20% increase in N_2O emissions in past 100 years due to the intensification of agriculture and the wide scale adoption of nitrogen fertilizers such as ammonium nitrate (AN) and urea (U) (Robinson et al, 2014). As well as contributing to greenhouse warming, N_20 is also the largest stratospheric ozone-depleting substance and is projected to remain so for duration of the century (Shcherbak et al, 2014).

It is imperative to reduce GHGs emission on a global scale, however the reduction of N_2O from the agricultural sector produces something of a dilemma between the need to mitigate climate change yet also the necessity of addressing world food insecurity. By 2050 global crop production is predicted to rise to 1343, 915 and 891 million tons for maize, rice and wheat respectively (53%, 27% and 32% higher than production in 2012) (Cui et al, 2014). A significant challenge lies in increasing the production to these levels whilst simultaneously reducing N_2O emissions. Research on the subject is therefore highly relevant.

1.1 Fertilizer form and N_2O emissions

In order to understand how fertilizer application results in an increased N_2O flux from agricultural soils one must understand the processes which produce N_2O in soils in general. Nitrification and denitrification are the two main processes which are responsible for N_2O production in soils. Nitrification is an aerobic process which involves the oxidation of ammonium (NH_4^+) to nitrate (NO_3^-) and denitrification is the anaerobic reduction of NO_3^- to dinitrogen gas (N_2) (Dobbie and Smith, 2003). N_2O is the gaseous intermediate of both as shown in figure 1. The rates of these processes are limited by the amount of mineral nitrogen within the soil ($NH_4^+$and NO_3^-), the water-filled pore space (WFPS) and the soil temperature (Dobbie and Smith, 2003).

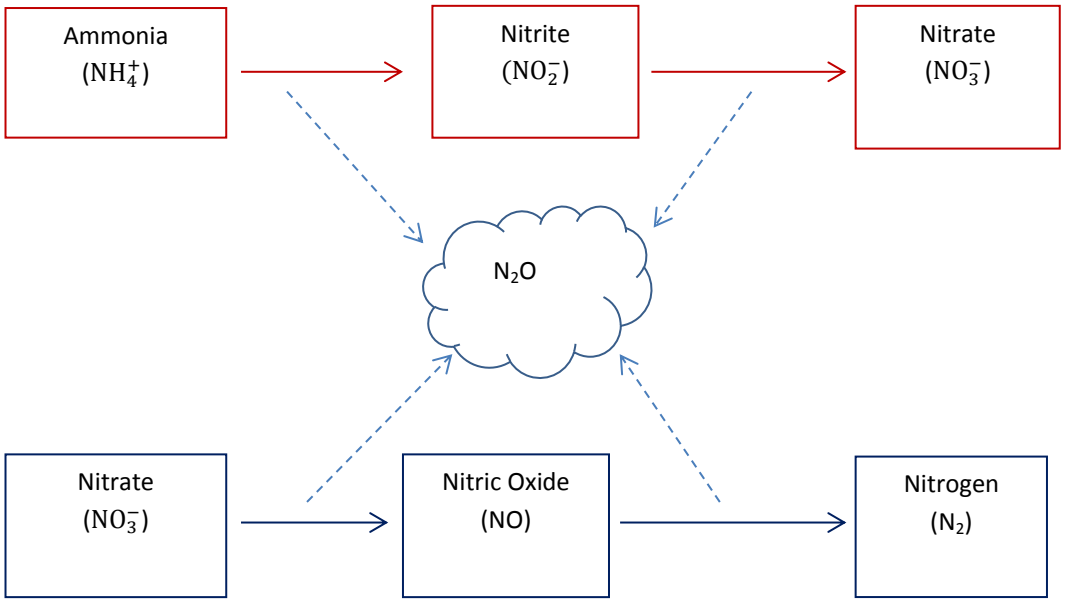

Figure 1 describes the form of nitrogen within aerobic nitrification (red) and anaerobic denitrification (blue).

Fertilizers promote nitrification and denitrification by providing an excess of mineral N (NO_3^- and NH_4^+). Thus the rates of both processes increase shortly after fertilizer application and produce large fluxes of N_2O. Fertilizer can be either organic (slurry, manures etc) or inorganic. AN and U are the predominant inorganic fertilizers used in the UK (Jones et al, 2007). Fertilizers are used to increase plant yield in agricultural systems and the decision to use one form of fertilizer over another may be influenced convenience, access, cost etc.

Although both organic and inorganic fertilizers promote N_2O emissions from soil, the effect of each is not the same. Organic manure treatments result in higher and longer lived peaks of N_2O than either AN or U. Emission peaks from AN and U are much shorter and generally subside to background values after much shorter time periods. Even when application of available N is the same between organic and inorganic fertilizers there is a five-fold higher NH_4^+ soil concentration on organically fertilized plots compared to AN or U treatments. This may partly explain why organic treatments increase N_2O more than inorganic fertilizers as NH_4^+ is a substrate for nitrification (Jones et al, 2007).

The difference between organic fertilizers and inorganic fertilizers is well understood. However, the difference between specific inorganic fertilizers (such as AN and U) is much smaller and at some points negligible. Applications of fertilizer in spring yield larger N_2O emissions from soils treated with AN compared with U yet in the summer months there is no difference between the two treatments. Average emission factors (percentage of nitrogen applied which is subsequently lost as N_2O) has been calculated for both AN and U in grassland soils across 13 sites. AN treated grasslands was found to have average emission factor of 2.75% (standard deviation = 0.56%) and U was found to have an emission factor of 2.12% (standard deviation = 0.44%). However, a t-test showed that the two treatments were not significantly different ($p > 0.01$) (Dobbie and Smith (2003) and Clayton et al (1996)).

1.2 Nitrification inhibitors and N_2O emissions.

Nitrification inhibitors (NI) work by slowing down the conversion of ammonium (NH_4^+) to nitrite, and the conversion from nitrite to nitrate (NO_3^-) (Misselbrook, 2014). This reduces N_2O emissions because the oxidation of ammonia and nitrite produces N_2O. Despite the name NIs work to inhibit denitrification as well as nitrification and have been proven in numerous studies to significantly reduce emissions where denitrification has been the dominant source of N_2O. For example, Clough et al (2007) found a significant reduction in emissions from waterlogged soil fertilized with urea when used together with a NI. NIs are available in many different forms and it is not always completely clear as to which one is the most effective (Sawamoto et al, 2010).

A meta-analysis of 113 datasets from 35 studies revealed that NIs are effective overall in reducing N_2O emissions (Akiyama et al, 2010). Inorganic fertilizers (AN and U) were applied to soil with and without the addition of a NI. A mean reduction of -38% was observed (with a range of 44% to -31%) in both fertilizers. The type of NI applied is dependent upon climatic

factors such as temperature and precipitation and also influenced by soil management practices such as application rate and by capital cost and availability.

The relationship between NI use and a reduction in mean N_2O emissions from fertilized land is well established yet the range of effectiveness has been somewhat contested. There is uncertainty regarding how NIs respond to changes in soil parameters. In order to ascertain how fertilizer type and NIs effect N_2O emissions from soil it is necessary to discuss which soil parameters are of importance with regard to N_2O emissions.

1.3 Soil variables and their effect on N_2O emissions

In order to draw meaningful conclusions on how fertilizer form and NIs impact on N_2O emissions, an understanding of the role of soil variables on N_2O emissions and their relative weight of influence is needed. Soil parameters have an impact on mineral N availability and therefore have significant effects on nitrification and denitrification.

Some soil parameters have been studied extensively and their effect on N_2O is well known whilst others are not so well established. For example, many studies have concluded that soil water content, temperature and mineral N availability are critical parameters on N_2O emissions (Jones et al 2011; Bremmer 1997; Dobbie and Smith 2003 and Laville et al 2011). Indeed it has been said showed that for N_2O fluxes to exceed 100g N_2O -N $h^{-1}d^{-1}$ it is necessary for soil water (WFPS) to be >65%, soil temperature to be >4.5 degrees and NO_3^- concentration to be > 5 mg kg^{-1} dry soil Dobbie and Smith (2003). The fact that threshold values exist suggest that N_2O is highly correlated with these variables. Soil texture was also said to have an important role despite there being fewer than anticipated papers regarding its influence (Buckingham et al, 2014).

The role of pH has been the subject of some division regarding its effect on N_2O emissions from soil. Cuhel et al (2010) did not find any differences in fluxes of N_2O between pH treatments, however this could be due to the relatively moderate changes in pH in their study. Having said that Buckingham et al (2014) also concluded that pH did not have a significant impact on N_2O emissions. In contrast to this several studies have concluded that pH does have a significant relationship with N_2O emissions. Weslien et al (2009), Robinson et al (2014), Barton et al (2013) and Mkhabela (2006) all found that lower (more acidic) pH decreased N_2O fluxes from soils. Robinson et al (2014) stated that lower pH results in a reduced N_2O flux from soils because the synthesis of crucial enzymes are inhibited at a lower pH, the study also observed that few papers have comprehensively determined the effect of

soil pH change on N_2O emissions. The split opinion suggests more research in the area is required.

From the review of literature it seems evident that there are some gaps in knowledge especially with regard to pH and its effect on N_2O flux from soil. It is also unclear as to how N_2O emissions from different forms of inorganic fertilizer will be effected by changed pH level. The results of an experiment on these interactions may help to shed light on the N_2O generating processes occurring within soil.

1.4 Project Aims

This project aims to extend the understanding of how soil fertilizer form and pH interact to effect N_2O emissions immediately after the application of inorganic fertilizer. The project will assess the impact of fertilizer form and pH separately and then go on to analyse how these two factors interact with each other and the result that this interaction may have on N_2O flux. Based on the research conducted by reviewing literature four hypothesis are provided below.

1. N_2O emissions from soil fertilized with AN will be significantly greater than emissions from soil fertilized with urea U(NI)
2. Emissions from cores of a lower pH will yield significantly reduced N_2O emissions than cores which have an ambient pH
3. There will be a significant interaction between fertilizer form and pH level whereby lower pH results in less N_2O emissions from both fertilizers.
4. N_2O emissions across all fertilizer treatments and pH levels will increase significantly with the passage of time.

2. Methodology

2.1 Experimental design

Soil samples used in this research were taken from *Westmill Organics* farm in Oxfordshire. In order to minimise differences in soil parameters (such as organic content, WFPS, mineral N content etc.) 30 cores were taken in close proximity to each other. Although the soil was taken from an agricultural setting the fertilizer application was assumed to be negligible prior to the core extraction.

As aforementioned the two main factors under investigation in this experiment are; 1) fertilizer form and 2) pH level. The first factor has three levels which are *AN*, *U* and *No fertilizer.* The second factor has two levels of *ambient pH* and *lowered ph*. 30 cores were taken from the site in total but only 24 used in the final experiment. The 6 extra cores were used for preliminary tests regarding pH alteration and the identification of appropriate gas chromatograph (GC) standards. The dividing of the 24 cores between the different factors is summarised in table 1.

Table 1. Within each fertilizer group there are eight cores, four of which are under ambient pH conditions and 4 had their pH lowered (with the addition of sulphuric acid)

	AN	**U(NI)**	**No fertilizer**
Ambient pH	IIII	IIII	IIII
Lowered pH	IIII	IIII	IIII

2.2 Application of fertilizer

The application rate of fertilizer to each core was determined based on a core area of 0.0802m^2 and a field nitrogen application rate of 240kg ha^{-1} yr^{-1}. The calculations for the amount of AN and U needed per core to approximate this application rate for the given area of 0.0804m^2 are described below.

$$(0.024 \text{ kg m}^2 \text{x } 0.0804 \text{ m}^2 \text{) x } 1000 \ = \ 1.9\text{g N/core}$$

AN contains 34% nitrogen and U(NI) contains 46%. The amount of fertilizer (either AN or U(NI)) applied to each core in order to provide 1.9g N per core is given by:

$$\frac{(\text{Molecular mass of chemical}) \text{ x } (1.92\text{g})}{\text{molecular mass of N in chemical}}$$

The result of running these calculations on a core area of 0.0804m^2 yielded application rates of 5.5g of AN and 4.1g of U(NI) to the respective cores. In order for the fertilizers to be fully incorporated by the cores they were first dissolved in water. Problems arose at this point as the fertilizers were unable to dissolve in a quantity of water which was sufficiently small to prevent the moisture from passing straight through the cores. As a result of this the amount of AN and U(NI) were both reduced by 45% to 3.03g and 2.27g respectively. This reduction corresponds to 1g of N applied to each of the cores receiving fertilizer treatment. Both the AN and U(NI) were dissolved in 4.5ml of water prior to application.

Regrettably the initial calculation of the core area was incorrect and this was first noticed post experiment. The core area is not 0.0804m^2 but rather 0.0008m^2. The primary implication of this mistake is that the application rate for both fertilizer treatments was significantly overestimated. The result of this error is that instead of 240kg of N per hectare the equivalent application rate for this experiment is 12,500kg of N per hectare, approximately 52 x as much. This is a significant error and clearly impacts on the results and their relevance to field conditions. However, the findings of this research will still serve to compare the differences between fertilizers at high application rates and, when compared to other studies, will further understanding on the nature of the relationship between fertilizer, pH and N_2O emission.

2.3 pH alteration

With the use of a pH meter, three of the extra cores were destructively analysed in order to establish their average pH. The determined value (7.8) was then used as the ambient pH value across all 24 samples. A target pH value of 5.8 pH was decided upon as the desired level for the *lowered pH* cores. pH was lowered using a solution of sulphuric acid (H_2SO_4) mixed with water. The strength of the final solution was determined by altering the ratio of H_2SO_4/H_2O and then applying the solutions to 10g samples taken from the practice cores. The result of this experimentation yielded a solution of 0.19ml of H_2SO4 mixed with 0.2ml of H_2O which resulted in a pH of 5.7 for the 10g samples. This equates to a solution of 1.9ml of H_2SO_4 mixed with 2ml of H_2O for each of the *lowered pH* cores.

2.4 Application summary

As denitrification is an anaerobic process the amount of moisture added to every core needed to be equal. This was achieved by adding water to cores which received no fertilizer to ensure equal distribution of moisture across all cores. A summary of all the treatments applied is displayed in table 2.

Table 2.

	AN	**U(NI)**	**No fertilizer**
Ambient pH	3.9ml H_2O + 4.5ml AN solution = 8.4ml total moisture	3.9ml H_2O + 4.5ml U(NI) solution = 8.4ml total moisture	3.9ml H_2O + 4.5ml H_2O = 8.4ml total moisture
Lowered pH	3.9ml acidic solution + 4.5ml H_2O = 8.4 total moisture	3.9ml acidic solution + 4.5ml H_2O = 8.4ml total moisture	3.9ml acidic solution + 4.5ml H_2O = 8.4ml total moisture

The application of moisture was undertaken over a period of four hours to reduce the likelihood of moisture leaking through the core. This was also done to ensure that all the fertiliser and pH moderating solution was absorbed by the soil. Once the application was complete the cores were left to incubate at 20°C for a period of 24 hours before sampling took place.

2.5 Sampling method

On each sampling day gas samples were taken at two time points (t=0 and t=1) to provide a flux measurement for that day. In order to ascertain an appropriate time gap, a practice run was conducted on two of the extra cores. One of which had been treated with AN at an ambient pH and the second had no fertilizer and had a lowered pH. These two treatments were chosen as they represented, what was expected to be, both extremes of N_2O emission. The practice run revealed that two hours was sufficient to observe a significant change in N_2O.

After incubation for 24 hours the cores were placed in 1L mason jars. The jars were progressively closed and the headspace was mixed by inserting a syringe through the septum, extracting 10ml of headspace and then immediately injecting the syringe back into the jar.

Following the mixing of the headspace the syringe was again filled to the 10ml point and this volume of extracted gas was then injected into a labelled pre-evacuated 3ml vial. The time of closing each jar was carefully noted to the nearest minute. After 2 hours the process was repeated (in the same order) and close attention was paid in making sure that the sampling of each jar occurred precisely 2 hours after t=0. The 24 vials for both time points were maintained at a constant temperature (20°C) away from sunlight. After t=1 on all sampling days the jars were placed back in an incubator at 20°C with the lids off. The time of t=0 on the first sampling day was noted and on the following three sample days t=0 was at the same time. This was to ensure sampling occurred precisely 24 hours apart.

2.6 Soil moisture content

After the sampling days the soil within each core was weighed prior to baking (wet weight) and then weighed once more after a period of 24 hours at 105°C. This was done to provide an insight into the relative moisture content of the soil cores as moisture content is crucial for denitrification. Although this does not provide a precise estimation of moisture content it may be useful within the analysis section if, for example, one core exhibits a larger flux of N_2O compared to a core under the same treatment conditions.

2.7 Gas measurement

After the four sampling days the gas samples were analysed using a gas chromatograph. Blanks were inserted between vials where the N_2O concentration was expected to be large to reduce carry over to the adjacent vial. The data were converted from the raw form (area mV.s) to ppm and then from ppm to ug N_2O-N m^{-2} hr^{-1} using the equations below.

ug N_2O-N m^{-2}:

$$\frac{\text{chamber headspace x } \Delta t0 - t1 \text{ (ppm)}}{\text{core area m}^{-2}}$$

ug N_2O-N m^{-2} hr^{-1}:

$$\frac{\text{ug } N_2O - \text{N m}^{-2}}{2}$$

2.8 Statistical Analysis

The flux data (in ug N_2O-N m^{-2} hr^{-1}) was analysed using IBM SPSS Statistic 21. T-tests were carried out to ascertain whether the difference in mean between the different sample groups were statistically significant ($P<0.05$). As shown in section 2.7 flux data was obtained by subtracting t=1 data from t=0.

Some of the treatments exhibited very little change between t=0 and t=1. Exclusion criteria was derived whereby a ppm flux would be presumed to be 0 if it did not exceed a certain ppm change for each day. The threshold ppm flux was derived by selecting a standard which had ppm values closest to the ppm values of the samples and then calculating 2 standard deviations away from the mean of said standard. For example, if on day 1 the 4000 ppm standard had a standard deviation value of 0.15ppm (2 standard deviations = 0.30) then a flux which was equal to or less than 0.3 ppm was excluded and presumed to be 0. This was done because, in normally distributed data, 95% of the data lies within 2 standard deviations of the mean. Therefore any flux equal to or below 0.3pmm may just be machine noise.

The interaction between pH and fertilizer, and the resultant effect on N_2O was analysed using a repeated measures two-way ANOVA test. The data used in this test was collapsed across all four sampling days to increase the number of data points within each group. This was done because of the observed lack of change over time and the realisation that greater numbers of sample would allow greater confidence to be placed in the eventual findings. Change over time was analysed by performing a Pearson's moment correlation test on each sample group for the four days.

Prior to the two way ANOVA test the data was subjected to tests to ensure that the assumptions of the ANOVA were met before analysis. These assumptions were met so a two way ANOVA test was deemed appropriate (See appendix A).

3. Results

The results are split in accordance with the hypothesis to clearly display the main findings of the experiment. The first hypothesis states that there will be a significant increase in N_2O flux from cores treated with AN compared to those treated with U(NI). The second hypothesis states that emissions from more acidic cores would result in recued N_2O and that this reduction will be significant ($p< 0.05$).

3.1 Is N_2O flux significantly larger from AN than U(NI)?

Figure 2 displays the mean flux in ug N_2O-N m^{-2} hr^{-1} for all the samples over the sampling period (between t=0 and t=4). The difference between AN and both U(NI) and the *no fertilizer* cores were found to be statistically significant at the 95% confidence interval (table 3). As a percentage the gas flux from the U(NI) cores are 10.8% the amount of the AN cores (under ambient pH conditions). The difference between the *U(NI) ambient* and the *no fertilizer ambient* cores was found to be not significant (p=.082).

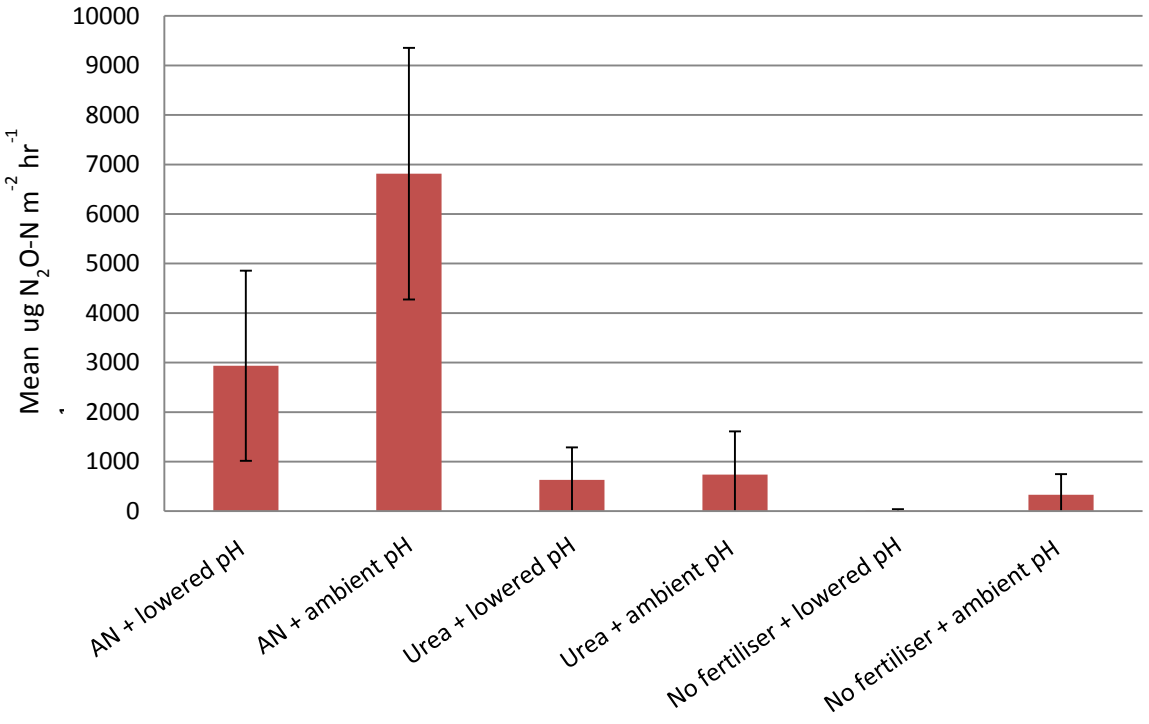

Figure 2. The mean difference between the samples across all four days of sampling. The error bars represent the standard deviation from the mean.

3.2 Does the flux from the ambient pH cores exceed the flux from the lowered pH cores?

Figure 2 also clearly indicates that N_2O flux decreased with a lowered pH. This difference was significant for both the AN and the *no fertilizer* cores whereby both treatments had $p < 0.05$. The U(NI) cores also exhibited a drop of emission at lower pH however this result was not statistically significant (p=.71). Table 3 displays the output from a paired t-test and demonstrates where the difference between the means was significant and where it was not.

Table 3 provides the significance of the differences in mean between treatments. An asterisk denotes a difference in mean which is not significant.

	Mean comparison	Significance
Pair 1	AN + U low pH	.00
Pair 2	AN + U	.00
Pair 3	AN low pH + AN	.01
Pair 4	U low pH + U	.71*
Pair 5	AN low pH + U	.00
Pair 6	AN low pH + U low pH	.00
Pair 7	U + No fertiliser	.08*
Pair 8	No fert + No fert low pH	.00

3.3 Is there a significant interaction between pH and fertilizer form on N_2O flux?

Figure 3 testifies to the significant difference in N_2O flux between AN and U(NI). Figure 3 also depicts the interaction of pH level and fertilizer type on N_2O flux. The difference in mean was found to be significant, at the 95% confidence interval, for the AN x pH interaction yet was not significant for the U(NI) x pH interaction. In other words, the magnitude of the effect of AN on N_2O flux depends significantly (in this experiment) on the pH level. This relationship was confirmed in both the factorial two way ANOVA test and in a t-test where only the relationship between AN and pH yielded significance.

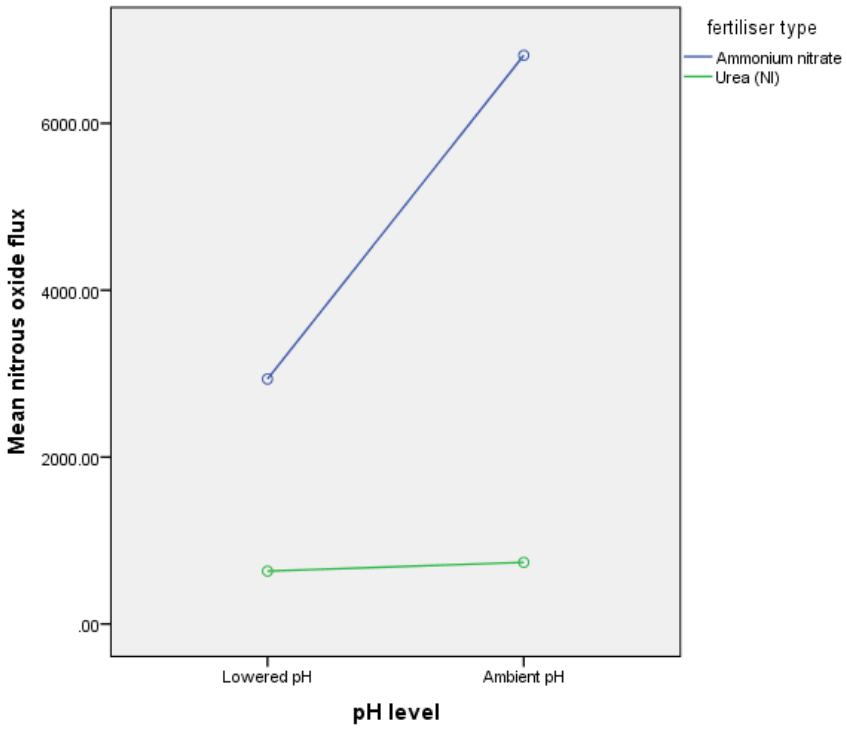

Figure 3 displays the relationship between fertilizer type and N_2O and also the interaction between pH and fertilizer and the resultant effect on N_2O. The graph was produced from SPSS which found the interaction between pH and fertiliser to be significant ($p<0.05$)

Figure 3 also demonstrates the lack of significant difference between U(NI) under ambient pH conditions and U(NI) under more acidic conditions. This lack of difference may be the result of the exclusion of data described in the methods section (thereby bringing the means closer together) or else may be indicative of an effect of U(NI) application on soil pH. Explanations will be discussed in section 4.4. The output from the two way ANOVA is located within B.

3.4 Is there a significant increase over time?

The final hypothesis of this research project states that N_2O emissions across all treatments will increase significantly over the sampling period. In order to test this a correlation analysis was carried out using Pearson's moment correlation coefficient. The results of which are shown in figure 4.

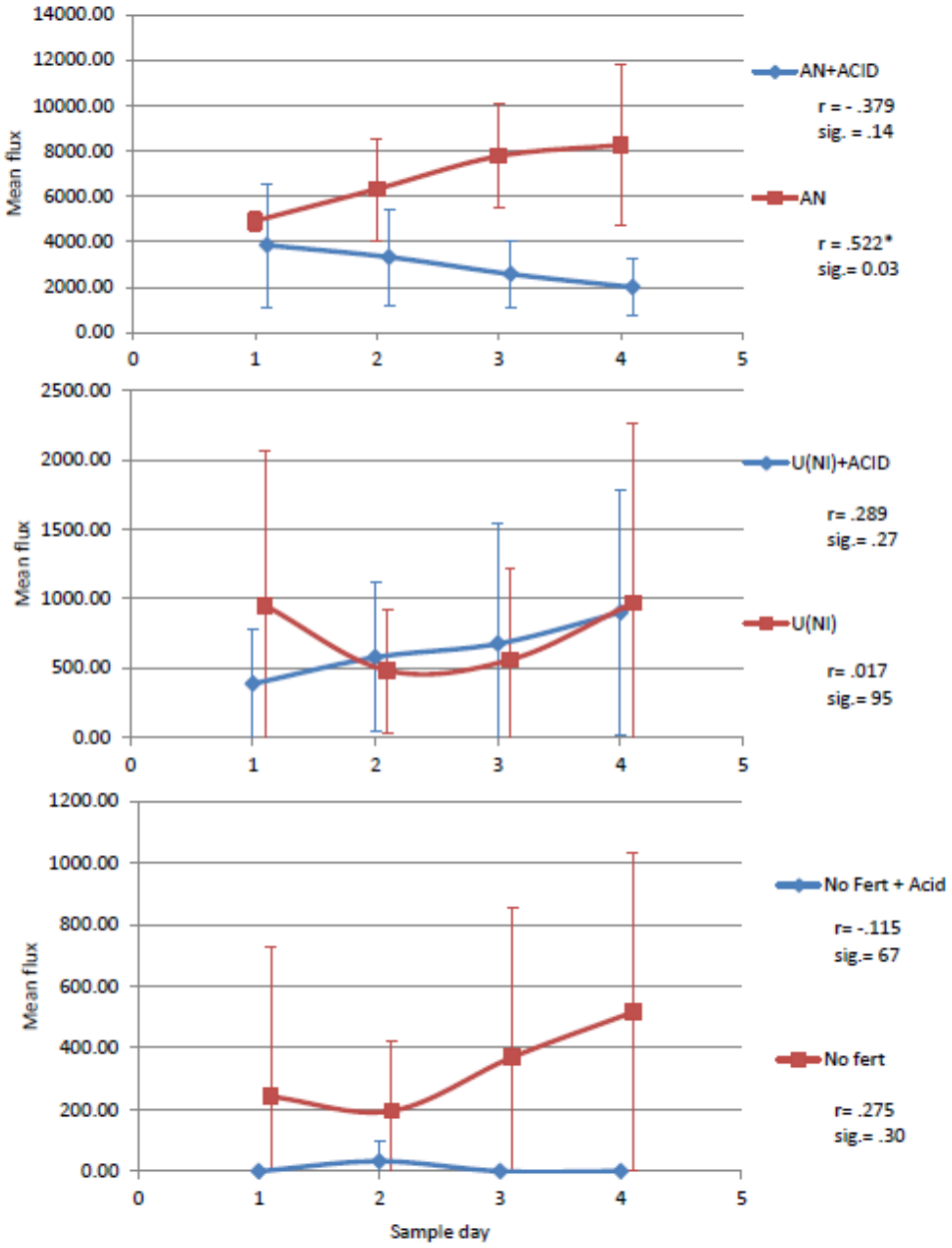

Figure 4. The graphs show mean N_2O flux (ug N_2O m^{-2} hr^{-1}) per day for the respective treatments. The error bars represent one standard deviation away from the mean. The *r* value represents the calculated Pearson's correlation value for each fertilizer over the course of the four sample days. An asterisk denotes a correlation which is significant ($p<0.05$).

Figure 4 shows the lack of significant change over time for any of the treatments with the exception of AN ($p = 0.03$).

3.5 Core moisture content

Samples were baked at 105°C for 24 hours after the experiment. This was done to ascertain the relative moisture content of the cores and to identify those which may have had anomalously low or high moisture content. The mean loss from the all cores after baking was 21.9g with a standard deviation of 3.6. The range for the samples was 15.5g – 30.09. The treatment group with the largest average loss (and therefore the presumed greatest moisture content) was *AN + lowered pH* with a mean loss of 25.83g. The treatment group which lost the least mass (18.56g) was the *no fertiliser + ambient pH* group. Appendix C contains a more detailed breakdown of core moisture results.

4. Discussion

4.1 Summary of main findings

1. There was a significantly greater N_2O flux from AN cores than either the U(NI) or the *no fertilizer cores.*
2. There was no significant difference in N_2O flux between U(NI) cores and the *no fertilizer* cores under ambient pH conditions.
3. More acidic cores resulted in reduced N_2O emissions with the exception of the U(NI) cores where the difference in pH was not significant.
4. The magnitude of the effect of AN on N_2O flux is to some extent dependent on soil pH with significantly larger mean N_2O flux from AN cores at ambient pH compared to the lowered pH cores.
5. Only cores treated with AN under ambient pH conditions changed significantly with time.

4.2 The N_2O flux from the AN was significantly greater than the N_2O flux from the U(NI)

The results demonstrate that gas flux over two hours was approximately 10x as great from AN cores as it was from U(NI) cores. The temperature of the experiment was maintained at a constant 20°C to mimic summertime conditions which, as discussed in the introduction, should result in negligible difference between AN and U. Therefore the significant difference in emissions between the two fertilizers is a result of either; the presence of the inhibitor or an unanticipated process which may have occurred as a result of the significantly over-estimated fertilizer application.

The application rate used in this project was clearly much too high (12,500kg ha^{-1}) however the effects of this error may not have manifested themselves on the timescale used in this experiment. Had the experiment been conducted over a period of weeks then the effects may have been more severe as emissions of N_2O have a tendency to peak 3-4 weeks after application of fertilizer. For example, it is known that over time an application rate of 200kg/ha^{-1} will be significantly different ($p<0.05$) from an application rate of 300kg/ha^{-1}.

However the differences between 200kg/ha^{-1} and 300kg/ha^{-1} is not statistically significant until twenty eight days after application of fertilizer (Sima at al, 2014). This provides a possible explanation as to why the U(NI) cores and the *no fertilizer* cores were not statistically different from each other under ambient pH conditions despite the large application rate. However, that it is not to say that the increased application rate was benign in its effects. Due to such large overestimations of application rate one must consider the possibly toxic impacts the addition of N (and inhibitor) may have had on the nitrifying and denitrifying populations.

It is unlikely that research has been carried out on fertilizer or inhibitor toxicity on a scale equitable to the application rate used in this experiment. However, Macadam et al (2003) reports that inhibitors such a DCD (dicyandiamide) can result in discoloration of some crops. Despite this, the effect of NIs are known to be an inhibitory rather than toxic. That is to say they slow down the breakdown down of substrate rather than destroy populations of bacteria. Although the application of fertilizers was far in excess of field applications one may assume that the addition of the excess fertilizer also did not have a toxic effect. It is more likely that nitrifiers and denitrifiers merely have an upper limit to the amount of substrate they can break down. Substrate levels above this limit, which is likely in this experiment, would therefore not increase the rate of nitrification or denitrification but just result in accumulation of $\mathrm{NH_4^+}$ and $\mathrm{NO_3^-}$ within the cores. Due to this and through comparison with literature it has been assumed that the excess application of fertilizer in this experiment did have any toxic effects.

The results of this research project indicate an N_2O reduction of 90% in soils treated with U(NI) compared to AN. Reductions of N_2O emissions, through the use of U(NI), on the scale observed within this study are not unprecedented occurrences. Khalil et al (2009) observed similar results in a lab experiment conducted at 20°C. Although the application rate was significantly lower (88kg N ha^{-1}), the reductions of N_2O were between 81% to 83% when U was applied with a nitrification inhibitor compared to normal urea. N_2O reductions on a similar scale were found by McTaggart et al (1997) with total reductions of up to 78% observed for soils treated with U(NI) compared to control plots. Examples of specific nitrification inhibitors include DCD and DMPP (dimethyl pyrazol phosphate). These inhibitors were found to reduce cumulative N_2O emissions by 80% and 89% respectively, when used in conjunction with urea, during the maize season (Liu et al, 2013).

The results of the experiment show that U(NI) reduced N_2O emissions in greater quantities than maximum reductions observed in other studies. A possible explanation for the high reduction value may be found by identifying the sources of N_2O in the soil. In other words, due to the effectiveness of the nitrification inhibitor treatment in lowering N_2O flux one may deduce that nitrification was important in producing the large N_2O flux observed with the AN. Carter (2007) observed a similar effect and found that an increased nitrification rate was the most important factor in explaining high initial N_2O from soils treated with urea fertiliser. In other words, the NI was very effective in reducing N_2O because nitrification is a prominent N_2O producing process when urea is broken down.

Confirming which process was dominant in producing N_2O in this experiment would require isotopic analysis of N emitted from the soils to determine whether the resultant N_2O originated from breakdown of $\mathrm{NH_4^+}$ or $\mathrm{NO_3^-}$. Although, the NI was no doubt influential in the difference between U(NI) and AN it seems somewhat unlikely that it was the only factor in such a large difference. One may speculate that denitrification (a common source of N_2O in soils treated with AN) played a part in increasing the gap between the two fertilizers. Identifying the primary source of N_2O in different agricultural settings is a vital step toward more targeted mitigation strategies.

4.3 More acidic soil produced significantly less N_2O than cores with an ambient pH with the exception of U(NI).

One of the aims of this project was to ascertain how pH effects N_2O emissions shortly after fertilizer application. The results show clearly that N_2O flux was significantly less in the soils with lower pH for both the AN fertilized cores and the cores where no fertilizer was applied ($P<0.05$). However, this difference was not observed in the U(NI) treatment where the difference in mean between the pH levels was found to be not significant ($P=0.82$). As previously stated the cores ambient pH was measured to be 7.8 and the target pH (for the reduced pH cores) was 5.7. In order to make sense of these findings, mechanisms to explain the results will be discussed. The precise mechanism responsible for the reduction in N_2O are made more complex with the addition of fertilizer. This section will discuss the effect of pH on N_2O emissions without taking into account the effect of fertilizer application (which will be discussed in section 4.4).

The effect of pH on N_2O emissions is not linear. The optimum pH for maximum N_2O emissions occurs at around 6.5. pH which is greater than 2 values away from this (8.5 or 4.5) is known to reduce N_2O emissions by inhibiting the ability of nitrifying bacteria. Altering pH also inhibits the ability of certain key enzymes to breakdown NO_3^- and NH_4^+. As well as enzymes which breakdown mineral N in the soil, very low pH can also effect the ability of some enzymes to breakdown soil N_2O which can result in a spike in N_2O emissions at very low pHs. For example, Stevens et al (1998) noted how N_2O became dominant at low pH values (3.5) as a result of the sensitivity of the N_2O-reductase enzyme to low pH levels. This enzymes function was impaired which resulted in N_2O accumulation and thus greater emission rates. In other words enzyme function is impaired in different ways across differing pH levels and the effect of pH on N_2O does not conform to a simple straight line relationship.

The experiment conducted for this study lowered the pH to 5.7 for the respective cores so the impairment of N_2O-reductase, observed by Stevens et al (1998), was likely to be negligible. However, a notable reduction in N_2O was evident. Strong inhibition of soil microbial nitrification and denitrification was found by Wang et al (2013) with soil pH altered from the optimum level. In their study, the greatest N_2O flux was observed from soils with a pH value of 8.55, the second largest from a soil pH of 5.90 and a consistently low flux from soils with a pH value of 3.65. The findings from Wang et al (2013) appear to contradict those of Stevens et al (1998). However in the former study fertilizer was added to each of the cores

which evidently had an effect on N_2O emissions. This suggests the N_2O flux may be influenced by an interaction between pH and fertilizer form (see section 4.4).

In addition to N_2O production through nitrification and denitrification, N_2O is also produced through dissimilatory NO_3^- reduction to NH_4^+ (figure 5) . In order to understand how pH effects soil N_2O flux overall it is necessary to discuss the effect of pH on each of these pathways individually. Nitrification is thought to be inhibited by pH however the exact mechanism is not known precisely. It is thought that pH controls NH_3 concentration within the soil. This is highly important for nitrification as NH_3 is the primary product of the nitrification process. It is worth noting that, although low pH reduces nitrification rate, the effect of pH is inhibitory rather than toxic. That is to say nitrifying bacteria were unlikely to have been directly effected by the pH change (Morkved et al, 2007).

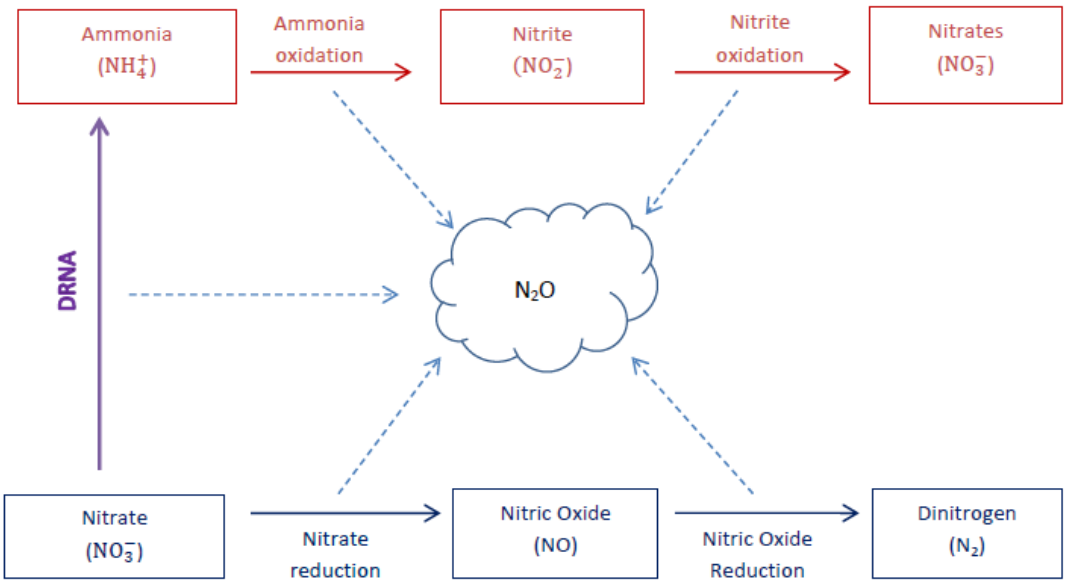

Figure 5 displays the main pools of nitrogen in both nitrification (red) and denitrification (blue). The diagram also depicts the dissimilatory NO_3^- reduction to NH_4^+ (DNRA).

As well as nitrification rate reduction (as a result of substrate limitation) it is also likely that the type of nitrifying bacteria is a factor. The experiment carried out in this study utilised H_2SO_4 to lower the pH of the soil which had an ambient pH of 7.8. The bacteria within the soil would therefore have been accustomed to a certain concentration of NH_3. After the application of the acid, the ambient microbial nitrifying population would have been unable to adjust to the new conditions in the 24 hour incubation period. Had the soil N_2O flux been ascertained over longer time periods it is likely that selection pressures favouring low

concentrations of NH_3 would have enabled nitrifying bacteria to adjust to the lower conditions and nitrification may have been less inhibited as a whole (Mokved et al, 2007).

The relationship between soil pH and denitrification is more complex than the relationship between soil pH and nitrification. In addition to the rate of denitrification being altered by pH, the ratio of its gaseous products (N_2O and N_2) also depends on pH. The rate of denitrification is affected by pH as the dynamics of the reduction enzymes is impaired in acidic soils. This was found by Yamulki at al (1997) where NO_3^- and NO_2^- reductases exhibited significant lag in soils where the pH was low. Although the rate of denitrification and the overall amount of N_2O produced in acidic soils is lower than ambient soils, the ratio of N_2O:N_2 increases as soil pH decreases (Simek and Cooper, 2002). This may be the result of the severe impairment of N_2O reductase which allows accumulation of N_2O within the soil at very low pH levels.

Dissimilatory NO_3^- reduction to NH_4^+ (DNRA) is the third process, influenced by pH, which generates N_2O within soils. DNRA is an anaerobic process which may occur simultaneously alongside denitrification yet very little is known how the process may be effected by soil pH. However, It is likely that DNRAs contribution to N_2O flux in this study was negligible as was the case in Stevens et al (1998).

It appears that results obtained in the experiment are supported by observations made in other studies. However, possible sources of error within the experiment may have had an impact on the findings relating to pH. For example, H_2SO_4 was applied to the soil in a solution with water which may not have permeated through the entire core but rather have remained in the top few centimetres. This may have resulted in the top layer of soil becoming saturated and therefore nitrification (an aerobic process) may have been inhibited. However (as described in the methods section) equal quantities of moisture were added to each core to avoid any extra moisture addition to one treatment compared with another.

The *AN* and *no fertilizer* cores both exhibited significant differences between the ambient and lowered pH cores. However, the same cannot be said about the U(NI) cores. This result will be analysed within the next section of discussion as the mechanisms responsible arise out of an interaction between fertilizer and pH. Therefore it is not easily explained when both factors are addressed individually as they have been thus far.

4.4 There is a significant interaction between fertilizer and pH

The interaction of pH and fertilizer type was tested using a repeated measures ANOVA (analysis of variance). The results of the analysis reveal that the magnitude of the effect of AN on N_2O flux was to some extent dependant on pH level for the sample ($P<0.05$). The results from the U(NI) show that between pH levels there was little difference between the means and that the difference was not significant ($P=0.71$). However, this does not mean that there is no interaction between U(NI) and pH level. On the contrary it is likely that U(NI) served to increase soil pH and thus bring the pH in the cores back toward ambient levels. These findings are discussed on the context of other studies within this section.

AN (which has a chemical formula of NH_4NO_3) works by providing an excess of the two substrates required for both nitrification ($\mathrm{ammonia}\ (\mathrm{NH_4^+})$) and denitrification ($\mathrm{nitrate}(\mathrm{NO_3^-})$) and thereby increases the rate of both processes. To understand how pH interacts with AN it is therefore necessary to discuss how pH level effects each of these substrates. Stimulation of N_2O by the breakdown of ammonia (nitrification) is optimised at soil pH of approximately 6.9. Above or below this optimum value will decrease the rate of N_2O production. However, stimulation of N_2O production by the breakdown of nitrates (denitrification) increases with increasing pH (as discussed in section 4.3). In both cases it is evident that at low pH the two constituents of AN are not broken down at optimum rates leading to an overall reduction in N_2O consistent with observations in this research (Wang et al, 2013). In essence AN at low pH does not increase N_2O emissions because the rate of substrate breakdown is the limiting factor rather than a reduction in the quantity of the substrate itself. As stated in section 4.2 the effect of pH is not to reduce the quantity of $\mathrm{NH_4^+}$ or $\mathrm{NO_3^-}$, but rather to inhibit the reduction of said substrates.

The interaction of U(NI) and pH on N_2O is not the same as the interaction of AN and pH. This is primarily because the hydrolysis of urea raises the pH of the soil (Cabrera at al, 1991). This is an important factor within this experiment as the application rate was several times larger than planned (12,500kg/ha^{-1}). The raising of soil pH, which occurred after the application of U, may have raised the pH of all the U(NI) cores well beyond the ambient pH of 7.8. The effect of this on the results are difficult to comprehend as, as discussed, pH raised above the optimum level for nitrifiers can reduce total N_2O produced. This may partially explain why the difference between AN and U(NI) (90%) was toward the upper estimates found in other studies (Khalil et al (2009) and McTaggart et al (1997)). Having said that, during trials of adjusting the pH it was discovered that the soil samples had a large buffering capacity. That is

to say it took a relatively strong solution of H_2SO_4 mixed with water to achieve the target pH. Therefore as a result of this one may conclude that it is unlikely the addition of U(NI) raised the pH too far beyond 7.8 without the buffering capacity returning the pH to ambient conditions.

Though the effect of the U(NI) addition may not have raised the pH in the ambient cores it is likely that the pH of the "*U(NI) + lowered pH"* cores was raised significantly. Due to the compaction of the soil within the cores it is possible that the dissolved treatments applied only penetrated a few centimetres within the core. Therefore U(NI) which received the acidic solution (along with the dissolved urea) may have had a mix of solutions towards the top of the cores. During the 24 hour incubation period it is likely that the combination of the native soil buffering capacity and the pH increasing property of urea served to bring the *"U(NI) + lowered pH"* cores back toward the ambient pH of 7.8. This would explain the lack of difference observed between the two pH levels of the U(NI) treatment. This property of urea may make it suitable in slightly acidic soils which require fertilizer.

4.5 No significant increase over time was observed with the exception of the AN soil cores.

The results show that there was little change over the 4 sampling days in any treatment group except AN at ambient pH which had a positive correlation of .522 (P=0.03). The standard deviation for samples which received AN was also smaller than the standard deviation of the U(NI) and the control cores. Had there been more than 4 repeats per treatment it is possible that the standard deviation may have been reduced across all the treatments. The limited number of repeats was down to both practical and monetary constraints. Also, more robust conclusions regarding change over time may have been reached had there been more resources to carrying out the experiment over a longer time period. Despite these drawbacks, this experiment still provides an insight into how fertilizer application effects N_2O emissions immediately after fertilizer application.

Gas emissions from fertilized plots in field experiments have revealed similarly pronounced reactions to fertilizer application. This suggests a good agreement between field measurements and laboratory experiments. Addition of AN has been observed to lead to a 3-fold increase in mean N_2O flux compared to control plots. Through the use of isotopic labelling it is possible to deduce which process (either nitrification or denitrification) are responsible for the initial AN induced N_2O spike immediately after the application of fertilizer. As aforementioned AN (NH_4NO_3) contains the substrate for both processes. Thus by isotopically labelling the different components it is possible to deduce the contribution of each to the observed N_2O increase. The result of said analysis has revealed that immediately after fertilizer application denitrification is the dominant N_2O generating process in both organically fertilized plots (slurry, manure etc) and chemically fertilized plots (Dittert et al, 2005).

The change over time data shows that the "*no fertilizer"* plots at ambient pH levels did show small change over the four sample days (r=.275). Although this increase was found to be insignificant (p=.30), one may speculate that the observed increase may have been the result of the addition of 8.4ml of moisture to each core. The additional moisture was required as fertilizer applied to cores would more evenly mixed if it were first dissolved in solution. A possible consequence of this was to increase the rate of anaerobic denitrification within the cores (even those which received no fertilizer). This effect seems to have had a small impact on the *no fertilizer* cores but, as stated, the increase was not significant.

The U(NI) cores did not change in their N_2O flux significantly over the four day sampling period. It is thought that N_2O, produced as a result of urea application, originates mainly from nitrifier activity (Koops et al, 1997 and Bol et al, 2004). This explains why nitrification inhibitors are commonly combined with urea (as is the case with this experiment). This may also explain why the U(NI) did not exhibit any significant change overtime yet the AN did. It seems that in this experiment the dominant form of N_2O generation came from denitrification. One may arrive at this conclusion because AN resulted in large fluxes of N_2O as it contains the substrate for both nitrification (NH_4^+) *and* denitrification (NO_3^-). Whereas smaller fluxes came from U(NI) because: a) urea favours nitrifiers and b) the presence of a nitrification inhibitor reduced the ability of the nitrifying bacteria.

5. Conclusion

The primary aim of this project was to extend the understanding of how soil fertilizer form and pH interact to effect N_2O emissions immediately after the application of inorganic fertilizer. The main conclusions to be drawn from the project are as follows:

A significant difference between AN and U(NI) was observed with the average U(NI) flux being approximately 10% as much as the mean AN flux collapsed across all four sample days. The primary reason for this difference was due to the presence of a nitrification inhibitor which slowed down the enzymatic reduction of the substrate for nitrification. This provides more evidence for the potential of NIs in addressing N_2O emissions from agriculture without having to reduce yield. However, the selection of appropriate fertilizer and inhibitor combinations should be undertaken with care to maximise the benefit.

pH alteration reduces the ability of enzymes to breakdown both $\mathrm{NH_4^+}$ and $\mathrm{NO_3^-}$ and thus limits the rate of both nitrification and denitrification. Assessing the effect of pH is challenging as soils often have high buffering capacities. Populations of nitrifiers and denitrifies may adapt to changes in pH via selection pressures over prolonged periods. More research needs to be undertaken on the effect of pH on N_2O production in soils. In particular, isotopic analysis of the source of emissions will aid understanding of how N_2O generating processes change at different pH levels.

The interaction of pH and fertilizer was a factor for both AN and U(NI). The breakdown of the two constituents of AN ($\mathrm{NH_4^+}$ and $\mathrm{NO_3^-}$) is impaired at low pH therefore N_2O production is reduced. U(NI) returned the pH of the acidified cores back toward ambient pH levels resulting in no difference between the two pH levels of the U(NI) samples. This suggest the use of U(NI) may be a prudent choice of fertilizer in slightly acidic soils.

The only treatment group in this study which increased in N_2O emissions over time was AN likely as a result of denitrification. This cannot be said for sure without isotopic labelling of N sources within the soil. However the observed slight, all be it insignificant, increase in emissions from the control cores suggests that the addition of moisture increased denitrification rates across all samples. The results of this experiment are not sufficient to conclusively state how the treatments change over time.

Further investigations are needed to establish how pH and fertilizer form interact over long time periods. Isotopic labelling of N sources within the soil may shed light on how the interactions effect N_2O producing processes. The work is important for developing targeted mitigation strategies across a wide range of soil types and conditions. It is clear that broad scale strategies to address N_2O emissions from agriculture will not be sufficient due to the wide variation in soil types and parameters which all impact N_2O emissions.

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8. Appendices

Additional data (not included within this appendix) is available by request:

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Appendix A: Two way ANOVA assumptions

Table 4 displays the assumptions for a two way ANOVA. The assumptions were tested in SPSS statistics 21.

	day 1	**day 2**	**day 3**	**day 4**
No outliers	OK	OK	OK	OK
Homogeneity of variance	OK	OK	OK	OK
Normal distribution	OK	OK	OK	OK

Outliers were identified by analysing box plot data outputs and homogeneity of variance was assessed through the Levene's test . The normality of the data was deduced numerically and visually. Numerically it was analysed by the Shapiro Wilks test for normality. Visually it was assessed by viewing histograms of the data distribution.

Appendix B: Two way ANOVA output

The table 5 displays the product of the two way ANOVA test. The significance values are all < 0.05 therefore a significant interaction between fertiliser form and pH level was deduced.

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
fertiliser_type	Pillai's Trace	.904	140.450[b]	1.000	15.000	.000	.904
	Wilks' Lambda	.096	140.450[b]	1.000	15.000	.000	.904
	Hotelling's Trace	9.363	140.450[b]	1.000	15.000	.000	.904
	Roy's Largest Root	9.363	140.450[b]	1.000	15.000	.000	.904
pH_level	Pillai's Trace	.591	21.715[b]	1.000	15.000	.000	.591
	Wilks' Lambda	.409	21.715[b]	1.000	15.000	.000	.591
	Hotelling's Trace	1.448	21.715[b]	1.000	15.000	.000	.591
	Roy's Largest Root	1.448	21.715[b]	1.000	15.000	.000	.591
fertiliser_type * pH_level	Pillai's Trace	.429	11.269[b]	1.000	15.000	.004	.429
	Wilks' Lambda	.571	11.269[b]	1.000	15.000	.004	.429
	Hotelling's Trace	.751	11.269[b]	1.000	15.000	.004	.429
	Roy's Largest Root	.751	11.269[b]	1.000	15.000	.004	.429

a. Design: Intercept
Within Subjects Design: fertiliser_type + pH_level + fertiliser_type * pH_level

b. Exact statistic

Appendix C: Relative soil moisture

Table 6 displays the loss of mass after baking at 105 degrees. The greater the loss in grams the higher the presumed moisture content

Core	Wet weight (grams)	Dry mass (grams)	loss (grams)
AN+ACID (1)	98	71	27
AN+ACID (2)	103	79	24
AN+ACID (3)	92	70	22
AN+ACID (4)	99	69	30
AN (1)	96	74	22
AN (2)	92	69	23
AN (3)	89	61	28
AN (4)	87	69	18
U+ACID (1)	118	93	25
U+ACID (2)	92	71	21
U+ACID (3)	79	59	20
U+ACID (4)	92	67	25
U (1)	86	65	21
U (2)	97	75	22
U (3)	84	63	21
U (4)	90	68	22
No Fertilizer + Acid (1)	84	66	18
No Fertilizer + Acid (2)	84	64	20
No Fertilizer + Acid (3)	90	67	23
No Fertilizer + Acid (4)	76	58	18
No fertilizer (1)	79	62	17
No fertilizer (2)	76	61	16
No fertilizer (3)	92	68	24
No fertilizer (4)	80	62	18