Appendix 1.2. Joint Experimental Protocols for Fertiliser, Organic Manures, and Urine and Dung.

ACO116

Joint ADAS, AFBI, Rothamsted, Rothamsted – North Wyke, and SAC experimental protocol for inorganic fertiliser experiments on arable and grassland sites (experimental design, N_2O, NH_3 flux and soil sampling)

Edition:02 **Effective from: 14.02.11**

Introduction

The following protocols have been prepared on behalf of the ACO116 consortium in order to harmonise the experimental approach used by research partners in order to achieve the highest possible quality of data from the research being undertaken. Wherever possible a standard approach has been adopted, however it is also necessary to accommodate minor differences in local practice providing that this does not compromise the quality of data being produced. This document aims to be consistent where possible with protocols developed in earlier projects, but it is also recognised that methodologies will be continuously reviewed, and where necessary updated (in consultation with consortium members) to ensure best practice and allow for the incorporation of new approaches and techniques.

Summary of Gas Flux Measurements

Equipment required

- Timer
- Needles and 50-60 ml glass syringe.
- 20-22 ml evacuated gas collection vials. These must be standard vials for gas analysis using an automated headspace sampler.

Complete a Site Details proforma (e.g. Appendix 1), describing the site, weather conditions, and any special features that may be of use when it comes to analysis of the results, e.g. rabbit droppings inside chamber, description of the soil conditions etc. Complete a Gas Sampling proforma (e.g. Appendix 2) during sampling.

Ensure that the chamber seal (water filled groove, rubber seal etc.) is undamaged and fully functioning so that a gas-tight seal will be formed. Place the lid on the chamber noting the exact time of enclosure in the proforma.

Each chamber (unstacked) should normally be enclosed using a lid for a **40 minute period**. Note any deviations on the proforma. The time of closing each chamber and the time of taking the gas sample should be recorded on a data sheet.

Pre-evacuated 20-22 ml glass vials should be filled with gas taken from each individual chamber by syringe as detailed below:

Take a 50 or 60 ml glass or plastic syringe fitted with a needle and either pierce the septum in the lid or connect to the chamber valve or connect to a 3-way tap inserted into the chamber (depending on chamber type) and slowly remove 50 or 60 ml of the headspace gas without withdrawing the needle or disconnecting the syringe. Depress the plunger to force this sample back into the chamber and to ensure that a representative gas sample is taken.

SLOWLY withdraw another 50 ml sample, hold at 50 ml until plunger stays fast then remove the syringe from the chamber. Watch that the plunger does not retract into the syringe body, meaning that the gas was sampled too quickly i.e. the gas is not at atmospheric pressure, and consequently, that the sample volume collected is less than 50 or 60 ml. Pierce the septum of the appropriate labelled gas vial, as the vial is pierced, the gas will automatically be withdrawn from the syringe to equalise the pressures in the vial and syringe. Push the plunger in as far as it will go and hold the syringe in position.
At some point prior to analysis (either in the field or lab) pierce the septum with another single needle (narrow bore) to allow excess pressure to be released. Note down the gas vial identifier, such that the vial and N_2O concentration in the particular chamber headspace can be matched following subsequent analysis by GC. Record the time that gas samples are taken on the proforma.

Select 3 chambers (one per block) at random from the treatment with the standard N input. From each of these 3 chambers take a time series of samples following closure; 6 samples at evenly spaced intervals (eg 0, 10, 20, 30, 50, 60 for the 40 minute closed chambers and 0, 15, 30, 45, 60, 75 and 90 for the 60 minute closures) and submit for analysis.

1. Experimental sites

Experiments will take place at or near the sites listed in the table below.

Zone Name	Soil Texture Group	Annual Rainfall (mm)	Experimental platform (planned)	Experimental land use and organisation responsible for the platform
Dry	Medium	0-750	Gilchristan, E Scotland	Arable (SAC)
Dry	Sandy/light	0-750	Wensum, E Anglia	Arable (ADAS)
Dry	Sandy/light	0-750	Woburn, Hertfordshire	Arable (RRes)
Medium	Medium	751-950	ADAS Rosemaund, Herefordshire	Arable (ADAS)
Dry	Heavy	0-750	ADAS Drayton, west Midlands	Grass (ADAS)
Wet	Medium /heavy	951+	North Wyke, Devon	Grass (RRes - NW)
Wet	medium	951+	ADAS Pwllpeiran, Wales	Grass (ADAS)
Wet	Sandy/light	951+	Crichton, SWScotland	Grass (SAC)
Wet	Medium	951+	Hillsborough, Northern Ireland	Grass (AFBI)

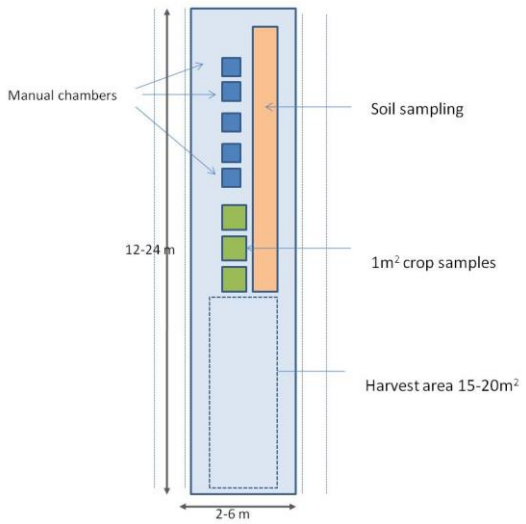
There should be no history of long term organic manure applications and no manure applications or grazing immediately prior to establishment of the experiment. Previous site history (over at least 12 months) should be recorded. A new site will be used for each experiment.

1.1 Crop/grass variety

Varieties of all crop types will be determined by the local research provider. The varieties should be typical of what is commercially grown in the area the site is located in. If possible, a short stemmed variety of cereals would be preferred for ease of N_2O sampling. In grassland plots the proportion of clover in plots should be recorded at the start and end of sampling

1.2 Plot sizes

For the fertiliser experiments, plots should be 2-6 m wide (depending on equipment available) and 12- 24 m long. A schematic diagram of a standard plot is illustrated below (not to scale).



Depending on the availability of space the harvest area could either be below the chambers (as illustrated) or to one side.

1.3 Farm operations

The host farmer or research provider will carry out:

- all soil cultivations and seed bed preparation for the test crop using equipment typical of the area and suitable for the soil type
- sowing of the test crop
- application of agro-chemicals to the test crop
- application of non- nitrogen fertilisers e.g. P, K, S etc. if required to the test crop. If the host farmer would normally apply non-N fertilisers at the same time as N fertiliser, non-N fertiliser will need to be applied by research staff. Rates of non-N fertiliser will be based on soil analysis and guidelines in 'The Fertiliser Manual (RB209) published 2010'. Such recommendations should be checked by a FACTS qualified advisor.
  The only nitrogen to be applied to the plots is to be by hand by research staff.
- all soil cultivations and seed bed preparation for the follow on crop using equipment typical of the area and suitable for the soil type. In some circumstances this would involve leaving the field in stubble throughout the winter period, but it could also include cultivation during this period, in accordance with standard practice
- sowing of the follow on crop
- application of agro-chemicals to the follow on crop

No nitrogen should be applied to the plots before the 12 month N_2O measurement period has finished. and 'NO NITROGEN SIGNS' must be put up around the perimeter of the experiment

1.4 Statistics

All nitrous oxide data will be statistically analysed by techniques appropriate to the experiments described. Cumulative emissions, emission factors and associated standard errors will be generated.

All crop data will be analysed by analysis of variance (Genstat 5 or later) provided that the assumptions of the test are met.

Advice from a trained statistician will be sought in the design and analysis of the experiments.

2. Treatments
2.1 Arable sites

2.1.1. WINTER WHEAT
The following treatments will be applied to winter wheat experiments

2.1.1.1. ADAS Rosemaund (winter wheat)

Treatment	Application rate & timing (kg/ha N)					Total applied (kg/ha N)
	Early March	Early April	Mid April	Late April	Early May	
1. Control	0	0	0	0	0	0
2. AN	20	20	0	20	0	60
3. AN	40	40	0	40	0	120
4. AN	40	70	0	70	0	180
5. AN	40	100	0	100	0	240
6. AN	40	130	0	130	0	300
7. AN (+NI)	34 (+6)	93 (+7)	0	93 (+7)	0	220 (+20)
8. Urea	40	100	0	100	0	240
9. Urea (+NI)	34 (+6)	93 (+7)	0	93 (+7)	0	220 (+20)
10. AN 5 splits	40	50	50	50	50	240
11. DCD Only	0 (+6)	0 (+7)	0	0 (+7)	0	0 (+20)

Notes:

(i) Fertiliser rates cover the range of "Fertiliser Manual (RB 209)" / SAC Technical Note recommendations for different soil types and 'typical' soil nitrogen supply indices.

(ii) Treatment 2 and 3 are a compromise as the "Fertiliser Manual (RB 209)" states that for applications of less than 120 kg/ha the recommended amount should be applied as a single dressing, but not before early April. In commercial systems this probably reflects situations with a high soil nitrogen supply and as a result there would be no need for an 'early March' dressing.

(iii) Treatments 7 to 10 apply the same total amount of nitrogen as treatment 5

On treatment 7, 9 and 11, DCD will be applied as a 2% solution (i.e 20g/1000ml) at a rate equivalent to 10kg/ha DCD (i.e. 500l/ha of 2% solution) after each fertiliser application.

The DCD solution should be applied within 1 hour after the fertiliser has been applied using calibrated spray equipment.

As DCD contains 65% N the nitrogen fertiliser applications will have to be reduced to ensure the same amount of plant available N is applied to treatments 7, and , 9.

2.1.1.2 Rothamsted Woburn (winter wheat)

Treatment	Application rate & timing (kg/ha N)					Total applied (kg/ha N)
	Early March	Early April	Mid April	Late April	Early May	
1. Control	**0**	**0**	**0**	**0**	**0**	**0**
2. AN	**20**	**20**	**0**	**20**	**0**	**60**
3. AN	**40**	**40**	**0**	**40**	**0**	**120**
4. AN	**40**	**70**	**0**	**70**	**0**	**180**
5. AN	**40**	**100**	**0**	**100**	**0**	**240**
6. AN	**40**	**130**	**0**	**130**	**0**	**300**
7. AN (+NI)	**34 (+6)**	**63 (+7)**	**0**	**63 (+7)**	**0**	**160 (+20)**
8. Urea	**40**	**70**	**0**	**70**	**0**	**180**
9. Urea (+NI)	**34 (+6)**	**63 (+7)**	**0**	**63 (+7)**	**0**	**160 (+20)**
10. AN 5 splits	**40**	**40**	**40**	**30**	**30**	**180**
11. DCD Only	**0 (+6)**	**0 (+7)**	**0**	**0 (+7)**	**0**	**0 (+20)**

Notes:

(i) Fertiliser rates cover the range of "Fertiliser Manual (RB 209)" / SAC Technical Note recommendations for different soil types and 'typical' soil nitrogen supply indices.

(ii) Treatment 2 and 3 are a compromise as the "Fertiliser Manual (RB 209)" states that for applications of less than 120 kg/ha the recommended amount should be applied as a single dressing, but not before early April. In commercial systems this probably reflects situations with a high soil nitrogen supply and as a result there would be no need for an 'early March' dressing.

(iii) Treatments 7 to 10 apply the same total amount of nitrogen as treatment 4

On treatment 7, 9 and 11, DCD will be applied as a 2% solution (i.e 20g/1000ml) at a rate equivalent to 10kg/ha DCD (i.e. 500l/ha of 2% solution) after each fertiliser application.

The DCD solution should be applied within 1 hour after the fertiliser has been applied using calibrated spray equipment.

As DCD contains 65% N the nitrogen fertiliser applications will have to be reduced to ensure the same amount of plant available N is applied to treatments 7, and 9.

2..1.1.3 SAC Gilchristan spring barley (malting)

Treatment	Application rate & timing (kg/ha N)			Total applied (kg/ha N)
	Seedbed	Mid/late March	Early/Mid Apr	
1. Control	0	0	0	0
2. AN	20	20	0	40
3. AN	40	40	0	80
4. AN	40	80	0	120
5. AN.	40	120	0	160
6. AN	40	160	0	200
7. AN (+ NI)	34 (+6)	73 (+7)	0	107 (+13)
8. Urea	40	80	0	120
9. Urea (+ NI)	34 (+6)	73 (+7)	0	107 (+13)
10*. AN 3 splits	40	40	40	120

For spring barley, "The Fertiliser manual (RB209)" recommends 2 splits (rather than 3) for the 'standard' timing treatments. Also it recommends that all the nitrogen should be applied before the end of March.

Notes:

(i) Fertiliser rates cover the range of "Fertiliser Manual (RB 209)" / SAC Technical Note recommendations for different soil types and 'typical' soil nitrogen supply indices.

(ii) Application timings are for guidance only and will depend on seasonal and local conditions

(iii) Treatments 7 to 10 apply the same total amount of nitrogen as treatment 4

On treatment 7 and 9, DCD will be applied as a 2% solution (i.e 20g/1000ml) at a rate equivalent to 10kg/ha DCD (i.e. 500l/ha of 2% solution) after each fertiliser application.

The DCD solution should be applied within 1 hour after the fertiliser has been applied using calibrated spray equipment.

As DCD contains 65% N the nitrogen fertiliser applications will have to be reduced to ensure the same amount of plant available N is applied to treatments 7, and 9.

2.2 Grassland sites

The following treatments will be applied at the grassland sites (Hillsborough Crichton ADAS Pwllpeiran, ADAS Drayton North Wyke**)**

Treatment	Application rate & timing (kg/ha N)						
	1st Cut				2nd Cut	3rd Cut	Total applied (kg/ha N)
	End Feb	Mid March	End March	Early April	End May	End June	
1.Control	**0**	**0**	**0**	**0**	**0**	**0**	**0**
2. AN*	**20**	**0**	**20**	**0**	**20**	**20**	**80**
3. AN*	**30**	**0**	**40**	**0**	**50**	**40**	**160**
4. AN*	**40**	**0**	**60**	**0**	**80**	**60**	**240**
5. AN.*	**70**	**0**	**70**	**0**	**100**	**80**	**320**
6. AN*	**90**	**0**	**90**	**0**	**120**	**100**	**400**
7. AN (+ NI)*	**64 (+6)**	**0**	**64 (+6)**	**0**	**93 (+7)**	**73 (+7)**	**294 (+26)**
8. Urea	**70**	**0**	**70**	**0**	**100**	**80**	**320**
9. Urea (+ NI)	**64 (+6)**	**0**	**64 (+6)**	**0**	**93 (+7)**	**73 (+7)**	**294 (+26)**
10. AN 4 splits (1st cut)*	**40**	**30**	**40**	**30**	**100**	**80**	**320**

Fertiliser application rates (and timings) cover the ranges recommended in Defra's "Fertiliser Manual (RB209)", for a 3 cut grass-silage system.

Notes:

(i) Treatments 7 to 10 apply the same total amount of nitrogen as treatment 5

(ii) Application timings are for guidance only and will depend on seasonal and local conditions

On treatment 7 and 9, DCD will be applied as a 2% solution (i.e 20g/1000ml) at a rate equivalent to 10kg/ha DCD (i.e. 500l/ha of 2% solution) after each fertiliser application.

The DCD solution should be applied within 1 hour after the fertiliser has been applied using calibrated spray equipment.

As DCD contains 65% N the nitrogen fertiliser applications will have to be reduced to ensure the same amount of plant available N is applied to treatments 7, and 9.

*Northern Ireland will use calcium ammonium nitrate.

3 Materials

3.1 Fertiliser application

Ammonium nitrate mineral N fertiliser (34.5% N) will be applied by hand.

To prevent any nutrient deficiencies, overall basal P, K and Mg fertilisers and S should be applied according to site requirements. Applications should follow recommendations in 'The Fertiliser Manual (RB209) published 2010' for sites in England or in SAC fertiliser recommendations for sites in Scotland. Such recommendations should be checked by a FACTS qualified advisor.

3.2 Agro-chemical applications

Apply other agro-chemicals as needed and according to good agricultural practice to control weeds, pests and diseases. Agro-chemicals should be applied by the host farmer.

Crop sampling

Arable sites

Crops are to be collected at harvest, and the weight of grain and straw should be recorded at each site. The ratio of grain to straw should be determined at harvest by sampling a minimum of 3 $1m^2$ subplots by hand and returning samples to the laboratory for weighing and analysis. Plot yields should be determined separately by harvesting a minimum area of 15 m^2 using a small plot harvester. Care should be taken not to walk on areas being used for crop harvests. The N content of grain and straw samples should be determined in order to calculate N offtake.

Grasslands

Grassland sites should be cut on 3 occasions during the year. Cuts would normally take place in May, June and July or August depending upon the location. Plot yields should be determined separately by harvesting a minimum area of 15 m^2 using a small plot harvester. The N content of grass samples should be determined in order to calculate N offtake.

4 Nitrous oxide emission measurements

The closed, static chamber method is used to measure the emission of N_2O from soil. A chamber box is inserted to a depth of approximately 5 cm or greater (i.e. sufficient to produce an adequate seal). Background measurements of the gases of interest are taken by manually sampling the atmosphere around the experimental plots. To begin flux measurements, a lid is placed on top of the chamber, enclosing the atmosphere above the soil and within the chamber. The time at which this is done is noted. The chamber lid is left on for a predetermined amount of time, usually 40 minutes. The accumulation of N_2O within the chamber is measured by manually taking a gas sample from the chamber, storing this in an evacuated glass vial and sending the sample for analysis by gas chromatography. The N_2O increase (ppmv) over the incubation period (minutes) is used to calculate the N_2O emission rate.

4.1 Time

Two members of staff can sample from 150 chambers (5 chambers x 6 plots x 3 blocks) in approximately 3hrs 15mins. This includes taking field and ambient samples. Extra time is required for vial labelling, packing and despatch, chamber maintenance.

4.2 Materials and Equipment

4.2.1.Chambers

These should be square/rectangular covering a surface area of *c*.0.16 m^2 (or circular and have a diameter in the range 25-50 cm to give a similar surface area). The minimum height above ground should be 15-20 cm over grass swards/ young arable crops. As the latter crops develop, the chambers may need to be replaced by taller ones, or stackable types (e.g. chambers with a water filled channel) where an "extension layer" can be added when necessary, to keep the chamber top above the height of the crop.

Two main methods of chamber installation are commonly used.

(a) Chambers that are essentially square or circular "sleeves": insert the bottom of the chamber wall into a 5 cm deep slot cut in the soil, and tamp the soil on the outside to make a good seal. Close the top with a gas-tight lid (using a rubber or water seal), leaving the lid in place only for the duration of the measurement. Leave the chamber body in situ between measurements (only remove if/when agronomic operations make it necessary).

(b) Chambers with soil "collars". In this type, a short sleeve or "collar" is sealed into the soil in a similar way as in (a), but the above-ground extension is only of the order of 4-5 cm. A closed chamber with 15-20 cm high walls and an integral top (i.e. in the form of an inverted bucket or box) is attached for the measurement period.

During the latter stages in the growth of cereal crops, chamber extensions will be required to accommodate the growing plants (normally in late May or June). These should be stacked above the existing chamber, providing a gas tight seal, and allowing gas flux measurements to be made throughout the growing season. Once in place, chamber extensions would remain until the crop is harvested.

4.2.2 Numbered pegs to identify the chambers.
4.2.3 Cutting tool/former.
4.2.4 Timer
4.2.5 Needles and 50-60 ml glass syringe.
4.2.6 20-22 ml evacuated gas collection vials. These must be standard vials for gas analysis using an automated headspace sampler.

- Crimp top glass vials
  Crimp caps
  Butyl rubber seals

4.3 Procedures

4.3.1 Safety considerations

The method involves bending to sample chambers at ground level, take care to avoid back strain/injury.

As hypodermic needles are used to sample ambient and chamber headspace gases, operators should take care not to "prick" themselves. Should blood be drawn following an accidental "pricking", the needle must be disposed of in a sharps bin to comply with health and safety regulations. Staff involved in these measurements should ensure that they have

up to date tetanus immunisation and are aware of local Risk Assessments for using needles. New needles should be used for each set of N_2O samples taken.

4.4 Autochambers

A single autochamber should be placed in the standard (Recommended RB209 ammonium nitrate treatment and timing) treatment. The autochamber should be programmed to take 4 samples per day in the 2 week period immediately after fertiliser application (at 04.00, 10.00, 16.00, 22.00). Between weeks 3-24 this could be reduced to 2 per day (10.00 and 22.00), and between weeks 24-52 this could be reduced to 1 per day (10.00). A training event will be arranged and notes created to provide guidance.

5 Measurements: planning

5.1 The flux of N_2O is determined by measuring the increase in N_2O concentration (above the concentration of the ambient air) in an enclosed chamber over a defined period of time. In most situations, the increase in N_2O concentration is linear over a 40-50 minute period, although may be in the range of 30-60 min depending on site conditions. Beyond this however, and depending on a variety of factors, the rate of increase tends to decline. As only one field measurement is made of the N_2O concentration, it is crucial to plan the timing of operations to allow for placing chambers and withdrawing the samples. Exceeding the suggested enclosure period greatly increases the risk of non-linearity and hence underestimating the flux.

5.2 Work out the timing of operations based on the experimental design outlined in the Study Protocol. As gas sampling takes the longest time, plan by working back from an enclosure period of 40-60 minutes per chamber. Allow 1 min between enclosing each chamber to ensure enough time for sampling.

5.3 Carry out operations by block, but randomise the order in which blocks are measured on different days, as N_2O fluxes do show some diurnal variation. If the experimental layout allows, on each measurement occasion the order of the plots and the order the chambers within each plot sampled should be **randomised daily** i.e. start at one end of the plot and work through the chambers in order or start at the other end of the plot and work through the chambers in that order.

5.4 Gas sampling should be carried out between 10:00 and 14:00 hours due to diurnal variation in N_2O emissions and if possible **between 10:00 and 12:00 hours** as indicated by IPCC good practice. For large experiments this might not be feasible. In all cases note the time of sampling on the proforma. The site details proforma (attached) and gas sampling proforma (attached) should be completed for each sample day.

5.5 Individual sample vials should be labelled with a unique sample identifier, which corresponds to that on the gas sampling proforma. The code should include a reference to the name of the site, experiment, plot, chamber and the project code. Both before and after collection it is good practice to place the gas vials in numerical order in a suitable container e.g. a labelled polystyrene tray.

6 Measurements from field plots

6.1 Complete a Site Details proforma (e.g. Appendix 1), describing the site, weather conditions, and any special features that may be of use when it comes to analysis of the results, e.g. rabbit droppings inside chamber, description of the soil conditions etc. Complete a Gas Sampling proforma (e.g. Appendix 2) during sampling. An example of a partially completed gas sampling proforma is shown in Appendix 3.

6.2 Measurements of N_2O should be made from randomly determined positions (avoiding features such as tramlines) in the appropriate field location identified in the Study Protocol.

6.3 **Five chambers** should be placed in each of the plots before collecting gas samples. Press the chambers into slots (previously made by a spade or by hammering in and removing a square steel frame) in the soil to ideally a depth of > 5 cm, but not less than 3 cm. Once the chamber is installed, the outside edge needs to be tamped down to ensure a good seal.

6.4 Ensure that the chambers are inserted into the ground to give a level top edge. This is important if the type of chamber used has a water filled channel, as the water in the channel running around the chamber top needs to make a gas-tight seal when the chamber lid is fitted.

The height of the chamber should be measured from the soil surface to the highest part of the chamber at all 4 corners and written on the proforma**.** Additionally, a stick/ruler should be positioned so that it rests on top of the chamber to enable a height measurement to be made from the centre of the chamber.

Chamber insertion should be done not less than 24 hours before a fertiliser/manure application.

6.5 Once the chamber is in position, ensure that the chamber seal (water filled groove, rubber seal etc.) is undamaged and fully functioning so that a gas-tight seal will be formed. Place the lid on the chamber noting the exact time of enclosure in the proforma.

6.6 The chambers should stay in place throughout the experiment (but the lids removed after each measurement), although they will need to be removed prior to farm operations e.g. cultivations, fertiliser/manure application etc. and replaced as soon as possible after. Number the chambers with a unique identifier e.g. plot number and chamber number (1 to 5). The location of each chamber needs to be marked e.g. with a wooden peg or magnetic marker so that the chamber is always inserted in the same place if the chamber is moved. **The long-term position of the chambers is critical and if removed need to be returned to exactly the same position**.

6.7 Each chamber (unstacked) should normally be enclosed using a lid for a **40 minute period**. Note any deviations on the proforma. The time of closing each chamber and the time of taking the gas sample should be recorded on a data sheet.

6.8 Pre-evacuated 20-22 ml glass vials should be filled with gas taken from each individual chamber by syringe as detailed below:

6.8.1 Take a 50 or 60 ml glass or plastic syringe fitted with a needle and either pierce the septum in the lid or connect to the chamber valve or connect to a 3-way tap inserted into the chamber (depending on chamber type) and slowly remove 50 or 60 ml of the headspace gas without withdrawing the needle or disconnecting the syringe.

6.8.2 Depress the plunger to force this sample back into the chamber and to ensure that a representative gas sample is taken.

6.8.3 SLOWLY withdraw another 50 ml sample, hold at 50 ml until plunger stays fast then remove the syringe from the chamber. Watch that the plunger does not retract into the syringe body, meaning that the gas was sampled too quickly i.e. the gas is not at atmospheric pressure, and consequently, that the sample volume collected is less than 50 or 60 ml.

6.8.4 Pierce the septum of the appropriate labelled gas vial, as the vial is pierced, the gas will automatically be withdrawn from the syringe to equalise the pressures in the vial and syringe.

6.8.5 Push the plunger in as far as it will go and hold the syringe in position.

6.8.6 At some point prior to analysis (either in the field or lab) pierce the septum with another single needle (narrow bore) to allow excess pressure to be released

6.8.7 Note down the gas vial identifier, such that the vial and N_2O concentration in the particular chamber headspace can be matched following subsequent analysis by GC.

6.8.8 Record the time that gas samples are taken on the proforma.

6.9 Nitrous oxide measurements should be made according to the sampling strategy outlined below:

Weeks before/after N input (e.g. manure/fertiliser application etc.)	Number of measurements
-1	1
0	4
1	4
2	2 (evenly spaced through the week)
3	2 (evenly spaced through the week)
4-7	2 (evenly spaced)
8-12	2 (evenly spaced)
13-16	2 (evenly spaced)
17-20	2 (evenly spaced)
21-24	2 (evenly spaced)
25-28	1
29-32	1
33-36	1
37-40	1
41-44	1
45-48	1
49-52	1
	Total c 30/year

Measurements will continue for **12 months** following the **FIRST fertiliser application** (or crop residue incorporation). If the experiment includes split fertiliser applications, after the first application of N fertiliser the above sampling strategy should be followed until the second fertiliser N application. **Following the second application, sampling should revert back to the start of the strategy** i.e. at 0 weeks after application and so on for any further fertiliser N applications. Also, it is essential that a N_2O sampling occurs prior to any second (or third etc.) application. This sampling should be as close as possible to the fertiliser addition and should **not be more than 3 days before** the fertiliser application.

On arable sites, N_2O measurements should be carried out once in the week before the main cultivation, once immediately after cultivation following harvest and if possible once after the first rain event (e.g. 5-10 mm) after this cultivation. The sampling before and after the first cultivation would not need to make measurements from individual plots, but instead have a reasonable replication across the site (c 20 chambers).

7 Linearity check

To check on the linearity of gas accumulation within a chamber's headspace (an underlying principle of the methodology), on every N_2O sampling occasion select 3 chambers at random from the treatment with the highest N input. From each of these 3 chambers take a time series of samples following closure; 6 samples at evenly spaced intervals (eg 0, 10, 20, 30, 50, 60 for the 40 minute closed chambers and 0, 15, 30, 45, 60, 75 and 90 for the 60 minute closures) and submit for analysis. The remaining chambers are measured using a terminal sampling only as above. If non-linear responses are obtained (e.g. when soil is particularly dry), a correction algorithm will need to be applied to adjust the flux values.

If the chambers are to be stacked in order to permit N_2O sampling from a growing crop, linearity checks should also be carried out prior to chamber stacking and N_2O measurement. The enclosure time may need to be increased as a result of the increase in chamber volume, but not too much so that N_2O accumulation is non-linear. At least 1 week before the addition of a stacked chamber is required, select two chambers at random from the treatment with the highest N input. From each chamber take 6 or 7 samples at evenly spaced intervals (0 min, 15, 30, 45, 60, 75 and 90 min) after closure. The determination of the enclosure time will be based on the resultant linearity graphs.

8 Measures to permit N_2O sampling throughout the growing season of arable crops

In order to sample N_2O throughout the growing season it is necessary to use a chamber type which allows chamber stacking e.g. chambers with a water filled channel.

8.1 The water seal allows a series of chambers to be stacked on top of each other as the crop increases in height. Once the crop has reached the top of the in-situ chamber i.e. the chamber lid can no longer be fitted without crop damage, fit another chamber onto the in-situ chamber for sampling. Fill both grooves with water i.e. between the 2 chambers and between the extra chamber and the lid. Put the lid on and sample as usual. Additional chambers can be added if the crop height necessitates it. After each gas sampling, the additional, stacked chambers that have been fitted can remain in

place so that the crop is not damaged. If the chambers are stacked 3 or 4 high it may be possible to remove the top chamber after sampling. Indicate on the proforma how many stacked chambers are left in-situ after sampling. If stacked chambers are removed, do not leave inside the plot on the soil surface, move to the discard area.

9 Ambient N_2O concentration at the site

Make an assessment of the ambient N_2O concentration of the experimental area, as follows:

9.1 Collect (in pre-evacuated gas vials) ten 20 to 22-ml ambient gas samples from the experimental plot area, 5 samples at the start of chamber sampling and 5 samples at the end of chamber sampling. These samples should be collected as in Section 3.8, away from any roads and the soil surface to avoid contamination from car exhausts or soil efflux respectively. Take the ambient samples from about one metre above the ground, i.e. approximately around waist height.

9.2 Vials should be labelled with the name of the field site and the project code and a unique sample identifier, such that these samples can be identified as ambient.

10. Completion and sample submission

10.1 Once all samples have been taken, remove the lids from the chambers and place each lid away from the chamber (approximately 1-2 m), so as to avoid a preferential rain shadow around the chamber.

10.2 The glass gas vials can be easily broken, so ensure that they are well packaged to avoid damage in transit. A photocopy of the proforma sheets should be kept in the working file used by the gas sampler for ready reference.

10.3 If there is no GC facility at the site where the experiment takes place, and samples are to be transported, the signed proforma sheets should be sent with the corresponding sample vials as **soon as possible** after collection to the laboratory for analysis. Prompt return of the gas vials is important since standards are added to each batch of vials at the analysis laboratory. The standards are essential to correct for gas loss if there is a delay in analysis by GC.

10.4 The gas samples will be analysed by GC.

10.5 Sample vials should be evacuated as close to N_2O sampling as possible. Do not use vials which have been evacuated for more than one week. Vials can be evacuated either using a manual or mechanical suction pump. Septa should be changed if there is any visible sign of wear, or after 4-5 sampling events. If there is no GC facility at the site where the experiment takes place, and you receive pre-evacuated vials and if you do not use the vials please return to the analysis laboratory.

11 Calculation of results

11.1 A designated person identified in the study protocol should always calculate the results.

11.2 Calculation of the N_2O flux

The automated GC equipment will analyse the N_2O concentration of the experimental field and ambient samples and the data will be copied from the PC and stored in an appropriate directory on the local computer system. certified gas stadards should be used and separate reference samples (AQCs) will be run with each ‘batch’.

11.1.1 A standard spreadsheet is available for calculation of the N_2O flux in g N_2O-N ha^{-1} d^{-1} (using the equations in Appendix 4). Input data required are: the chamber enclosure start and end times; the chamber height; and the average air temperature over the sampling period.

11.1.2 The fluxes from the 5 individual chambers on each plot should be averaged to produce a mean estimate of the plot N_2O flux rate.

11.1.3 A treatment N_2O flux rate should be calculated by averaging the replicate plot mean values (usually 3 or 4) for each individual treatment including the untreated control.

11.1.4 Cumulative N_2O fluxes should be calculated using the **trapezoidal rule** (area under the curve) to interpolate fluxes between sampling points. For each treatment, cumulative fluxes should be calculated using the plot mean values in order to calculate a mean cumulative emission value and associated standard error.

11.1.5 The IPCC equivalent N_2O-N soil emission factor should be calculated using the below equation:

$$\frac{\text{Cumulative } N_2O \text{ flux from N applied/incorporated (kg } N_2O\text{-N)} - \text{Cumulative } N_2O \text{ flux from control (kg } N_2O\text{-N)}}{\text{N applied/incorporated (kg N)}} * 100$$

12. Quality control

An exchange of samples of chamber air and standard gas mixtures between laboratories (i.e. ADAS, AFBI, NW-Res, SAC) operating the GCs should be carried out, to avoid the possibility of any bias in the results towards high or low values. Each institute should follow local QA protocols, e.g. on use of reference samples in each GC run.

13. Soil measurements

Ancillary soil measurements should be made to enable comparisons between sites, to get an overall picture of the responses of different N forms/rates etc., provide essential data to the modellers and to be able to relate the work to previous studies. These measurements should be:

(a) gravimetric soil moisture content (in order to derive % water-filled pore space, WFPS)

(b) soil temperature

13.1 Soil moisture and bulk density

At every N_2O measurement occasion, soil samples (0-10 cm) will be taken from each **block** for the determination of gravimetric soil moisture. Samples should be labelled with a unique identifier and should be stored in a cold room prior to analysis, within 28 days of sampling.

Following the receipt of soil moisture results, the data should be checked, copied into the working file at each site and, entered into an excel spreadsheet on the local computer system. File records of laboratory analyses must include identification of the laboratory and analytical methods used.

It is necessary to measure the soil dry bulk density (mass per unit volume of dry soil in its undisturbed state) of the topsoil (0.-10 cm) in order to convert the gravimetric moisture content to water filled pore space (WFPS). This is an extremely useful measure since it provides an indication of potential gas movement and aerobicity of the soil, and therefore may be useful in explaining the N_2O emission pattern. Dry bulk

density should be measured using the core cutter method, which uses small cylinders (min 10 cm diameter) that are hammered into the soil. The weight of soil within the core is determined after careful trimming. Bulk density should be measured using one core per plot, on the untreated 'control' and highest N input treatment plots (i.e. 6 samples in total, assuming each treatment is replicated 3 times):

- in the 1st week after the first (or perhaps only) N application.
- after c.4 weeks following N application (spring sown crops only where soil settling after sowing is likely).
- following autumn soil cultivations (arable sites only).

13.2 Soil temperature

Soil temperature should be measured continuously using 3 automatic temperature loggers (e.g. TinyTalk logger, USB interface temperature data logger etc.) set to log at hourly intervals for the length of the experiment i.e. one per block. Each logger should be sealed in a polythene bag and secured with tape to prevent damage by moisture. A piece of string should be strongly attached at one end to the bag and the other to a peg on the soil surface marking the location of the logger burial. Loggers should be buried at a depth of 5 cm on the same selected treatment. Following the last N_2O sampling on each experiment the loggers should be dug up and promptly downloaded. For security, down loaded data should be immediately copied to the local computer system at each site.

13.3 Soil Mineral N

Soil samples (0-10 cm) will be taken at regular intervals (see Table below) from selected treatments for the determination of topsoil SMN and soil moisture. All treatments will be sampled **except treatment 2 and treatment 4.** The sample should be made up from 5 randomly selected subsamples within the plot. Thus where there are 10 treatments, 8 of these would be sampled (excluding the intermediate fertiliser treatments) in each of 3 replicate plots, generating 24 samples. The soil samples should be labelled with a unique identifier. The soil samples are to be analysed fresh within 5 days so must be kept cool i.e. in a cold store or fridge.

Mineral N sampling schedule

Weeks before/after N input (e.g. manure/fertiliser application etc.)	Number of measurements
-1	1
0	1
1	1
2	1
3	1
4-7	1
8-12	1
13-16	1
17-20	1
21-24	1
25-30	1
30-37	1
37-46	1
46-52+	1
	Total c 14 samplings/year

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Analysis

Soil samples are passed through a 4mm sieve. The stone content of the site should be determined separately as a one off measurement in order to allow the concentration of N to be calculated on a site basis.

A fresh sample of the sieved soil is accurately weighed (to 2 dps) into a plastic pot and 2M KCl is added to give a soil to extractant ratio of 1:2 (eg 100 g soil added to 200 ml KCl), this mixture is shaken on an orbital shaker for 1 hour.

After shaking allow the mixture to settle for 2-3 minutes before transferring approx. 15ml into a 16ml disposable centrifuge tube; centrifuge at 400rpm for 10 min. Transfer centrifuged supernatant into a 10ml polystyrene test tube, cap then store the extracts in a fridge until analysis. An alternative approach is to filter samples through a Whatman No 40 filter paper. A 15ml sample of the 2M KCI is also centrifuged or filtered providing a blank for analysis.

Determination of NH_4-N and NO_3-N carried out using an autoanalyser. The top standard for NH_4-N and NO_3-N is 2 ppm. Some samples may need to be diluted - use 2M KCI solution.

Solutions

2M KCl – Dissolve 149.1 KCl (KCl, mol. Wt. 74.55) in de-ionised water in a volumetric flask and make up to one litre with de-ionised water. Use high purity KCl.

Results are expressed as mg/kg soil (OD) i.e. ppm (OD).

Following the receipt of soil analysis results, the data should be checked, copied into the working file at each site, entered into an excel spreadsheet

14 Meteorological measurements

At each experimental site, daily rainfall and air and soil (5cm depth) temperature (min and max) and atmospheric air pressure should be measured.

Appendix 1.

Proforma for gas sampling for analysis by GC

Experiment Code:	
Experiment Title:	
Site Name:	
Field Name:	
Sampling Date:	
Number of Ambients and Time Taken:	
Number of stored samples and concentration (ppm): (To be entered by lab staff)	
Weather during sampling:	**Raining / Dry** **Hot / Warm / Cool / Cold** **Calm / Light breeze / Windy**
Soil conditions:	**Water on surface / Soil wet / Soil moist / Soil dry**
General Comments:	

NORTH WYKE ROTHAMSTED RESEARCH

afbi Agri-Food and Biosciences Institute

SAC

ADAS

Sampled by:…… Certified by:.....……..

Appendix 2.

Sample Identifier	Plot Number	Chamber Number	Chamber and lid equipment numbers	Chamber Height (cm)	Time Chamber On	Time Gas Sampling	Notes

Sampled by:…… Certified by:.....……..

Appendix 3.

Example data for N_2O gas sampling sheet

Sample Identifier	Plot Number	Chamber Number	Chamber and lid equipment numbers	Chamber Height (cm)	Time Chamber On	Time Gas Sampling	Notes
TT-NT2605-001	6	1	BOX/CH487 BOX/LD206	15.5, 19.5 15.2, 19.3	10:54	11:34	
TT-NT2605-002	6	2	BOX/CH488 BOX/LD207	20.3, 20.4 20.2, 19.9	10:54:30	11:34:30	
TT-NT2605-003	6	3	BOX/CH489 BOX/LD208	15.5, 19.5 15.2, 19.3	10:54	11:34	
TT-NT2605-004	6	4	BOX/CH490 BOX/LD209	20.3, 20.4 20.2, 19.9	10:55	11:35	
TT-NT2605-005	6	5	BOX/CH491 BOX/LD210	15.5, 19.5 15.2, 19.3	10:55:30	11:35:30	
TT-NT2605-006	9	2	BOX/CH492 BOX/LD211	20.3, 20.4 20.2, 19.9	10:55	11:35	
TT-NT2605-007	9	2	BOX/CH493 BOX/LD212	17.6, 22.5 17.6, 21.3	10:56	11:36	
etc.	etc.	etc.	Etc.	etc.	etc.	etc.	

Sampled by:…… Certified by:.....……..

Appendix 4.

N_2O flux calculation from a static closed chamber:

$$N_2O \text{ flux (g } N_2O\text{-N ha}^{-1} \text{ d}^{-1}) = \frac{\text{increase in } N_2O \text{ concentration (ppm) x chamber height (cm)}}{\text{Chamber enclosure time (min)}} \text{ x Conversion factor}$$

Derivation of the factor with which to convert the increase in N_2O concentration (ppm) inside a chamber to a N_2O flux rate (g N_2O-N ha^{-1} d^{-1}).

Where increase in concentration = 1 ppm min^{-1}

1 ppm $\equiv$ 1 ml m^{-3} min^{-1}

$\equiv$ 1 x 44/23.63 (mg N_2O m^{-3} min^{-1}), assumes that at 1 atmosphere of pressure the molar volume of an ideal gas at 15°C occupies 23.63 litres of space

$\equiv$ product of the above x 28/44 x $1/10^3$ (g N_2O-N m^{-3} min^{-1})

$\equiv$ product of the above x $1/10^6$ (g N_2O-N cm^{-3} min^{-1})

Therefore flux in **g N_2O-N ha^{-1} d^{-1}** is as follows:

$\equiv$ 1 x 44/23.63 x 28/44 x $1/10^3$ x $1/10^6$ x 10^8 (cm^2 to ha) x 60 (min to hr) x 24 (hr to days)

Factor to convert the increase in N_2O concentration over time (in ppm min^{-1}) is:

$\equiv$ 170.6306

Note the volume that an ideal gas occupies will vary depending on temperature. The figure used can be altered using the values in the table below:

Air temperature (°C)	Molar volume (l/mol)	Conversion factor
-10	21.58	186.8397
-9	21.66	186.1324
-8	21.74	185.4305
-7	21.83	184.7338
-6	21.91	184.0424
-5	21.99	183.3561
-4	22.07	182.6749
-3	22.15	181.9987
-2	22.24	181.3276
-1	22.32	180.6613
0	22.40	180.0000
1	22.48	179.3435
2	22.56	178.6917
3	22.65	178.0447
4	22.73	177.4023
5	22.81	176.7646
6	22.89	176.1314
7	22.97	175.5027
8	23.06	174.8786
9	23.14	174.2588
10	23.22	173.6434
11	23.30	173.0324
12	23.38	172.4256
13	23.47	171.8231
14	23.55	171.2247
15	23.63	170.6306
16	23.71	170.0405
17	23.79	169.4545
18	23.88	168.8725
19	23.96	168.2945
20	24.04	167.7205
21	24.12	167.1503
22	24.20	166.5840
23	24.29	166.0216
24	24.37	165.4629
25	24.45	164.9080
26	24.53	164.3568
27	24.61	163.8092
28	24.70	163.2653
29	24.78	162.7250
30	24.86	162.1883
31	24.94	161.6550
32	25.02	161.1253
33	25.11	160.5991
34	25.19	160.0762
35	25.27	159.5568

ACO116

Joint ADAS, AFBI and Rothamsted Research North Wyke experimental protocol for livestock manure experiments on arable and grassland sites (experimental design, N_2O flux, NH_3 flux, NO_3 leaching and soil sampling)

Edition:02 **Effective from: 14.12.11**

Introduction

The following protocol has been prepared on behalf of the ACO116 consortium in order to harmonise the experimental approach used by research partners in order to achieve the highest possible quality of data from the research being undertaken. Wherever possible a standard approach has been adopted, however it is also necessary to accommodate minor differences in local practice providing that this does not compromise the quality of data being produced. This document aims to be consistent where possible with protocols developed in earlier projects, but it is also recognised that methodologies will be continuously reviewed, and where necessary updated (in consultation with consortium members) to ensure best practice and allow for the incorporation of new approaches and techniques.

2. EXPERIMENTAL PLATFORMS AND TREATMENTS

1.1. Experimental Sites

Experiments will take place at or near the sites listed in the table below.

Zone Name	Soil Texture Group	Annual Rainfall (mm)	Experimental platform (planned)	Experimental land use and organisation responsible for the platform
Dry	Sandy/light	0-750	Wensum, East Anglia	Arable (ADAS)
Medium	Medium	751-950	ADAS Rosemaund, Herefordshire	Arable (ADAS)
Dry	Heavy	0-750	ADAS Drayton, West Midlands	Grass (ADAS)
Wet	Medium /heavy	951+	North Wyke, Devon	Grass (RRes - NW)
Wet	Medium	951+	ADAS Pwllpeiran, Wales	Grass (ADAS)
Wet	Medium	951+	Hillsborough, Northern Ireland	Grass (AFBI)

There should be no history of long term organic manure applications and no manure applications or grazing 6 months prior to establishment of the experiment. Previous site history (over at least 12 months) should be recorded.

2. Treatments

2.1 Arable sites

The following treatments will be applied at the arable sites: ADAS Rosemaund (cattle slurry/FYM to winter wheat) and Wensum (pig slurry/FYM to winter barley).

1	Autumn control
2	Autumn applied slurry surface broadcast (incorporated within 24 hrs)
3	Autumn applied slurry bandspread
4	Autumn applied FYM (incorporated within 24 hrs))
5	Autumn applied poultry litter (incorporated within 24 hrs)
6	Autumn applied layer manure (incorporated within 24 hrs)
7	Spring control
8	Spring applied slurry surface broadcast
9	Spring applied slurry bandspread
10	Spring applied FYM (topdressed)
11	Spring applied poultry litter (topdressed)
12	Spring applied layer manure (topdressed)
13	Control- Manufactured fertiliser at 0 kg/ha
14	Manufactured fertiliser at 50 kg/ha
15	Manufactured fertiliser at 100 kg/ha
16	Manufactured fertiliser at 150 kg/ha
17	Manufactured fertiliser at 200 kg/ha
18	Manufactured fertiliser at 250 kg/ha

2.2 Grassland sites

The following treatments will be applied at ADAS Pwllpeiran, North Wyke and Hillsborough.

1	Control (autumn)
2	Autumn applied cattle slurry surface broadcast
3	Autumn applied cattle slurry bandspread
4	Autumn applied cattle slurry +DCD surface broadcast
5	Autumn applied cattle slurry +DCD bandspread
6	Autumn applied cattle FYM
7	Control (spring)
8	Spring applied cattle slurry surface broadcast
9	Spring applied cattle slurry bandspread
10	Spring applied cattle slurry +DCD surface broadcast
11	Spring applied cattle slurry +DCD bandpsread
12	Spring applied cattle FYM (topdressed)
13	Control - Manufactured fertiliser at 0 kg/ha grass (first cut)
14	Manufactured fertiliser at 40 kg/ha grass (first cut)
15	Manufactured fertiliser at 80 kg/ha grass (first cut)
16	Manufactured fertiliser at 120 kg/ha grass (first cut)
17	Manufactured fertiliser at 160 kg/ha grass (first cut)
18	Manufactured fertiliser at 200 kg/ha grass (first cut)

At ADAS Drayton the treatments will be:

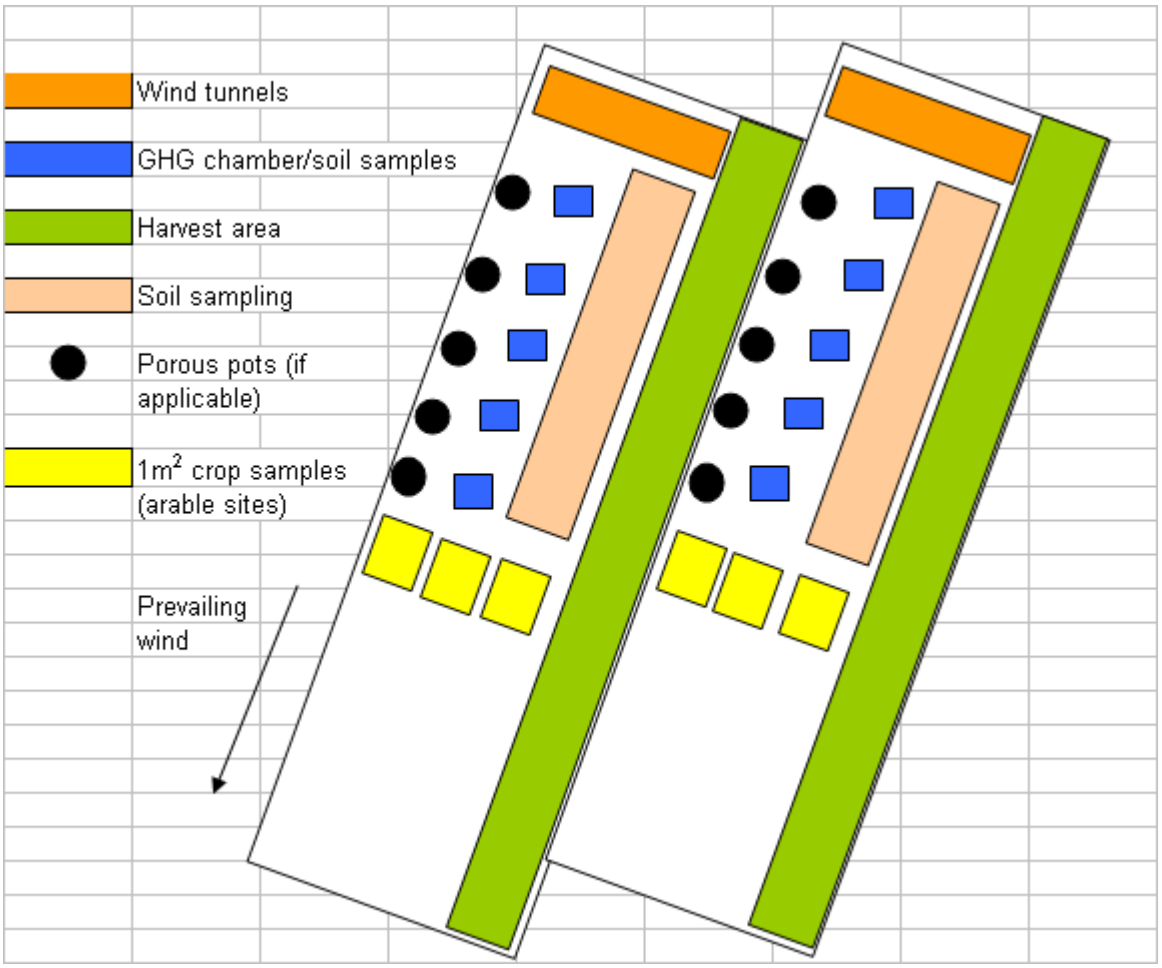
1	Control (autumn)
2	Autumn applied pig slurry surface broadcast
3	Autumn applied pig slurry bandspread
4	Autumn applied pig slurry +DCD surface broadcast
5	Autumn applied pig slurry +DCD bandspread
6	Autumn applied pig FYM
7	Control (spring)
8	Spring applied pig slurry surface broadcast
9	Spring applied pig slurry bandspread
10	Spring applied pig slurry +DCD surface broadcast
11	Spring applied pig slurry +DCD bandpsread
12	Spring applied pig FYM (topdressed)
13	Control - Manufactured fertiliser at 0 kg/ha grass (first cut)
14	Manufactured fertiliser at 40 kg/ha grass (first cut)
15	Manufactured fertiliser at 80 kg/ha grass (first cut)
16	Manufactured fertiliser at 120 kg/ha grass (first cut)
17	Manufactured fertiliser at 160 kg/ha grass (first cut)
18	Manufactured fertiliser at 200 kg/ha grass (first cut)

3. EXPERIMENTAL DESIGN AND ANALYSIS

3.1 Experimental design

There will be three replicates of each treatment. For practical reasons associated with the use of wind tunnels for measuring ammonia emissions, the spring, autumn and fertiliser treatments will be grouped together (each group with an untreated control), but arranged in a randomised block design within each group.

Plots will be 2-6m wide and 12-24m long. The livestock manure plots will be orientated at 20 degrees to the 'vertical' to allow for correct placement of the wind tunnels for measuring ammonia emissions. An example schematic diagram of 2 standard plots is illustrated below (not to scale).



Depending on the availability of space, the harvest area could either be to one side of (as illustrated) or below the chambers

3.2. Statistical analysis

All nitrous oxide, ammonia and nitrate leaching data will be statistically analysed by techniques appropriate to the experiments described. Cumulative emissions/losses, emission factors and associated standard errors will be generated.

All crop data will be analysed by analysis of variance (Genstat 5 or later) provided that the assumptions of the test are met.

Advice from a trained statistician will be sought in the design and analysis of the experiments.

4. SITE MANAGEMENT

4.1 Crop/grass variety

Varieties of all crop types will be determined by the local research provider. The varieties should be typical of what is commercially grown in the area the site is located in. If possible, a short stemmed variety of cereals would be preferred for ease of N_2O sampling.

4.2. Crop management

To prevent any nutrient deficiencies, overall basal P, K and Mg fertilisers and S should be applied according to site requirements. Applications should follow recommendations in 'The Fertiliser Manual (RB209) published 2010' for sites in England. Such recommendations should be checked by a FACTS qualified advisor.

Apply other agro-chemicals as needed and according to good agricultural practice to control weeds, pests and diseases. Agro-chemicals should be applied by the host farmer.

4.3. Livestock manure management

Livestock manures will be sourced locally by the relevant site manager and stored on site over-winter so that the same manures can be applied at the autumn and spring timings. Slurries will be stored in tanks and solid manures in heaps either in an undercover storage area or covered by plastic sheeting

Livestock manure application rates will be based on the total N content of the supplied materials ensuring that the crop available N applied should not exceed crop N requirements (the same application rate will be used for the autumn and spring applications). The storage and land spreading of the manures will be compliant with Good Agricultural Practice (i.e. application rates will not exceed 250 kg total N/ha).

4.4 Farm operations

The host farmer or research provider will carry out:

- all soil cultivations and seed bed preparation for the test crop using equipment typical of the area and suitable for the soil type
- sowing of the test crop
- application of agro-chemicals to the test crop
- application of non-nitrogen fertilisers e.g. P, K, S etc. if required to the test crop. If the host farmer would normally apply non-N fertilisers at the same time as N fertiliser, non-N fertiliser will need to be applied by research staff. Rates of non-N fertiliser will be based on soil analysis and guidelines in 'The Fertiliser Manual (RB209) published 2010'. Such recommendations should be checked by a FACTS qualified advisor.
  The only nitrogen to be applied to the plots is to be by hand by research staff.
- all soil cultivations and seed bed preparation for the follow on crop using equipment typical of the area and suitable for the soil type. In some circumstances this would involve leaving the field in stubble throughout the winter period, but it could also include cultivation during this period, in accordance with standard practice
- sowing of the follow on crop
- application of agro-chemicals to the follow on crop

No additional nitrogen should be applied to the plots before the 12 month N_2O measurement periods have finished and 'NO NITROGEN SIGNS' must be put up around the perimeter of the experiment.

5. SITE CHARACTERISATION

5.1 Sample collection

In order to characterise the experimental site, baseline topsoil samples (0-7.5cm for grassland sites; 0-15cm for arable sites) should be taken from each block prior to any organic material applications using a 'cheese' corer, pot corer, tubular or screw auger as appropriate.

Each sample should consist of not less than 25 cores of soil taken from separate points, evenly distributed across the block using a "W"-shaped or regular grid pattern traverse. The soil collected should be sub-sampled after thorough mixing to provide 2 subsamples for laboratory analysis as described below.

5.2 Sample analysis

5.2.1 Routine soil analysis

Approximately 500 g of soil should be dispatched to the laboratory for the following analyses:

- pH (in water)
- extractable P, K, S, Mg
- total N
- total organic carbon by modified Walkley Black and loss on ignition (LOI) methods
- particle size distribution (PSD) – sand, silt, clay

5.3 Bulk density

At each site, soil bulk density should be measured using the core cutter method in which small cylinders (min 10 cm diameter) are hammered into the soil. Ten cores (0-7.5cm for grassland sites; 0-15cm for arable sites) should be taken per block and the weight of soil within the cores determined after careful trimming.

Samples should be taken when soils are at or close to field capacity.

5.4. Available water capacity

Ten undisturbed soil cores per block (30 cores in total) should be taken from the topsoil using the small cylinders used for bulk density measurements. These samples will be sent to ADAS Gleadthorpe for field capacity (0.05bar) moisture content determination. An additional bulked sample of disturbed topsoil (0-10 cm) will be taken from each block (10 cores per sample) and sent to ADAS Gleadthorpe for the determination of permanent wilting point (15 bar).

5.5 Soil mineral N

At the same time as the sampling described above, an additional soil mineral N sample (0-90cm) should also be taken from each block, with 10 cores per block and the samples split 0-30 cm, 30-60 and 60-90 cm (or to soil depth, in 30 cm increments). Samples should be taken using either Eijkelkamp "Stepwise" Gouge Augers of 40, 30, and 20 mm diameter; EJH Danish augers of 19 and 22 mm diameter or JMC "Backsaver" equipment. Samples should be kept cool in a coolbox containing icepacks and dispatched the **same day** to the laboratories for analysis (samples should not be taken on a Friday to ensure they reach the laboratory the next day). Soil mineral N analysis is described in Appendix II.

6. LIVESTOCK MANURE APPLICATION AND ANALYSIS

6.1 Livestock manure application

Livestock manure applications should follow the NVZ rules for high readily available N materials (*i.e.* poultry manure and slurry) applied outside the closed periods. All livestock manures should be applied at the same time either manually (solids) or using suitable application machinery (liquids). The exact livestock manure application timings will depend on seasonal and local conditions

Slurry, FYM and poultry manure application rates should be based on manure analysis**.** All manure application rates must be agreed in advance with a FACTS qualified adviser.

Target application rates on arable land are: 40m^3/ha for slurry, 30t/ha for FYM, 8 t/ha for broiler litter and 13 t/ha for layer manure. For the poultry manure it will be necessary to base the application rate on an analysis of the applied material

Target application rates on grassland are: 30m3/ha for slurry and 25 t/ha for FYM

The slurry must be agitated before application to ensure that material with a similar dry matter content is applied to each plot When applying slurry using tractor powered application equipment the tanker output should be calibrated by measuring the machine output over a measured time to ensure accurate measurement of the application rate. If practically possible weighing the tanker before and after application should be used to check the application rate..

Trailing hose applications to arable land will be applied at 30 cm spacings and trailing shoe applications to grassland will be applied at 20 cm spacings.

For each application photographic records (i.e. digital images) of the applications will be made to record the distance between the bands so that the proportion of ground covered with slurry can be calculated for each application method and rate. Visual assessments will be made of the time taken for slurry to infiltrate into the soil (i.e. until there are no obvious signs of 'ponded' slurry on the soil surface)

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When slurry is applied using watering cans the volume of slurry added to each can should be accurately measured using a calibrated measuring cylinder. The number of cans used per plot should be recorded so that the application rate can be quantified.

6.2 Use of DCD

At the grassland sites, DCD will be applied to treatments 4, 5, 10 and 11 as a 1% solution (i.e. 10g/1000ml) at a rate equivalent to 10kg/ha DCD (i.e. 1000l/ha of 1% solution). The DCD solution should be applied using calibrated spray equipment within 1 hour after the slurry has been applied.

6.3 Livestock manure analysis

Take a representative sample of each livestock manure from each block at spreading (*c.*2 litres of liquid per block for each liquid manure type and *c.*2 kg of solid per block for each solid manure type). The liquid samples should be taken in 2 labelled 1 litre plastic bottles and the solid samples in clean plastic bags. All samples should be analysed 'fresh' (i.e. not frozen).

- dry matter
- pH
- total N, P, K, S, Mg & Ca
- ammonium N (NH_4-N) and nitrate N (NO_3-N) *i.e.* readily available N
- total organic carbon (Modified Walkley-Black method)

7. MANUFACTURED FERTILISER APPLICATIONS

Ammonium nitrate mineral N fertiliser (34.5% N) will be applied by hand to the manufactured fertiliser N response plots (treatments 13-18) at appropriate timings and splits as advised by the Work Package Manager.

8. CROP ASSESSMENTS

8.1 Yield measurements

8.1.1 Arable sites

It is necessary to quantify the whole crop N uptake i.e. in grain and straw.

A few days before harvest, take about 100 hundred tillers at random from across the plot. Weigh a representative sample of the whole crop. Thresh the sample and weigh the grain to determine how much of the crop is grain and how much is straw and chaff. Take one representative sample of mixed straw and chaff per plot and analyse for % dry matter and total N content.

Plot yields should be determined separately by harvesting a minimum area of 15 m^2 using a small plot harvester. Record the yield and the harvest area. Take a representative sample of grain off the combine and send for % dry matter and total N content.

The dry matter and N content of grain and straw samples should be determined in order to calculate N offtake.

Care should be taken not to walk on areas being used for crop harvests.

8.1.2 Grassland sites

Grassland sites should be cut on at least 3 occasions during the year. Cuts would normally take place in May, June and July or August depending upon the location. Plot yields should be determined by harvesting a minimum area of 15 m^2 using a small plot harvester. The dry matter and N content of grass samples should be determined in order to calculate N offtake.

9. N_2O MEASUREMENTS

The closed, static chamber method is used to measure the emission of N_2O from soil. A chamber box is inserted to a depth of approximately 5 cm or greater (i.e. sufficient to produce an adequate seal). Background measurements of the gases of interest are taken by manually sampling the atmosphere around the experimental plots. To begin flux measurements, a lid is placed on top of the chamber, enclosing the atmosphere above the soil and within the chamber. The time at which this is done is noted. The chamber lid is left on for a predetermined amount of time, usually 40 minutes. The accumulation of N_2O within the chamber is measured by manually taking a gas sample from the chamber, storing this in an evacuated glass vial and sending the sample for analysis by gas chromatography. The N_2O increase (ppmv) over the incubation period (minutes) is used to calculate the N_2O emission rate.

Nitrous oxide measurements should be made according to the sampling strategy outlined below:

Weeks after application	Number of GHG measurements
1 week before application (ambient)	1
0-2	10
2-4	4
4-8	2
8-12	2
12-16	2
16-20	2
20-24	2
24-28	1
28-32	1
32-36	1
36-40	1
40-44	1
44-48	1
48-52	1
	Total = 32

Measurements will continue for **12 months** following the **autumn and spring manure application timings**.

On arable sites, N_2O measurements should be carried out once in the week before the main cultivation, once immediately after cultivation following harvest and if possible once after the first rain event (e.g. 5-10 mm) after this cultivation.

Details of the method to be used are given in Appendix II.

10. AMMONIA MEASUREMENTS

Ammonia losses will be measured from the control plots and all livestock manure treated plots using small-scale windtunnels (see Appendix IV)., placed on the plots as shown in the diagram above.

Ammonia emissions will be measured for up to 7 days from the slurry and FYM treatments and up to 21 days from the poultry manure treatments. Bubbler samples will be changed at the following times after livestock manure application: 1 hour, 3 hours, 6 hours, 24 hours and then daily until the end of the measurement period.

Sub-samples (20ml) of 0.02M orthophosphoric acid should be sent to the laboratory for analysis of ammonium-N. A duplicate 20ml sub-sample should be retained in the fridge in case reanalysis is required at a later date.

11. LOSSES TO WATER

Nitrate-N and ammonium-N losses to water will be measured using Prenart SuperQuartz soil water samplers from the autumn livestock manure application timings at the Wensum site only. Five samplers will be installed on each plot with samples taken every 2 weeks or after 25mm of rainfall, whichever is sooner. Samples of leachate will be analysed for nitrate-N and ammonium-N. The IRRIGUIDE model will be

used to calculate overwinter drainage volumes. The nitrate-N and ammonium-N concentrations will be combined with over winter drainage volumes to calculate the amount of N leached from the contrasting manure treatments.

12 ONGOING SOIL MEASUREMENTS

Ancillary soil measurements should be made to enable comparisons between sites, to assist our understanding of the responses of different N forms/rates etc., provide essential data to the modellers and to be able to relate the work to previous studies. These measurements should be:
(a) gravimetric soil moisture content (in order to derive % water-filled pore space, WFPS)
(b) soil temperature

12.1 Soil moisture and bulk density

At every N_2O measurement occasion, soil samples (0-10 cm) will be taken from each **block** for the determination of gravimetric soil moisture. Samples should be labelled with a unique identifier and should be stored in a cold room prior to analysis, within 28 days of sampling.

Following the receipt of soil moisture results, the data should be checked, copied into the working file at each site and, entered into an excel spreadsheet on the local computer system. File records of laboratory analyses must include identification of the laboratory and analytical methods used.

It is necessary to measure the soil dry bulk density (mass per unit volume of dry soil in its undisturbed state) of the topsoil (0.-10 cm) in order to convert the gravimetric moisture content to water filled pore space (WFPS). This is an extremely useful measure since it provides an indication of potential gas movement and aerobicity of the soil, and therefore may be useful in explaining the N_2O emission pattern. Dry bulk density should be measured as described in Section 5.3 above. Bulk density should be measured using one core per plot, on the untreated 'control' (i.e. treatment 1) and highest N input treatment plots (i.e. treatment 18) giving 6 samples in total, assuming each treatment is replicated 3 times:

On the arable sites bulk density measurements should also be taken:

- in the 1st week after the manure applications.
- after c.4 weeks following manure application (spring sown crops only where soil settling after sowing is likely).
- following autumn soil cultivations (arable sites only).

On the grassland experiments, soil bulk density measurements should be taken 1 week after the manure applications.

12.2 Soil temperature

Soil temperature should be measured continuously using 3 automatic temperature loggers (e.g. TinyTalk logger, USB interface temperature data logger etc.) set to log at hourly intervals for the length of the experiment i.e. one per block. Each logger should be sealed in a polythene bag and secured with tape to prevent damage by moisture. A piece of string should be strongly attached at one end to the bag and the other to a peg on the soil surface marking the location of the logger burial. Loggers should be buried at a depth of 5 cm on the same selected treatment. Following the last N_2O sampling on each experiment the loggers should be dug up and promptly downloaded. For security, down loaded data should be immediately copied to the local computer system at each site.

12.3 Soil Mineral N

Soil samples (0-10 cm) will be taken at regular intervals (see Table below) from all the livestock manure treatments (treatments 1-18) for the determination of topsoil SMN and soil moisture. The sample should be made up from 5 randomly selected subsamples within the plot. The soil samples should be labelled with a unique identifier. The soil samples are to be analysed fresh within 5 days so must be kept cool i.e. in a cold store or fridge. See Appendix 1 for details of the analytical technique to be used.

Mineral N sampling schedule:

Weeks before/after N input (e.g. manure/fertiliser application etc.)	Number of measurements
-1	1
0	1
1	1
2	1
3	1
4-7	1
8-12	1
13-16	1
17-20	1
21-24	1
25-30	1
30-37	1
37-46	1
46-52+	1
	Total c 14 samplings/year

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13 Meteorological measurements

At each experimental site, daily rainfall and air and soil (5cm depth) temperature (min and max) should be measured.

APPENDIX I: Analysis of soil samples for soil mineral N.

1. Analysis

Soil samples are passed through a 4mm sieve. The stone content of the site should be determined separately as a one off measurement in order to allow the concentration of N to be calculated on a site basis.

A fresh sample of the sieved soil is accurately weighed (to 2 dps) into a plastic pot and 2M KCl is added to give a soil to extractant ratio of 1:2 (eg 100 g soil added to 200 ml KCl), this mixture is shaken on an orbital shaker for 1 hour.

After shaking allow the mixture to settle for 2-3 minutes before transferring approx. 15ml into a 16ml disposable centrifuge tube; centrifuge at 400rpm for 10 min. Transfer centrifuged supernatant into a 10ml polystyrene test tube, cap then store the extracts in a fridge until analysis. An alternative approach is to filter samples through a Whatman No 40 filter paper. A 15ml sample of the 2M KCI is also centrifuged or filtered providing a blank for analysis.

Determination of NH_4-N and NO_3-N carried out using an autoanalyser. The top standard for NH_4-N and NO_3-N is 2 ppm. Some samples may need to be diluted - use 2M KCI solution.

2. Solutions

2M KCl – Dissolve 149.1 KCl (KCl, mol. Wt. 74.55) in de-ionised water in a volumetric flask and make up to one litre with de-ionised water. Use high purity KCl.

Results are expressed as mg/kg soil (OD) i.e. ppm (OD).
Following the receipt of soil analysis results, the data should be checked, copied into the working file at each site, entered into an excel spreadsheet

APPENDIX II: Nitrous oxide emission measurements

1 Time

Two members of staff can sample from 150 chambers (5 chambers x 6 plots x 3 blocks) in approximately 3hrs 15mins. This includes taking field and ambient samples. Extra time is required for vial labelling, packing and despatch, chamber maintenance.

2 Materials and Equipment

2.1.Chambers

These should be square/rectangular covering a surface area of *c*.0.16 m^2 (or circular and have a diameter in the range 25-50 cm to give a similar surface area). The minimum height above ground should be 15-20 cm over grass swards/ young arable crops. As the latter crops develop, the chambers may need to be replaced by taller ones, or stackable types (e.g. chambers with a water filled channel) where an "extension layer" can be added when necessary, to keep the chamber top above the height of the crop.

Two main methods of chamber installation are commonly used.

(a) Chambers that are essentially square or circular "sleeves": insert the bottom of the chamber wall into a 5 cm deep slot cut in the soil, and tamp the soil on the outside to make a good seal. Close the top with a gas-tight lid (using a rubber or water seal), leaving the lid in place only for the duration of the measurement. Leave the chamber body in situ between measurements (only remove if/when agronomic operations make it necessary).

(b) Chambers with soil "collars". In this type, a short sleeve or "collar" is sealed into the soil in a similar way as in (a), but the above-ground extension is only of the order of 4-5 cm. A closed chamber with 15-20 cm high walls and an integral top (i.e. in the form of an inverted bucket or box) is attached for the measurement period.

During the latter stages in the growth of cereal crops, chamber extensions will be required to accommodate the growing plants (normally in late May or June). These should be stacked above the existing chamber, providing a gas tight seal, and allowing gas flux measurements to be made throughout the growing season. Once in place, chamber extensions would remain until the crop is harvested.

2.2. Other equipment

- Numbered pegs to identify the chambers.
- Cutting tool/former.
- Timer
- Needles and 50-60 ml glass syringe.
- 20-22 ml evacuated gas collection vials. These must be standard vials for gas analysis using an automated headspace sampler.
- Crimp top glass vials
- Crimp caps
- Butyl rubber seals

3 Procedures

3.1 Safety considerations

The method involves bending to sample chambers at ground level, take care to avoid back strain/injury.

As hypodermic needles are used to sample ambient and chamber headspace gases, operators should take care not to "prick" themselves. Should blood be drawn following an accidental "pricking", the needle must be disposed of in a sharps bin to comply with health and safety regulations. Staff involved

in these measurements should ensure that they have up to date tetanus immunisation and are aware of local Risk Assessments for using needles. New needles should be used for each set of N_2O samples taken and old needles always disposed of in a sharps bin.

3.2 Measurements: planning

The flux of N_2O is determined by measuring the increase in N_2O concentration (above the concentration of the ambient air) in an enclosed chamber over a defined period of time. In most situations, the increase in N_2O concentration is linear over a 40-50 minute period, although may be in the range of 30-60 min depending on site conditions. Beyond this however, and depending on a variety of factors, the rate of increase tends to decline. As only one field measurement is made of the N_2O concentration, it is crucial to plan the timing of operations to allow for placing chambers and withdrawing the samples. Exceeding the suggested enclosure period greatly increases the risk of non-linearity and hence underestimating the flux.

Work out the timing of operations based on the experimental design outlined in the Study Protocol. As gas sampling takes the longest time, plan by working back from an enclosure period of 40-60 minutes per chamber. Allow 1 min between enclosing each chamber to ensure enough time for sampling.

Carry out operations by block, but randomise the order in which blocks are measured on different days, as N_2O fluxes do show some diurnal variation. If the experimental layout allows, on each measurement occasion the order of the plots and the order the chambers within each plot sampled should be **randomised daily** i.e. start at one end of the plot and work through the chambers in order or start at the other end of the plot and work through the chambers in that order.

Gas sampling should be carried out between 10:00 and 14:00 hours due to diurnal variation in N_2O emissions and if possible **between 10:00 and 12:00 hours** as indicated by IPCC good practice. For large experiments this might not be feasible. In all cases note the time of sampling on the proforma. The site details proforma (Annex 1) and gas sampling proforma (Annex 2) should be completed for each sample day.

Individual sample vials should be labelled with a unique sample identifier, which corresponds to that on the gas sampling proforma. The code should include a reference to the name of the site, experiment, plot, chamber and the project code. Both before and after collection it is good practice to place the gas vials in numerical order in a suitable container e.g. a labelled polystyrene tray.

3.3 Measurements from field plots

Complete a Site Details proforma (e.g. Annex 1), describing the site, weather conditions, and any special features that may be of use when it comes to analysis of the results, e.g. rabbit droppings inside chamber, description of the soil conditions etc. Complete a Gas Sampling proforma (e.g. Annex 2) during sampling. An example of a partially completed gas sampling proforma is shown in Annex 3.

Measurements of N_2O should be made from randomly determined positions (avoiding features such as tramlines) in the appropriate field location.

Five chambers should be placed in each of the plots before collecting gas samples. Press the chambers into slots (previously made by a spade or by hammering in and removing a square steel frame) in the soil to ideally a depth of > 5 cm, but not less than 3 cm. Once the chamber is installed, the outside edge needs to be tamped down to ensure a good seal.

Ensure that the chambers are inserted into the ground to give a level top edge. This is important if the type of chamber used has a water filled channel, as the water in the channel running around the chamber top needs to make a gas-tight seal when the chamber lid is fitted.

The height of the chamber should be measured from the soil surface to the highest part of the chamber at all 4 corners and written on the proforma**.** Additionally, a stick/ruler should be positioned so that it rests on top of the chamber to enable a height measurement to be made from the centre of the chamber.

Chamber insertion should be done not less than 24 hours before a fertiliser/manure application.

Once the chamber is in position, ensure that the chamber seal (water filled groove, rubber seal etc.) is undamaged and fully functioning so that a gas-tight seal will be formed. Place the lid on the chamber noting the exact time of enclosure in the proforma.

The chambers should stay in place throughout the experiment (but the lids removed after each measurement), although they will need to be removed prior to farm operations e.g. cultivations, fertiliser/manure application etc. and replaced as soon as possible after. Number the chambers with a unique identifier e.g. plot number and chamber number (1 to 5). The location of each chamber needs to be marked e.g. with a wooden peg or magnetic marker so that the chamber is always inserted in the same place if the chamber is moved. **The long-term position of the chambers is critical and if removed need to be returned to exactly the same position**.

Each chamber (unstacked) should normally be enclosed using a lid for a **40 minute period**. Note any deviations on the proforma. The time of closing each chamber and the time of taking the gas sample should be recorded on a data sheet.

Pre-evacuated 20-22 ml glass vials should be filled with gas taken from each individual chamber by syringe as detailed below:

- Take a 50 or 60 ml glass or plastic syringe fitted with a needle and either pierce the septum in the lid or connect to the chamber valve or connect to a 3-way tap inserted into the chamber (depending on chamber type) and slowly remove 50 or 60 ml of the headspace gas without withdrawing the needle or disconnecting the syringe.
- Depress the plunger to force this sample back into the chamber and to ensure that a representative gas sample is taken.
- SLOWLY withdraw another 50 ml sample, hold at 50 ml until plunger stays fast then remove the syringe from the chamber. Watch that the plunger does not retract into the syringe body, meaning that the gas was sampled too quickly i.e. the gas is not at atmospheric pressure, and consequently, that the sample volume collected is less than 50 or 60 ml.
- Pierce the septum of the appropriate labelled gas vial, as the vial is pierced, the gas will automatically be withdrawn from the syringe to equalise the pressures in the vial and syringe.
- Push the plunger in as far as it will go and hold the syringe in position.
- At some point prior to analysis (either in the field or lab) pierce the septum with another single needle (narrow bore) to allow excess pressure to be released
- Note down the gas vial identifier, such that the vial and N_2O concentration in the particular chamber headspace can be matched following subsequent analysis by GC.
- Record the time that gas samples are taken on the proforma.

3.4 Linearity check

To check on the linearity of gas accumulation within a chamber's headspace (an underlying principle of the methodology), on every N_2O sampling occasion select 3 chambers at random from the treatment with the highest N input. From each of these 3 chambers take a time series of samples following closure; 6 samples at evenly spaced intervals (eg 0, 10, 20, 30, 50, 60 for the 40 minute closed chambers and 0, 15, 30, 45, 60, 75 and 90 for the 60 minute closures) and submit for analysis. The remaining chambers are measured using a terminal sampling only as above. If non-linear responses are

obtained (e.g. when soil is particularly dry), a correction algorithm will need to be applied to adjust the flux values.

If the chambers are to be stacked in order to permit N_2O sampling from a growing crop, linearity checks should also be carried out prior to chamber stacking and N_2O measurement. The enclosure time may need to be increased as a result of the increase in chamber volume, but not too much so that N_2O accumulation is non-linear. At least 1 week before the addition of a stacked chamber is required, select two chambers at random from the treatment with the highest N input. From each chamber take 6 or 7 samples at evenly spaced intervals (0 min, 15, 30, 45, 60, 75 and 90 min) after closure. The determination of the enclosure time will be based on the resultant linearity graphs.

3.5 Measures to permit N_2O sampling throughout the growing season of arable crops

In order to sample N_2O throughout the growing season it is necessary to use a chamber type which allows chamber stacking e.g. chambers with a water filled channel.

The water seal allows a series of chambers to be stacked on top of each other as the crop increases in height. Once the crop has reached the top of the in-situ chamber i.e. the chamber lid can no longer be fitted without crop damage, fit another chamber onto the in-situ chamber for sampling. Fill both grooves with water i.e. between the 2 chambers and between the extra chamber and the lid. Put the lid on and sample as usual. Additional chambers can be added if the crop height necessitates it. After each gas sampling, the additional, stacked chambers that have been fitted can remain in place so that the crop is not damaged. If the chambers are stacked 3 or 4 high it may be possible to remove the top chamber after sampling. Indicate on the proforma how many stacked chambers are left in-situ after sampling. If stacked chambers are removed, do not leave inside the plot on the soil surface, move to the discard area.

3.6 Ambient N_2O concentration at the site

Make an assessment of the ambient N_2O concentration of the experimental area, as follows:

- Collect (in pre-evacuated gas vials) ten 20 to 22-ml ambient gas samples from the experimental plot area, 5 samples at the start of chamber sampling and 5 samples at the end of chamber sampling. These samples should be collected as in Section 3.8, away from any roads and the soil surface to avoid contamination from car exhausts or soil efflux respectively. Take the ambient samples from about one metre above the ground, i.e. approximately around waist height.

- Vials should be labelled with the name of the field site and the project code and a unique sample identifier, such that these samples can be identified as ambient.

3.7. Completion and sample submission

- Once all samples have been taken, remove the lids from the chambers and place each lid away from the chamber (approximately 1-2 m), so as to avoid a preferential rain shadow around the chamber.
- The glass gas vials can be easily broken, so ensure that they are well packaged to avoid damage in transit. A photocopy of the proforma sheets should be kept in the working file used by the gas sampler for ready reference.
- If there is no GC facility at the site where the experiment takes place, and samples are to be transported, the signed proforma sheets should be sent with the corresponding sample vials as **soon as possible** after collection to the laboratory for analysis. Prompt return of the gas vials is important since standards are added to each batch of vials at the analysis laboratory. The standards are essential to correct for gas loss if there is a delay in analysis by GC.
- The gas samples will be analysed by GC.

- Sample vials should be evacuated as close to N_2O sampling as possible. Do not use vials which have been evacuated for more than one week. Vials can be evacuated either using a manual or mechanical suction pump. Septa should be changed if there is any visible sign of wear, or after 4-5 sampling events. If there is no GC facility at the site where the experiment takes place, and you receive pre-evacuated vials and if you do not use the vials please return to the analysis laboratory.

3.8 Calculation of results

- A designated person identified in the study protocol should always calculate the results.
- The automated GC equipment will analyse the N_2O concentration of the experimental field and ambient samples and the data will be copied from the PC and stored in an appropriate directory on the local computer system. certified gas standards should be used and separate reference samples (AQCs) will be run with each 'batch'
- Calculation of the N_2O flux. A standard spreadsheet is available for calculation of the N_2O flux in g N_2O-N ha^{-1} d^{-1} (using the equations in Annex 4). Input data required are: the chamber enclosure start and end times; the chamber height; and the average air temperature over the sampling period.
- The fluxes from the 5 individual chambers on each plot should be averaged to produce a mean estimate of the plot N_2O flux rate.
- A treatment N_2O flux rate should be calculated by averaging the replicate plot mean values (usually 3 or 4) for each individual treatment including the untreated control.
- Cumulative N_2O fluxes should be calculated using the **trapezoidal rule** (area under the curve) to interpolate fluxes between sampling points. For each treatment, cumulative fluxes should be calculated using the plot mean values in order to calculate a mean cumulative emission value and associated standard error.
- The IPCC equivalent N_2O-N soil emission factor (% N applied) should be calculated using the following equation:

$$\frac{\text{Cumulative } N_2O \text{ flux from N applied (kg } N_2O\text{-N)} - \text{Cumulative } N_2O \text{ flux from control (kg } N_2O\text{-N)} * 100}{\text{N applied (kg N)}}$$

3.9 Quality control

An exchange of samples of chamber air and standard gas mixtures between laboratories (i.e. ADAS, AFBI, NW-Res) operating the GCs should be carried out, to avoid the possibility of any bias in the results towards high or low values. Each institute should follow local QA protocols, e.g. on use of reference samples in each GC run.

Annex 1.

Proforma for gas sampling for analysis by GC

Experiment Code:	
Experiment Title:	
Site Name:	
Field Name:	
Sampling Date:	
Number of Ambients and Time Taken:	
Number of stored samples and concentration (ppm): (To be entered by lab staff)	
Weather during sampling:	**Raining / Dry** **Hot / Warm / Cool / Cold** **Calm / Light breeze / Windy**
Soil conditions:	**Water on surface / Soil wet / Soil moist / Soil dry**
General Comments:	

Sampled by:…… Certified by:.....……..

Annex 2.

Sample Identifier	Plot Number	Chamber Number	Chamber and lid equipment numbers	Chamber Height (cm)	Time Chamber On	Time Gas Sampling	Notes

Sampled by:…… Certified by:.....……..

Annex 3.

Example data for N_2O gas sampling sheet

Sample Identifier	Plot Number	Chamber Number	Chamber and lid equipment numbers	Chamber Height (cm)	Time Chamber On	Time Gas Sampling	Notes
TT-NT2605-001	6	1	BOX/CH487 BOX/LD206	15.5, 19.5 15.2, 19.3	10:54	11:34	
TT-NT2605-002	6	2	BOX/CH488 BOX/LD207	20.3, 20.4 20.2, 19.9	10:54:30	11:34:30	
TT-NT2605-003	6	3	BOX/CH489 BOX/LD208	15.5, 19.5 15.2, 19.3	10:54	11:34	
TT-NT2605-004	6	4	BOX/CH490 BOX/LD209	20.3, 20.4 20.2, 19.9	10:55	11:35	
TT-NT2605-005	6	5	BOX/CH491 BOX/LD210	15.5, 19.5 15.2, 19.3	10:55:30	11:35:30	
TT-NT2605-006	9	2	BOX/CH492 BOX/LD211	20.3, 20.4 20.2, 19.9	10:55	11:35	
TT-NT2605-007	9	2	BOX/CH493 BOX/LD212	17.6, 22.5 17.6, 21.3	10:56	11:36	
etc.	etc.	etc.	Etc.	etc.	etc.	etc.	

Sampled by:…… Certified by:.....…….

Annex 4.

N_2O flux calculation from a static closed chamber:

$$N_2O \text{ flux (g } N_2O\text{-N ha}^{-1} \text{ d}^{-1}) = \frac{\text{increase in } N_2O \text{ concentration (ppm) x chamber height (cm)}}{\text{Chamber enclosure time (min)}} \text{ x Conversion factor}$$

Derivation of the factor with which to convert the increase in N_2O concentration (ppm) inside a chamber to a N_2O flux rate (g N_2O-N ha^{-1} d^{-1}).

Where increase in concentration = 1 ppm min^{-1}

1 ppm $\equiv$ 1 ml m^{-3} min^{-1}

$\equiv$ 1 x 44/23.63 (mg N_2O m^{-3} min^{-1}), assumes that at 1 atmosphere of pressure the molar volume of an ideal gas at 15°C occupies 23.63 litres of space

$\equiv$ product of the above x 28/44 x $1/10^3$ (g N_2O-N m^{-3} min^{-1})

$\equiv$ product of the above x $1/10^6$ (g N_2O-N cm^{-3} min^{-1})

Therefore flux in **g N_2O-N ha^{-1} d^{-1}** is as follows:

$\equiv$ 1 x 44/23.63 x 28/44 x $1/10^3$ x $1/10^6$ x 10^8 (cm^2 to ha) x 60 (min to hr) x 24 (hr to days)

Factor to convert the increase in N_2O concentration over time (in ppm min^{-1}) is:

$\equiv$ 170.6306

Note the volume that an ideal gas occupies will vary depending on temperature. The figure used can be altered using the values in the table below:

Air temperature (°C)	Molar volume (l/mol)	Conversion factor
-10	21.58	186.8397
-9	21.66	186.1324
-8	21.74	185.4305
-7	21.83	184.7338
-6	21.91	184.0424
-5	21.99	183.3561
-4	22.07	182.6749
-3	22.15	181.9987
-2	22.24	181.3276
-1	22.32	180.6613
0	22.40	180.0000
1	22.48	179.3435
2	22.56	178.6917
3	22.65	178.0447
4	22.73	177.4023
5	22.81	176.7646
6	22.89	176.1314
7	22.97	175.5027
8	23.06	174.8786
9	23.14	174.2588
10	23.22	173.6434
11	23.30	173.0324
12	23.38	172.4256
13	23.47	171.8231
14	23.55	171.2247
15	23.63	170.6306
16	23.71	170.0405
17	23.79	169.4545
18	23.88	168.8725
19	23.96	168.2945
20	24.04	167.7205
21	24.12	167.1503
22	24.20	166.5840
23	24.29	166.0216
24	24.37	165.4629
25	24.45	164.9080
26	24.53	164.3568
27	24.61	163.8092
28	24.70	163.2653
29	24.78	162.7250
30	24.86	162.1883
31	24.94	161.6550
32	25.02	161.1253
33	25.11	160.5991
34	25.19	160.0762
35	25.27	159.5568

APPENDIX IV. Measuring ammonia emissions using small scale wind tunnels.

1. Materials and equipment

1.1 Field equipment

A wind tunnel unit consisting of:
A transparent polycarbonate canopy (2.0m x 0.5m) and inlet air sampler
Galvanised sheet steel duct housing:
- Fan unit (small or large)
- Anemometer
- Outlet air sampler

A control box for each wind tunnel, housing:
- Diaphragm pump
- Platon flow meter
- Inlet and outlet 'bubbler' heads
- Two critical orifices (one for inlet and outlet air lines)
- Counter
- Timer
- Re-set button

Also required for each wind tunnel and control box system
- **Two air lines** for inlet and outlet bubblers
- **One anemometer cable**
- **One ratchet strap** to hold canopy onto tunnel
- If required, a control box stand
- 2 x 250 ml conical flask 'bubblers' per tunnel (inlet and outlet) containing 80 mls of 0.02 M orthophosphoric acid and a spare set of two bubblers for changeover.

Each set of flasks should be labelled with 'plot number' and 'inlet' or 'outlet'.
Flasks **must always** be used at the labelled position.
A Generator and transformer (or mains supply) to power all equipment.
Power cables to connect the tunnels:
- One 5 m cable per tunnel to connect each wind tunnel to its control box
- Sufficient 25 m extension cables to connect each control box to the generator (see 'Field equipment layout' of power cables between wind tunnels and generator').

Also required in the field
- Key to open control boxes
- De-ionised water used for rinsing bubbler heads in the field (if required)
- Field recording sheets (see Annex 1 & 2)

1.2 Laboratory materials

- De-ionised water for diluting bubbler samples.
- 100 ml volumetric flasks and small plastic funnels for preparing bubbler samples.
- Pre-labelled samples vials for storing 20 ml sub-sample (labelled with the study code, date, sampling period, plot number and inlet/outlet position).
- 0.02 M orthophosphoric acid for charging bubblers (80 mls per bubbler per sampling period)

To make 1 litre of 0.02M orthophosphoric acid:
Orthophosphoric acid (H_2PO_4) has a molecular weight of 98.00.
Concentrated H_3PO_4 is approximately 88% H_3PO_4 w/w.

Thus 1 litre of 0.02M H_3PO_4 requires 1.96g H_3PO_4 (i.e. 98x0.02).
Therefore the amount of concentrated H_3PO_4 required is (100/88) x1.96 = 2.227g
(made up to I litre with de-ionised water).

2. Time

Two people can set up a 15 wind tunnel system in one day. Treatment applications will take additional time.

One person will take approximately 3 hours to renew 30 bubbler flasks and prepare the 30 samples for analysis.

3. Health & Safety

- The Generator MUST be earthed prior to turning on (see 'Field equipment layout')
- Take care to avoid back injury when lifting and transporting the wind tunnels
- When moving/transporting the wind tunnels protect hands and take care to avoid cuts from the sharp edges of the metal skirting
- Be careful when using the 110 V power source in the field, particularly in wet conditions. If using mains power always connect the cable through a suitable circuit breaker.
- Ensure that power cables laid across the ground surface are not damaged by vehicles driving over them
- Fully unwind all power cables before use
- Always switch off fans before moving the tunnels
- Always wear protective gloves and safety glasses when measuring out concentrated H_3PO_4. Only pour out in a well-ventilated area or fume cabinet.

4. Procedure

Field equipment maintenance & calibration

4.1 General maintenance

General maintenance of the wind tunnels includes:
Fan calibration and flow setting
Anemometer calibration
Critical orifice calibration
Checking diaphragm in diaphragm pump and changing if necessary
Changing filter between bubblers and critical orifice.

4.1.1 Fan calibration and flow setting

The airflow through the tunnels is set at approximately 1 m/s. Generally there is no need for users to check this setting.
Changes to the airflow velocity and calibration must only be carried out by FIG.
Airflow velocity is changed by adjusting the bypass opening to the fan. The control ring can be slid along the duct to expose more or less of the bypass opening. When the required tunnel flow rate has been set the control ring is clamped and locked in place with a self-tapping screw.
Place the tunnel to be calibrated on the air flow test rig.
(The calibration rig comprises a standard inlet cone. When air flows into the duct there is a pressure reduction due to the acceleration of the flow. The pressure reduction is given by;
$\mathbf{Q = \alpha A \sqrt{(2\delta P \rho)}}$, where
Q = mass flow rate (kg/s)
A = area of duct (0.07068 m^2)
α = coefficient of discharge for the inlet (0.953)
δP = pressure reduction at D/4 down stream (Pa)
ρ = air density (kg/m^3)

The mass flow rate required to produce 1 m/s in the canopy cross section is 0.279 kg/s (at standard temperature and pressure this is equivalent to 0.228 m^3/s). This flow rate will generate a depression of 7.0 Pa. Allowing for some blockage due to crop a pressure of 5.8 Pa was used to set the flow rate. The anemometer frequency at this flow rate is c.108 hz.)
Connect the fan power supply and the anemometer to the control box.
Connect the 110 v ac power supply to the control box.
Connect a frequency meter to the anemometer calibration socket (pin1=ground; pin3=output).
Connect a reference manometer to the inlet cone on the test rig.
Run the tunnel fan and adjust the bypass ring to obtain the desired flow.

4.1.2 Anemometer calibration

Anemometers should be calibrated following installation and their calibration checked annually.
Equipment needed:

- Wind tunnel air flow test rig
- Wind tunnel
- Control box
- Manometer (the manometer should be calibrated annually and a record of the calibration kept with the wind tunnel calibration records)
- Air flow meter.

Calibration procedure:

- Put the wind tunnel onto the airflow test rig.
- Connect up the wind tunnel and control box as you would in the field, i.e. connect the anemometer and control box using the connecting cable and connect the power cable to the control box and wind tunnel.
- Zero the timer and counter in the control box.
- Turn on the power starting the fan.
- Run for approximately 200 seconds, reading the manometer 4 times during the 200 second period at c.25, 75, 125 and 175 seconds. Record the 4 manometer readings in mm of water gauge.
- After approximately 200 seconds turn the power off and record the number of counts and time (from control box).
- Use calibration spreadsheet (copy held at Boxworth) to collate recorded data (4 manometer readings, time period and number of counts) and calculate the grams of air per pulse. (Note: the anemometer measures approximately 108 hertz or pulses per second – check this is approximately what has been calculated).
- When re-calibrating an anemometer check agreement between the old and new calibrations. If the calibration has changed significantly re-do the calibration. If the 2 new calibrations vary remove the anemometer from the tunnel and send it back to the manufacturer to check.

Note: The airflow meter is a readout instrument that comes with the anemometers. It is useful for use in the field as a check if a problem is suspected. During anemometer calibration plug the airflow meter into the box (next to the tunnel plug in) and record the velocity. This reading can be used as a cross check in the field.

4.1.3 Critical orifice calibration

Critical orifices should be calibrated following installation and the calibration checked annually.
Equipment for calibration:

- Control box fitted with 2 critical orifices
- Accurate gas flow meter (e.g. Zeal wet-type gas flow meter, which should be calibrated annually and a record of the calibration kept with the wind tunnel calibration records)

Calibration procedure:

- Connect the control box to a power source
- To calibrate the critical orifice connected to the inlet air line, connect the gas flow meter to the exhaust of the inlet air line
- Turn the power on for a few second to fill line with air
- Turn the power off. Zero the timer and record the gas flow meter reading
- Turn power on and run for approximately 200 seconds
- Turn power off. Record the time (from timer) and the gas flow meter reading
- Calculate the volume of air pumped in litres per minute;
- [(Gas flow meter reading at end – gas flow meter reading at start)/ time] x 60
- To calibrate the critical orifice connected to the outlet air line, connect the gas flow meter to the exhaust of the outlet air line and repeat steps iii-viii.

4.2 Field equipment layout

Plot layout: Align plots, wherever possible, to the prevailing wind to avoid air being drawn into the tunnel canopy from adjacent plots (or other major sources of NH_3). Before treatment application place the wind tunnels/canopies next to their plots.

Generator must be approximately level, and sited centrally to the study area. **It MUST be earthed prior to turning on.** Securely attach the earthing wire to the generator and drive the earthing rod well into the ground. ADAS has two large generators and two transformers which have been modified to provide eight sockets, both have built-in circuit breakers.

An external circuit breaker will ALWAYS be required when using Mains electricity.

Cable layout: Plan BEFORE the start of the trial, ensuring:

Each socket on the transformer should NOT carry more than 16 amps The large fan units plus control box draw c.5 amps each and the small fan units plus control box draw c.2.6 amps each. It is recommended that a single 16 amp socket should not power more than 4 small fan units plus control boxes or 3 small and 1 large fan unit plus control boxes

To avoid power loss, use no more than 3 x 25 m extension cables between the control box and the generator (i.e. no more than 75 m of cable in total).

To ensure a consistent voltage drop always use the same length of cable between the generator and each tunnel.

Spread the power load evenly across the eight sockets:

a. i.e. for 30 tunnels: 6 sockets should power 4 wind tunnels, plus control boxes,
b. and 2 sockets should power 3 wind tunnels plus control boxes;
c. for 15 tunnels: 7 sockets should power 2 wind tunnels plus control boxes
d. and 1 socket should power 1 wind tunnel plus control box.

Cable requirements:

For a 30 wind tunnel system:
30 x 5 m cables (to connect each wind tunnel to its control box) PLUS 53 x 25 m extension cables are needed.
For a 15 wind tunnel system:
- Using a maximum of 50 m of cable (between control box and generator) 15 x 5 m cables (to connect each wind tunnel to its control box) and 23 x 25 m extension cables are need;

- Using a maximum of 75 m of cable (between control box and generator) 15 x 5 m cables and 31 x 25 m extension cables are needed (length of cable used in a 15 wind tunnel experiment would depend on distance between generator and plots).

4.3 Starting measurement

Check that power is switched OFF on all the control boxes. Turn the power on at the generator.

4.3.1 Apply treatments to plots.

When applying liquid treatments to plots:

- Avoid positioning the tunnel canopy over a surface depression or slope where liquid may flow in from outside the monitoring area, or out.
- Take care to avoid any large spillage of liquid treatment near the plots. If this occurs the affected area should be immediately covered with soil or sand in order to reduce excess ammonia volatilisation and possible contamination.

4.3.2 Initiating measurements

Immediately after applying the treatment move the canopy onto the treated area with its inlet at the front edge of the treated area. Insert the tunnel fan unit into the tunnel canopy and connect each wind tunnel unit up as follows;

- Connect each control box to the power source (generator or mains through 110 V transformer) using a **maximum of 3** x 25 m extension cables.
- Using the 5 m power lead connect the power source from the control box to the wind tunnel.
- Connect the anemometer cable from the wind tunnel to the control box.
- Connect the inlet and outlet air lines from the wind tunnel to the control box.
- It is recommended that the left bubbler is connected to the back port on the control box and connected to the outline air line and the right bubbler is connected to the front port and connected to the inlet air line.
- Position the inlet air sampler through the hole at the end of the canopy ensuring that the inlet air sampling holes are pointing away from the canopy.

Position bubblers at the inlet and outlet sampling positions and connect them to the corresponding wind tunnel inlet/outlet airlines.

Seal the canopy to the wind tunnel fan unit with the ratchet strap and fill in any spaces where the canopy is not flush with the soil surface with soil or sand.

Zero the counter and timer in the control box (hold down top reset button and press reset button on timer/counter).

Start air sampling by turning the control box on. Record the time air sampling started on the field recording sheet (Annex 1).

Also record on a separate field recording sheet for each plot the wind tunnel number, the control box number, and the inlet and outlet critical orifice numbers (Annex 2).

Check that air is flowing freely through the bubblers.

4.4 Changing bubblers

- Before changing bubblers check them visually. If any appear to be bubbling less than the others investigate further (as far as is possible) and record any problems identified on the field recording sheet (Annex 2).
- Stop air sampling by turning the control box OFF.
- For each bubbler: Remove the bubbler head and (if stated in study protocol) rinse the stem into the flask with de-ionised water. Insert a stopper into the flask. Ensure the flask is labelled with plot number and inlet/outlet position and sampling period.
- Insert rinsed bubbler head into a fresh labelled bubbler flask containing 0.02 M orthophosphoric acid.
- At each bubbler change, record the timer and counter readings in each control box on the recording sheet. Note: The counter will run for approximately 7-10 days before re-setting. At the start of the study decide whether the counter and timer will be (i) re-set at each bubbler change, or (ii) left to run for the whole study. Either option is acceptable.
- Re-start air sampling by turning the control box ON.
- Perform a second visual check on the bubblers to ensure they are bubbling correctly.

4.5 Blank measurement

The wind tunnel method calculates NH_3 loss by subtracting NH_3 trapped from the air of the inlet sampler from that trapped from air of the outlet sampler.

Additionally it is good practice to use blank samples to quantify any NH_3 contamination during all stages of sample handling. Three blank samples should be included during one of the sampling periods.

- For one measurement period prepare an additional 3 x 80 ml samples of orthophosphoric acid in conical flasks. Stopper the flasks and mark them 'blank'.
- Transport the blanks to the field site along with the other flasks. During the measurement period the blank flasks should be left stoppered in a safe place in the field. The blank samples should be prepared and analysed in the same way as all other samples.
- Incorporate the results of the blank determinations into the data analysis.

4.6 Bubbler sample preparation for analysis

- Discharge the exposed sample into 100 ml volumetric flask.
- Rinse the bubbler flask into the volumetric flask with de-ionised water, making up to 100 ml with de-ionised water.
- Shake the flask well and pour a 20 ml sub-sample into a labelled sample vial.

N**ote:** Where the same 100 ml volumetric flask is used for all samples, prepare all inlet samples from one run before the outlet samples. Between samples rinse the volumetric flask 3 times with 10-20 mls of deionised water.

- Store samples under refrigerated conditions (at <4°C) pending analysis. (Samples may be stored refrigerated for a maximum of 21 days before analysis.).
- Transport samples to the laboratory in a cool-box containing an ice-pack.
- Analyse samples for NH_3 by colourometric analysis using flow injection.

4.7 Ending the study

- Remove the bubblers for the final time
- Turn the power OFF at the generator
- Carefully coil all airlines, power cables and anemometer cables and store under cover

N.B. When coiling up the cables check for, and note, any damage caused by animals or other cause.

- Remove wind tunnels and canopies from plots and store undercover

4.8 General information relating to wind tunnel operation

- The airflow meter is a readout instrument that comes with the anemometers. It can be used as a check in the field if a problem is suspected with the anemometers. Plug in the airflow meter to the control box (next to the tunnel plug). Read the velocity. See if the velocity seems to be fluctuating a lot and compare velocity measurement to that made with the airflow meter when the anemometers were calibrated. Do not leave the air flow meter plugged into the control box for more than a few minutes while the study is in progress as it can cause the counter to stop.
- The Platon flow meters in the control boxes are not calibrated, but can be used as a visual indicator in the field of potential problems. The value given by the air flow meter should stay approximately constant over time (within approximately 1 l/min variation over the course of a study). Each orifice should pump approximately 3.5 to 4.0 l/min, therefore 2 should pump approximately 7 to 8 l/min and this should be the approximate value given by the Platon flow meter.
- The generator should be serviced before the start of each experiment. Check oil level before starting experiment and re-check oil level once a week while the experiment is running.

4.9 Data processing

NH_3 emissions from each plot for each period are calculated from:

- The total mass of air in period through tunnel
- The litres of air pumped through the bubblers during period
- NH_4-N analysis of the orthophosphoric acid.

The simplified calculation of NH_3 emission from each plot involves the following steps:

- For each sampling period the following data is needed
  A – mass of air sampled by each bubbler (Volume of air sampled calculated as time in minutes multiplied by litres per minute pumped through each critical orifice – from critical orifice calibration. Volume of air converted to mass of air).
  B – amount of NH_4-N trapped by each bubbler over the sampling period.
  C- mass of air drawn through each tunnel during the sampling period (total number of counts in period multiplied by the grams of air per pulse – from anemometer calibration).
- NH_4-N loss from the plot for the sampling period is then calculated as:
  - $((B/A)_{\text{outlet bubbler}} - (B/A)_{\text{inlet bubbler}}) \times C$
- This gives the NH_4-N loss for the sampling period from the 1 m^2 area under the wind tunnel canopy. This result should then be converted to kg/ha loss.
- Cumulative NH_3 loss can be calculated by summing NH_3 loss for all time period.
- Anemometer and critical orifice calibrations can be obtained from ADAS.
- An example Excel spreadsheet for calculating NH_3 emissions from wind tunnel data can be obtained from ADAS.

ANNEX 1.

WIND TUNNEL FIELD RECORDING SHEET – 1

PLOT NUMBER	TIME AIR SAMPLING STOPPED	TIME AIR SAMPLING STARTED	COUNTER READING	TIMER READING

Recorded by: **Date:**

Verified by:

Other comments:

ANNEX 2.

WIND TUNNEL FIELD RECORDING SHEET – 2

PLOT NUMBER	BLOCK NUMBER	TREATMENT NAME	WIND TUNNEL NUMBER	CONTOL BOX NUMBER	INLET ORIFICE NUMBER	OUTLET ORIFICE NUMBER

Recorded by: **Date:**

ACO116

Joint ADAS, AFBI, Rothamsted Research, SAC experimental protocol for experiments to quantify nitrous oxide emissions from grazing returns (dung and urine)

Edition:02 **Effective from: 16.03.12**

Introduction

This protocol has been prepared on behalf of the ACO116 consortium in order to harmonise the experimental approach used by research partners in order to achieve the highest possible quality of data from the research being undertaken. Wherever possible a standard approach has been adopted, however it is also necessary to accommodate minor differences in local practice providing that this does not compromise the quality of data being produced. This document aims to be consistent where possible with protocols developed in earlier projects, but it is also recognised that methodologies will be continuously reviewed, and where necessary updated (in consultation with consortium members) to ensure best practice and allow for the incorporation of new approaches and techniques.

3. EXPERIMENTAL PLATFORMS AND TREATMENTS

1.1. Experimental Sites

Experiments will take place at or near the sites listed in the table below.

Zone Name	Soil Texture Group	Annual Rainfall (mm)	Experimental platform (planned)	Experimental land use and organisation responsible for the platform
Dry	Heavy	0-750	ADAS Drayton, West Midlands	Grass (ADAS)
Wet	Sandy/light	951+	Crichton, SWScotland	Grass (SAC)
Wet	Medium /heavy	951+	North Wyke, Devon	Grass (RRes - NW)
Wet	Medium	951+	ADAS Pwllpeiran, Wales	Grass (ADAS)
Wet	Medium	951+	Hillsborough, Northern Ireland	Grass (AFBI)

There should be no history of long term organic manure applications and no manure applications or grazing 6 months prior to establishment of the experiment. Previous site history (over at least 12 months) should be recorded.

2. Treatments

1. Untreated control
2. Urine – spring (March-May)
3. Urine – summer (June-August)
4. Urine – autumn (September-October)
5. Urine – spring +DCD*
6. Urine – summer + DCD*
7. Urine – autumn +DCD*
8. Synthetic urine – spring (March-May)
9. Synthetic urine – summer (June-August)
10. Synthetic urine – autumn (September-October)
11. Dung - spring
12. Dung – summer
13. Dung - autumn

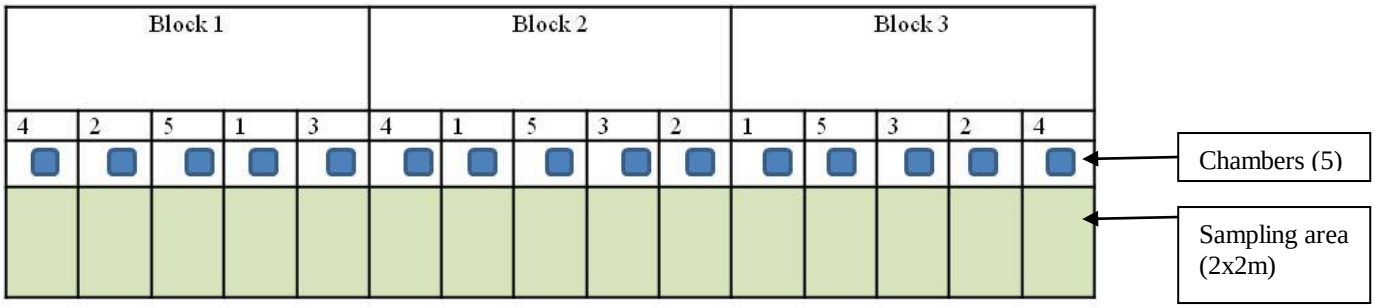
3. EXPERIMENTAL DESIGN AND ANALYSIS

3.1 Experimental design

There will be three replicates of each treatment. Plot will be 3m x 6m, with separate areas set aside for nitrous oxide emission measurements, soil mineral nitrogen sampling and crop N uptake measurements.

The spring summer and autumn experiments will be treated as separate experiments, so each should be blocked and randomised and include a zero N control.

Example of an experimental design for one of the 3 experiments (spring summer or autumn)



Treatments

1 zero N control
2 Urine
3 Urine plus DCD
4 Synthetic urine
5 Dung

3.2. Statistical analysis

All nitrous oxide data will be statistically analysed by techniques appropriate to the experiments described. Cumulative emissions/losses, emission factors and associated standard errors will be generated.

Advice from a trained statistician will be sought in the design and analysis of the experiments.

4. SITE MANAGEMENT

4.1 Site History

The experiment should be set up on grassland where there has been no recent history of animal grazing or manure applications in order to minimise spatial heterogeneity.

4.2. Crop management

To prevent any nutrient deficiencies, overall basal P, K and Mg fertilisers and S should be applied according to site requirements. Applications should follow recommendations in 'The Fertiliser Manual (RB209) published 2010' for sites in England. Such recommendations should be checked by a FACTS qualified advisor.

Apply other agro-chemicals as needed and according to good agricultural practice to control weeds, pests and diseases. Agro-chemicals should be applied by the host farmer.

No additional nitrogen should be applied to the plots before the 12 month N_2O measurement periods have finished. and 'NO NITROGEN SIGNS' must be put up around the perimeter of the experiment.

5. SITE CHARACTERISATION

5.1 Soil Sample collection

In order to characterise the experimental site, baseline topsoil samples (0-7.5cm) should be taken from each block prior to any organic material applications using a 'cheese' corer, pot corer, tubular or screw auger as appropriate.

Each sample should consist of not less than 25 cores of soil taken from separate points, evenly distributed across the block using a "W"-shaped or regular grid pattern traverse. The soil collected should be sub-sampled after thorough mixing to provide 2 subsamples for laboratory analysis as described below.

5.2 Chemical analysis

Approximately 500 g of soil should be dispatched to the laboratory for the following analyses:

- pH (in water)
- extractable P, K, S, Mg
- total N
- total organic carbon - modified Walkley-Black, or by an elemental carbon analyser
- Particle size distribution (PSD) – sand, silt, clay

5.3 Bulk density

Soil bulk density should be measured using the core cutter method in which small cylinders (min 10 cm diameter) are hammered into the soil. Ten cores (0-7.5cm) should be taken per block and the weight of soil within the cores determined after careful trimming.

Samples should be taken when soils are at or close to field capacity.

5.4. Available water capacity

Ten undisturbed soil cores per block (30 cores in total) should be taken from the topsoil using the small cylinders used for bulk density measurements. These samples will be sent to ADAS Gleadthorpe for field capacity (0.05bar) moisture content determination. An additional bulked sample of disturbed topsoil (0-10 cm) will be taken from each block (10 cores per sample) and sent to ADAS Gleadthorpe for the determination of permanent wilting point (15 bar).

5.5 Soil mineral N

At the start of the study, a soil mineral N sample (0-90cm) should also be taken from each block, with 10 cores per block and the samples split 0-30 cm, 30-60 and 60-90 cm (or to soil depth, in 30 cm increments). Samples should be taken using either Eijkelkamp "Stepwise" Gouge Augers of 40, 30, and 20 mm diameter; EJH Danish augers of 19 and 22 mm diameter or JMC "Backsaver" equipment. Samples should be kept cool in a coolbox containing icepacks and dispatched the **same day** to the laboratories for analysis (samples should not be taken on a Friday to ensure they reach the laboratory the next day). Soil mineral N analysis is described in Appendix II.

6. Applications

6.1. Urine and dung

Fresh dung and urine from dairy or beef cattle (ideally collected within 7 days of the start of the experiment) will be used. The urine should be stored in a refrigerator at < 4°C and NOT FROZEN before application. In total *c.*200 litres of fresh urine and 300 kg of dung will be required for each application timing.

Synthetic urine will be prepared using the formulation described for "R2" in the paper by Kool et al 2006 (*Soil Biol Biochem 38, 1757-1763*).

Real 2, R2	g N /L	formula	m.w.	no. N's	% N	g compound/ L	g in 90L	g for 3 applications
Urea	7.92	CH_4N_2O	60.06	2	46.6	17.0	1,529	**4,587**
Hippuric Acid	0.53	$C_9H_9NO_3$	179.17	1	7.8	6.8	610	**1,831**
Allantoin	1.46	$C_4H_6N_4O_3$	158.12	4	35.4	4.1	371	**1,113**
Uric Acid	0.08	$C_5H_4N_4O_3$	168.11	4	33.3	0.2	22	**65**
Creatinine	0.33	$C_4H_7N_3O$	113.12	3	37.1	0.9	80	**240**
$KHCO_3$						14	1,260	**3,780**
KCl						10.5	945	**2,835**
$CaCl_2.2H_2O$						0.4	36	**108**
$MgCl.5H_2O$						1.2	108	**324**
Na_2SO_4						3.7	333	**999**

Urine treatments
On each plot, there will be five urine patches each measuring 60 cm x 60 cm located randomly.

Each urine patch will receive 1.8 litres of urine (equivalent to 5litres/m^2) which will be applied using a watering can fitted with a rose. The application area will be bordered by a frame to prevent the urine running off the patch during application. The frame will be removed once the urine has soaked into the soil.

On each plot an additional area, 2m x 2m, will be treated with 20 litres of urine and used for soil mineral N and grass N uptake measurements.

The volumes of urine required for each treatment and application timing are:

	No Plots	No Patches	Patch Area, m2	Total Area	
Gas Areas per Plot (0.6mx0.6m)	3	5	0.36	5.4	
Soil Areas per Plot (2mx2m)	3	1	4	12	
Total Area				17.4	m2
Rate				5	L/m2
Volume Required per Application				**87**	**L**

Therefore collect *c.*200 litres of fresh urine and prepare *c.*100 litres of synthetic urine for each application timing.

Dung treatments
On each plot there will be 5 dung patches each measuring 40cm x 40cm. Each dung patch will receive 3.2kg of fresh dung (equivalent to 20kg/m^2), spread to an even thickness across the 40cm x 40cm area so that the patch is completely enclosed by a nitrous oxide chamber. Each plot will require 16 kg of dung for the nitrous oxide measurements.

On each plot, an additional area 2m x 2m will be treated with 80 kg of dung. This area will be used for soil mineral N and grass N uptake measurements.

Therefore collect 300 kg of dung per application timing.

6.2 DCD

For treatments 5,6 and 7 DCD will be mixed with the urine in the watering cans before application at a rate equivalent to10 kg/ha equivalent to 36ml of 1% DCD solution for each nitrous oxide 'patch' and 400ml of 1% DCD solution for each soil sampling and grass harvesting area. (DCD is being mixed with the urine to enable uniform distribution of the very small amount of product over the treatment area).

6.3 Urine and dung analysis

Take representative samples of dung, fresh and synthetic urine from the bulked dung, urine real and urine synthetic at spreading. (c.1 litre of real urine,1 litre of synthetic urine and 1 kg of dung). Therefore for each site there will be 2 samples of urine and 1 of dung at spring, summer and autumn (6 urine and 3 dung). The urine and dung samples should be analysed 'fresh' where possible for:

- dry matter
- pH
- total N
- ammonium N (NH_4-N) and nitrate N (NO_3-N) *i.e.* readily available N
- total organic carbon (modified Walkley-Black, or analysis by a TOC analyser (uv persulphate oxidation)

It will be necessary to calculate the total N and available N applied by the urine and dung applications.

Additional urine analysis

From the bulked synthetic and real urine at each site in spring, summer, and autumn take two 30 ml sub-samples. The subsamples should be diluted 1:3 with HPLC grade deionised water and one subsample should be preserved by adding 1M H2SO4 or 12 M HCl to reduce the pH to 3 (using a pH meter) and the other subsample preserved by adding chloroform.(100 ul per litre). Label and store both sub-samples in a freezer at (minus) -20°C. The frozen samples should be packed in ice and when required sent by express delivery to:

Dr. Ronnie Laughlin, AFBI,
18a Newforge Lane
Belfast, Northern Ireland
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The samples will be analysed for:

- Urea
- Hippuric Acid
- Allantoin
- Uric acid
- Creatinine

7. N_2O MEASUREMENTS

The closed, static chamber method is used to measure the emission of N_2O from soil. A chamber box is inserted to a depth of approximately 5 cm or greater (i.e. sufficient to produce an adequate seal). Background measurements of the gases of interest are taken by manually sampling the atmosphere around the experimental plots. To begin flux measurements, a lid is placed on top of the chamber,

enclosing the atmosphere above the soil and within the chamber. The time at which this is done is noted. The chamber lid is left on for a predetermined amount of time, usually 40 minutes. The accumulation of N_2O within the chamber is measured by manually taking a gas sample from the chamber, storing this in an evacuated glass vial and sending the sample for analysis by gas chromatography. The N_2O increase (ppmv) over the incubation period (minutes) is used to calculate the N_2O emission rate. Nitrous oxide measurements should be made according to the sampling strategy outlined below:

Weeks before/after N input (e.g. manure/fertiliser application etc.)	Number of measurements
-1	1
0	5
1	5
2	2 (evenly spaced through the week)
3	2 (evenly spaced through the week)
4-7	2 ie one sampling every other week until week 25 (evenly spaced)
8-12	2 (evenly spaced)
13-16	2 (evenly spaced)
17-20	2 (evenly spaced)
21-24	2 (evenly spaced)
25-28	1 ie one sampling monthly from here
29-32	1
33-36	1
37-40	1
41-44	1
45-48	1
49-52	1
	Total = 32 samplings per year

Measurements will continue for **12 months** following each **application**.

Details of the method to be used are given in Appendix II.

8 ONGOING SOIL MEASUREMENTS

Ancillary soil measurements should be made to enable comparisons between sites, to assist our understanding of the responses of different N forms/rates etc., provide essential data to the modellers and to be able to relate the work to previous studies. These measurements should be:

(a) gravimetric soil moisture content (in order to derive % water-filled pore space, WFPS)
(b) soil temperature

8.1 Soil moisture and bulk density

At every N_2O measurement occasion, soil samples (0-10 cm) will be taken from each **block** for the determination of gravimetric soil moisture. Samples should be labelled with a unique identifier and should be stored in a cold room prior to analysis, within 28 days of sampling.

It is necessary to measure the soil dry bulk density (mass per unit volume of dry soil in its undisturbed state) of the topsoil (0.-10 cm) in order to convert the gravimetric moisture content to water filled pore space (WFPS). This is an extremely useful measure since it provides an indication of potential gas

movement and aerobicity of the soil, and therefore may be useful in explaining the N_2O emission pattern. Dry bulk density should be measured as described in Section 5.3 above. Bulk density should be measured at the start of the experiment using one core per plot, on the untreated 'control' (i.e. treatment 1) and on the urine treatment plots giving 6 samples in total.

8.2 Soil Mineral N

Soil samples (0-10 cm) will be taken at regular intervals (see Table below) from all treatments for the determination of topsoil SMN and soil moisture. The sample should be made up from 5 randomly selected subsamples form the 2m x2m urine or dung treated area set aside for soil sampling. The soil samples should be labelled with a unique identifier. The soil samples should be analysed fresh within 5 days so must be kept cool (and not frozen) i.e. in a cold store or fridge. See Appendix 1 for details of the analytical technique to be used.

Mineral N sampling schedule:

Weeks before/after N input (e.g. manure/fertiliser application etc.)	Number of measurements
-1	1
0	1
1	1
2	1
3	1
4-7	1
8-12	1
13-16	1
17-20	1
21-24	1
25-30	1
30-37	1
37-46	1
46-52+	1
	Total c 14 samplings/year

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8.3 Soil temperature

Soil temperature should be measured continuously using 3 automatic temperature loggers (e.g. TinyTalk logger, USB interface temperature data logger etc.) set to log at hourly intervals for the length of the experiment i.e. one per block. Each logger should be sealed in a polythene bag and secured with tape to prevent damage by moisture. A piece of string should be strongly attached at one end to the bag and the other to a peg on the soil surface marking the location of the logger burial. Loggers should be buried at a depth of 5 cm on the same selected treatment. Following the last N_2O sampling on each experiment the loggers should be dug up and promptly downloaded. For security, down loaded data should be immediately copied to the local computer system at each site.

9. Harvest measurements

Grass yield should be determined each time the grass reaches the top of the static chambers used for measuring nitrous oxide emissions. A 1 m x 1 m quadrat should be harvested within the 2 m x 2 m treated patch and from the untreated control plots. The remaining area should also be cut and the grass cuttings removed. Representative samples of grass will be analysed for dry matter and total nitrogen content so that N uptake can be determined.

10 Meteorological measurements

At each experimental site, daily rainfall and air and soil (5cm depth) temperature (min and max) should be measured.

APPENDIX I: Analysis of soil samples for soil mineral N.

1. Analysis

Soil samples are passed through a 4mm sieve. The stone content of the site should be determined separately as a one off measurement in order to allow the concentration of N to be calculated on a site basis.

A fresh sample of the sieved soil is accurately weighed (to 2 dps) into a plastic pot and 2M KCl is added to give a soil to extractant ratio of 1:2 (eg 100 g soil added to 200 ml KCl), this mixture is shaken on an orbital shaker for 1 hour.

After shaking allow the mixture to settle for 2-3 minutes before transferring approx. 15ml into a 16ml disposable centrifuge tube; centrifuge at 400rpm for 10 min. Transfer centrifuged supernatant into a 10ml polystyrene test tube, cap then store the extracts in a fridge until analysis. An alternative approach is to filter samples through a Whatman No 40 filter paper. A 15ml sample of the 2M KCI is also centrifuged or filtered providing a blank for analysis.

Determination of NH_4-N and NO_3-N carried out using an autoanalyser. The top standard for NH_4-N and NO_3-N is 2 ppm. Some samples may need to be diluted - use 2M KCI solution.

2. Solutions

2M KCl – Dissolve 149.1 KCl (KCl, mol. Wt. 74.55) in de-ionised water in a volumetric flask and make up to one litre with de-ionised water. Use high purity KCl.

Results are expressed as mg/kg soil (OD) i.e. ppm (OD).
Following the receipt of soil analysis results, the data should be checked, copied into the working file at each site, entered into an excel spreadsheet

APPENDIX II: Nitrous oxide emission measurements

1 Time

Two members of staff can sample from 150 chambers (5 chambers x 6 plots x 3 blocks) in approximately 3hrs 15mins. This includes taking field and ambient samples. Extra time is required for vial labelling, packing and despatch, chamber maintenance.

2 Materials and Equipment

2.1.Chambers

These should be square/rectangular covering a surface area of *c*.0.16 m^2 (or circular and have a diameter in the range 25-50 cm to give a similar surface area). The minimum height above ground should be 15-20 cm over grass swards/ young arable crops. As the latter crops develop, the chambers may need to be replaced by taller ones, or stackable types (e.g. chambers with a water filled channel) where an "extension layer" can be added when necessary, to keep the chamber top above the height of the crop.

Two main methods of chamber installation are commonly used.

(a) Chambers that are essentially square or circular "sleeves": insert the bottom of the chamber wall into a 5 cm deep slot cut in the soil, and tamp the soil on the outside to make a good seal. Close the top with a gas-tight lid (using a rubber or water seal), leaving the lid in place only for the duration of the measurement. Leave the chamber body in situ between measurements (only remove if/when agronomic operations make it necessary).

(b) Chambers with soil "collars". In this type, a short sleeve or "collar" is sealed into the soil in a similar way as in (a), but the above-ground extension is only of the order of 4-5 cm. A closed chamber with 15-20 cm high walls and an integral top (i.e. in the form of an inverted bucket or box) is attached for the measurement period.

During the latter stages in the growth of cereal crops, chamber extensions will be required to accommodate the growing plants (normally in late May or June). These should be stacked above the existing chamber, providing a gas tight seal, and allowing gas flux measurements to be made throughout the growing season. Once in place, chamber extensions would remain until the crop is harvested.

2.2. Other equipment

- Numbered pegs to identify the chambers.
- Cutting tool/former.
- Timer
- Needles and 50-60 ml glass syringe.
- 20-22 ml evacuated gas collection vials. These must be standard vials for gas analysis using an automated headspace sampler.
- Crimp top glass vials
- Crimp caps
- Butyl rubber seals

3 Procedures

3.1 Safety considerations

The method involves bending to sample chambers at ground level, take care to avoid back strain/injury.

As hypodermic needles are used to sample ambient and chamber headspace gases, operators should take care not to "prick" themselves. Should blood be drawn following an accidental "pricking", the

needle must be disposed of in a sharps bin to comply with health and safety regulations. Staff involved in these measurements should ensure that they have up to date tetanus immunisation and are aware of local Risk Assessments for using needles. New needles should be used for each set of N_2O samples taken and old needles always disposed of in a sharps bin.

3.2 Measurements: planning

The flux of N_2O is determined by measuring the increase in N_2O concentration (above the concentration of the ambient air) in an enclosed chamber over a defined period of time. In most situations, the increase in N_2O concentration is linear over a 40-50 minute period, although may be in the range of 30-60 min depending on site conditions. Beyond this however, and depending on a variety of factors, the rate of increase tends to decline. As only one field measurement is made of the N_2O concentration, it is crucial to plan the timing of operations to allow for placing chambers and withdrawing the samples. Exceeding the suggested enclosure period greatly increases the risk of non-linearity and hence underestimating the flux.

Work out the timing of operations based on the experimental design outlined in the Study Protocol. As gas sampling takes the longest time, plan by working back from an enclosure period of 40-60 minutes per chamber. Allow 1 min between enclosing each chamber to ensure enough time for sampling.

Carry out operations by block, but randomise the order in which blocks are measured on different days, as N_2O fluxes do show some diurnal variation. If the experimental layout allows, on each measurement occasion the order of the plots and the order the chambers within each plot sampled should be **randomised daily** i.e. start at one end of the plot and work through the chambers in order or start at the other end of the plot and work through the chambers in that order.

Gas sampling should be carried out between 10:00 and 14:00 hours due to diurnal variation in N_2O emissions and if possible **between 10:00 and 12:00 hours** as indicated by IPCC good practice. For large experiments this might not be feasible. In all cases note the time of sampling on the proforma. The site details proforma (Annex 1) and gas sampling proforma (Annex 2) should be completed for each sample day.

Individual sample vials should be labelled with a unique sample identifier, which corresponds to that on the gas sampling proforma. The code should include a reference to the name of the site, experiment, plot, chamber and the project code. Both before and after collection it is good practice to place the gas vials in numerical order in a suitable container e.g. a labelled polystyrene tray.

3.3 Measurements from field plots

Complete a Site Details proforma (e.g. Annex 1), describing the site, weather conditions, and any special features that may be of use when it comes to analysis of the results, e.g. rabbit droppings inside chamber, description of the soil conditions etc. Complete a Gas Sampling proforma (e.g. Annex 2) during sampling. An example of a partially completed gas sampling proforma is shown in Annex 3.

Measurements of N_2O should be made from randomly determined positions (avoiding features such as tramlines) in the appropriate field location.

Five chambers should be placed in each of the plots before collecting gas samples. Press the chambers into slots (previously made by a spade or by hammering in and removing a square steel frame) in the soil to ideally a depth of > 5 cm, but not less than 3 cm. Once the chamber is installed, the outside edge needs to be tamped down to ensure a good seal.

Ensure that the chambers are inserted into the ground to give a level top edge. This is important if the type of chamber used has a water filled channel, as the water in the channel running around the chamber top needs to make a gas-tight seal when the chamber lid is fitted.

The height of the chamber should be measured from the soil surface to the highest part of the chamber at all 4 corners and written on the proforma**.** Additionally, a stick/ruler should be positioned so that it rests on top of the chamber to enable a height measurement to be made from the centre of the chamber.

Chamber insertion should be done not less than 24 hours before a fertiliser/manure application.

Once the chamber is in position, ensure that the chamber seal (water filled groove, rubber seal etc.) is undamaged and fully functioning so that a gas-tight seal will be formed. Place the lid on the chamber noting the exact time of enclosure in the proforma.

The chambers should stay in place throughout the experiment (but the lids removed after each measurement), although they will need to be removed prior to farm operations e.g. cultivations, fertiliser/manure application etc. and replaced as soon as possible after. Number the chambers with a unique identifier e.g. plot number and chamber number (1 to 5). The location of each chamber needs to be marked e.g. with a wooden peg or magnetic marker so that the chamber is always inserted in the same place if the chamber is moved. **The long-term position of the chambers is critical and if removed need to be returned to exactly the same position**.

Each chamber (unstacked) should normally be enclosed using a lid for a **40 minute period**. Note any deviations on the proforma. The time of closing each chamber and the time of taking the gas sample should be recorded on a data sheet.

Pre-evacuated 20-22 ml glass vials should be filled with gas taken from each individual chamber by syringe as detailed below:

- Take a 50 or 60 ml glass or plastic syringe fitted with a needle and either pierce the septum in the lid or connect to the chamber valve or connect to a 3-way tap inserted into the chamber (depending on chamber type) and slowly remove 50 or 60 ml of the headspace gas without withdrawing the needle or disconnecting the syringe.
- Depress the plunger to force this sample back into the chamber and to ensure that a representative gas sample is taken.
- SLOWLY withdraw another 50 ml sample, hold at 50 ml until plunger stays fast then remove the syringe from the chamber. Watch that the plunger does not retract into the syringe body, meaning that the gas was sampled too quickly i.e. the gas is not at atmospheric pressure, and consequently, that the sample volume collected is less than 50 or 60 ml.
- Pierce the septum of the appropriate labelled gas vial, as the vial is pierced, the gas will automatically be withdrawn from the syringe to equalise the pressures in the vial and syringe.
- Push the plunger in as far as it will go and hold the syringe in position.
- At some point prior to analysis (either in the field or lab) pierce the septum with another single needle (narrow bore) to allow excess pressure to be released
- Note down the gas vial identifier, such that the vial and N_2O concentration in the particular chamber headspace can be matched following subsequent analysis by GC.
- Record the time that gas samples are taken on the proforma.

3.4 Linearity check

To check on the linearity of gas accumulation within a chamber's headspace (an underlying principle of the methodology), on every N_2O sampling occasion select 3 chambers at random from the urine treatment. From each of these 3 chambers take a time series of samples following closure; 6 samples at evenly spaced intervals (eg 0, 10, 20, 30, 50, 60 for the 40 minute closed chambers and 0, 15, 30, 45, 60, 75 and 90 for the 60 minute closures) and submit for analysis. The remaining chambers are

measured using a terminal sampling only as above. If non-linear responses are obtained (e.g. when soil is particularly dry), a correction algorithm will need to be applied to adjust the flux values.

If the chambers are to be stacked in order to permit N_2O sampling from a growing crop, linearity checks should also be carried out prior to chamber stacking and N_2O measurement. The enclosure time may need to be increased as a result of the increase in chamber volume, but not too much so that N_2O accumulation is non-linear. At least 1 week before the addition of a stacked chamber is required, select two chambers at random from the treatment with the highest N input. From each chamber take 6 or 7 samples at evenly spaced intervals (0 min, 15, 30, 45, 60, 75 and 90 min) after closure. The determination of the enclosure time will be based on the resultant linearity graphs.

3.5 Ambient N_2O concentration at the site

Make an assessment of the ambient N_2O concentration of the experimental area, as follows:

- Collect (in pre-evacuated gas vials) ten 20 to 22-ml ambient gas samples from the experimental plot area, 5 samples at the start of chamber sampling and 5 samples at the end of chamber sampling. These samples should be collected as in Section 3.8, away from any roads and the soil surface to avoid contamination from car exhausts or soil efflux respectively. Take the ambient samples from about one metre above the ground, i.e. approximately around waist height.

- Vials should be labelled with the name of the field site and the project code and a unique sample identifier, such that these samples can be identified as ambient.

3.6. Completion and sample submission

- Once all samples have been taken, remove the lids from the chambers and place each lid away from the chamber (approximately 1-2 m), so as to avoid a preferential rain shadow around the chamber.
- The glass gas vials can be easily broken, so ensure that they are well packaged to avoid damage in transit. A photocopy of the proforma sheets should be kept in the working file used by the gas sampler for ready reference.
- If there is no GC facility at the site where the experiment takes place, and samples are to be transported, the signed proforma sheets should be sent with the corresponding sample vials as **soon as possible** after collection to the laboratory for analysis. Prompt return of the gas vials is important since standards are added to each batch of vials at the analysis laboratory. The standards are essential to correct for gas loss if there is a delay in analysis by GC.
- The gas samples will be analysed by GC.
- Sample vials should be evacuated as close to N_2O sampling as possible. Do not use vials which have been evacuated for more than one week. Vials can be evacuated either using a manual or mechanical suction pump. Septa should be changed if there is any visible sign of wear, or after 4-5 sampling events. If there is no GC facility at the site where the experiment takes place, and you receive pre-evacuated vials and if you do not use the vials please return to the analysis laboratory.

3.7 Calculation of results

- A designated person identified in the study protocol should always calculate the results.
- The automated GC equipment will analyse the N_2O concentration of the experimental field and ambient samples and the data will be copied from the PC and stored in an appropriate directory on the local computer system. certified gas standards should be used and separate reference samples (AQCs) will be run with each 'batch'
- Calculation of the N_2O flux. A standard spreadsheet is available for calculation of the N_2O flux in g N_2O-N ha^{-1} d^{-1} (using the equations in Annex 4). Input data required are: the chamber

enclosure start and end times; the chamber height; and the average air temperature over the sampling period.

- The fluxes from the 5 individual chambers on each plot should be averaged to produce a mean estimate of the plot N_2O flux rate.
- A treatment N_2O flux rate should be calculated by averaging the replicate plot mean values (usually 3 or 4) for each individual treatment including the untreated control.
- Cumulative N_2O fluxes should be calculated using the **trapezoidal rule** (area under the curve) to interpolate fluxes between sampling points. For each treatment, cumulative fluxes should be calculated using the plot mean values in order to calculate a mean cumulative emission value and associated standard error.
- The IPCC equivalent N_2O-N soil emission factor (% N applied) should be calculated using the following equation:

$$\frac{\text{Cumulative } N_2O \text{ flux from N applied (kg } N_2O\text{-N)} - \text{Cumulative } N_2O \text{ flux from control (kg } N_2O\text{-N)} * 100}{\text{N applied (kg N)}}$$

3.8 Quality control

An exchange of samples of chamber air and standard gas mixtures between laboratories (i.e. ADAS, SAC, AFBI, NW-Res) operating the GCs should be carried out, to avoid the possibility of any bias in the results towards high or low values. Each institute should follow local QA protocols, e.g. on use of reference samples in each GC run.

Annex 1.

Proforma for gas sampling for analysis by GC

Experiment Code:	
Experiment Title:	
Site Name:	
Field Name:	
Sampling Date:	
Number of Ambients and Time Taken:	
Number of stored samples and concentration (ppm): (To be entered by lab staff)	
Weather during sampling:	**Raining / Dry** **Hot / Warm / Cool / Cold** **Calm / Light breeze / Windy**
Soil conditions:	**Water on surface / Soil wet / Soil moist / Soil dry**
General Comments:	

Sampled by:…… Certified by:.....……..

Annex 2.

Sample Identifier	Plot Number	Chamber Number	Chamber and lid equipment numbers	Chamber Height (cm)	Time Chamber On	Time Gas Sampling	Notes

Sampled by:…… Certified by:.....……..

Annex 3.

Example data for N_2O gas sampling sheet

Sample Identifier	Plot Number	Chamber Number	Chamber and lid equipment numbers	Chamber Height (cm)	Time Chamber On	Time Gas Sampling	Notes
TT-NT2605-001	6	1	BOX/CH487 BOX/LD206	15.5, 19.5 15.2, 19.3	10:54	11:34	
TT-NT2605-002	6	2	BOX/CH488 BOX/LD207	20.3, 20.4 20.2, 19.9	10:54:30	11:34:30	
TT-NT2605-003	6	3	BOX/CH489 BOX/LD208	15.5, 19.5 15.2, 19.3	10:54	11:34	
TT-NT2605-004	6	4	BOX/CH490 BOX/LD209	20.3, 20.4 20.2, 19.9	10:55	11:35	
TT-NT2605-005	6	5	BOX/CH491 BOX/LD210	15.5, 19.5 15.2, 19.3	10:55:30	11:35:30	
TT-NT2605-006	9	2	BOX/CH492 BOX/LD211	20.3, 20.4 20.2, 19.9	10:55	11:35	
TT-NT2605-007	9	2	BOX/CH493 BOX/LD212	17.6, 22.5 17.6, 21.3	10:56	11:36	
etc.	etc.	etc.	Etc.	etc.	etc.	etc.	

Sampled by:…… Certified by:.....…….

Annex 4.

N_2O flux calculation from a static closed chamber:

$$N_2O \text{ flux (g } N_2O\text{-N ha}^{-1} \text{ d}^{-1}) = \frac{\text{increase in } N_2O \text{ concentration (ppm) x chamber height (cm)}}{\text{Chamber enclosure time (min)}} \text{ x Conversion factor}$$

Derivation of the factor with which to convert the increase in N_2O concentration (ppm) inside a chamber to a N_2O flux rate (g N_2O-N ha^{-1} d^{-1}).

Where increase in concentration = 1 ppm min^{-1}

1 ppm $\equiv$ 1 ml m^{-3} min^{-1}

$\equiv$ 1 x 44/23.63 (mg N_2O m^{-3} min^{-1}), assumes that at 1 atmosphere of pressure the molar volume of an ideal gas at 15°C occupies 23.63 litres of space

$\equiv$ product of the above x 28/44 x $1/10^3$ (g N_2O-N m^{-3} min^{-1})

$\equiv$ product of the above x $1/10^6$ (g N_2O-N cm^{-3} min^{-1})

Therefore flux in **g N_2O-N ha^{-1} d^{-1}** is as follows:

$\equiv$ 1 x 44/23.63 x 28/44 x $1/10^3$ x $1/10^6$ x 10^8 (cm^2 to ha) x 60 (min to hr) x 24 (hr to days)

Factor to convert the increase in N_2O concentration over time (in ppm min^{-1}) is:

$\equiv$ 170.6306

Note the volume that an ideal gas occupies will vary depending on temperature. The figure used can be altered using the values in the table below:

Air temperature (°C)	Molar volume (l/mol)	Conversion factor
-10	21.58	186.8397
-9	21.66	186.1324
-8	21.74	185.4305
-7	21.83	184.7338
-6	21.91	184.0424
-5	21.99	183.3561
-4	22.07	182.6749
-3	22.15	181.9987
-2	22.24	181.3276
-1	22.32	180.6613
0	22.40	180.0000
1	22.48	179.3435
2	22.56	178.6917
3	22.65	178.0447
4	22.73	177.4023
5	22.81	176.7646
6	22.89	176.1314
7	22.97	175.5027
8	23.06	174.8786
9	23.14	174.2588
10	23.22	173.6434
11	23.30	173.0324
12	23.38	172.4256
13	23.47	171.8231
14	23.55	171.2247
15	23.63	170.6306
16	23.71	170.0405
17	23.79	169.4545
18	23.88	168.8725
19	23.96	168.2945
20	24.04	167.7205
21	24.12	167.1503
22	24.20	166.5840
23	24.29	166.0216
24	24.37	165.4629
25	24.45	164.9080
26	24.53	164.3568
27	24.61	163.8092
28	24.70	163.2653
29	24.78	162.7250
30	24.86	162.1883
31	24.94	161.6550
32	25.02	161.1253
33	25.11	160.5991
34	25.19	160.0762
35	25.27	159.5568