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Supplementary Information

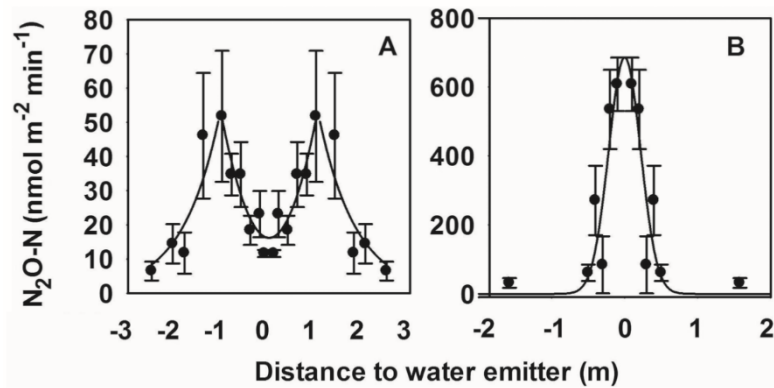
1. Additional site selection considerations for grazed pastures

For example, bovine urine has been found to alter the soil microbial community, significantly increasing the abundance of *amoA* genes (nitrification) and *nosZ* (nitrous oxide reduction to nitrogen gas) over a period of 57 days following application (Wakelin et al., 2013). Repeated urine application after 57 days resulted in further significant increases in the abundances of these genes, with a clear effect from the previous addition. Exclusion of animals from the experimental area prior to its use (for at least three months to allow 90% of deposited urine N₂O emissions to have occurred [Vangeli et al., in prep], but preferably longer) is recommended to reduce background spatial variability resulting from urine and dung patches (the exclusion period required will depend on the time taken for deposition effects to return to baseline). Recently developed remote sensing technologies may also prove useful in identifying homogenous areas and, for example, urine affected patches (e.g. Roten et al., 2017 and Maire et al., 2018).

2. Capturing the spatial variability of drip irrigated crops

Irrigation mainly occurs in summer when rainfall is low and evapotranspiration rates are high (sometimes > 5 – 7 mm/day from crops, A. Vallejo, personal communication). Considerable amounts of water may therefore be frequently applied to crops (and often in conjunction with relatively high N inputs to match the high crop growth rates under the warm conditions). There is a wide range of irrigation systems which deliver water to crops in different spatial distributions, intensities and frequencies, for example: sprinklers, micro-sprinklers, furrow irrigators, ranger

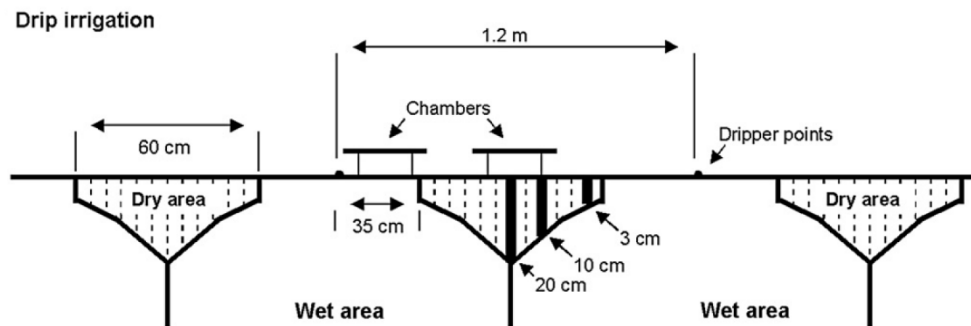
irrigators, flood irrigators, surface drip irrigators and subsurface drip irrigators. The system used thus strongly affects the spatial and temporal variability of soil moisture contents, and consequently, of N₂O fluxes. This must be captured by the experimental design chamber layout (and sampling frequency; Section 4.3. in main text).



Supplementary Figure 1. N₂O fluxes with distance from the source on one sampling day for A) microsprinkler irrigation, and B) drip irrigation. Error bars represent the standard error of the mean of replicates in a randomized complete blocks design (SEM; n=3). (Adapted from Alsina et al., 2013).

The spatial variability of water application is low in total wet surface irrigation systems, such as sprinkler and ranger irrigation systems, but is very high in partial wet irrigation systems, such as surface or subsurface drip irrigation (or even in furrow irrigation). In drip irrigation systems, for example, water is applied from perforated lines of emitters (drippers), spaced typically 0.25 – 2 m apart, running over the soil surface. Water is emitted from each dripper at a low flow rate (< 8 l hour⁻¹) and it takes several hours to complete an irrigation event. In a field experiment conducted to assess N₂O emissions from drip irrigated and fertigated systems, Vallejo et al. (2014) found that soil moisture contents decreased with the distance to the dripper on most of the sampling dates. Near the source, the % WFPS was commonly over 70%, while further away (20 – 50 cm), remained below 50% most of the time. Other areas of the soil surface, between dripper holes/lines, remained dry (< 20% WFPS), but N₂O

emissions, presumably from wetter soil layers at depth, were still recorded from these areas. Where drip fertigation was used, there was additionally a high spatial variability in N concentrations in the wetted areas.



Supplementary Figure 2. Schematic distribution of wet and dry areas for drip irrigation systems showing the location of static chambers for soil N₂O sampling. (Adapted from Sánchez-Martín et al., 2008).

Overall, a gradient in N₂O fluxes with distance from dripper points was observed, supporting the findings of other drip irrigation studies (e.g. Alsina et al., 2013; Supplementary Figure 1; Abalos et al., 2014). Chambers covering both the wet and dry areas were therefore included (e.g. Sánchez-Martín et al., 2008; Supplementary Figure 2) and calculations to spatially integrate N₂O fluxes were weighted by the relative proportions of each area.

3. Strategic chamber placement and calculating N₂O emissions from grazed pastures

In grazed pasture systems, where the majority of the N₂O emissions come from animal urine patches, stratifying the sampling into two distinct statistical populations, such as 'urine patch' and 'non-urine patch' areas, is recommended. This can be done by applying known amounts of urine N to specific areas, then measuring the emissions from these patches and the urine-free areas between

them. Field scale emissions can then be calculated based on urine patch area coverage:

$$N_t = (N_1 \times P_1) + (N_2 \times P_2) \quad (1)$$

where N_t is the total N_2O emission from a grazed field, N_1 and N_2 are the N_2O emissions from the urine and non-urine patch areas, respectively, as measured using the NSS chambers, P_1 and P_2 are the proportion of the field covered by urine and non-urine patch areas, respectively. The values of P_1 and P_2 will vary, depending on the stocking rate and the urine patch area coverage. Finally, the spatial structure in gas emission pattern may change during the growing season (Rochette et al., 1991) and flux sampling strategies need to be tailored accordingly.

4. Using soil temperature to guide the timing of NSS chamber deployments

Using soil temperature to determine the timings for NSS chamber deployment is not always straightforward. Ideally, the occurrence of the daily mean soil temperature at the depth of maximum N_2O production should be used, but this depth is difficult to determine and variable. In addition, soil surface N_2O emissions lag N_2O production at each depth by vertical transport times via gas diffusion, which varies with soil edaphic conditions (Clough et al., 1999). Thus, there can be a delay in emissions relative to temperature (Hatch et al., 2005).

5. Capturing the temporal variability of N_2O fluxes from irrigated crops

Irrigation is usually used only when soils are dry (e.g. % WFPS < 40%; A. Vallejo, personal communication). Large volumes of water (e.g. sometimes > 40 mm water per week; A. Vallejo, personal communication) may then be applied, rapidly increasing the soil % WFPS (sometimes to values close to 100% WFPS, depending

on the irrigation system). Rewetting of dry soils can lead to considerable releases of N₂O within hours to days (Bergstermann et al., 2011), which are often quantitatively important in terms of cumulative annual N₂O emissions. Moreover, the coincidence of high evaporation and evapotranspiration rates with irrigation events means that % WFPS levels in the upper parts of the soil often fall quickly. Thus, sampling under such circumstances should ideally be undertaken daily. When daily sampling is not possible after irrigation, representative samplings need to be taken to capture the temporal variability of fluxes as best as possible – targeting two periods, the first when soil has been recently wetted and second as it is drying is suggested (Guardia et al., 2017).

6. Alternative, non-destructive pore-water sampling

Miniature suction cups or Rhizon samplers may also be installed beneath chambers for non-destructive soil pore-water sampling (e.g. Marsden et al., 2019) in some soil types where soil moisture is sufficient. However, care should be taken not to remove too much soil water via the miniature suction cup, especially if repeatedly sampling. Care must also be taken to ensure sampler installation does not materially disturb the soil beneath the chamber and installation well in advance of gas sampling is recommended (Section 4.4. and 5.1.).

7. Experimental design process guiding questions

This section aims to guide experimenters through the experimental design process via a series of questions and decisions that need to be made to achieve a well thought out experimental design. A formal decision tree tool to guide experimenters directly to the design that minimises overall uncertainty was not developed, as small differences in the individual circumstances of each

experiment/site can have a big impact on the decisions taken. Moreover, it was suggested that such a tool could, in fact, adversely affect the design of future experiments through inappropriate use of (or over-reliance on) the tool, which would be unlikely to be effective in the wide range of situations in which static chambers are used to determine soil N₂O emissions. Guidance that asks the right questions but requires experimenters to provide the pertinent answers for their experiment was therefore deemed more appropriate. Ideally, proposed experiment designs should be discussed with an experienced applied statistician at the earliest possible stage but, unfortunately, not all experiments have such resources.

Experimenters must start by determining the main aim of the experiment. For simplicity, this guiding process focuses on two different, broad N₂O emission experiment types: i) experiments to investigate representative N₂O emissions from particular treatment(s) (e.g. fertiliser, animal urine, etc.) at selected site(s)/conditions (e.g. on that crop/soil type/field/local area), often to determine representative EFs (Supplementary Figure 3); and ii) experiments to evaluate the spatial/temporal variability of N₂O emissions at selected site(s) (either in general, or in response to a particular treatment; Supplementary Figure 4). While, all of this information (different treatment responses and spatial and temporal variability) is desirable, it is usually difficult to thoroughly investigate all aspects in the same experiment and usually one or two aspects needs to be prioritised. It is hoped this guidance will be of use for a broad range of experiments, but it may be less relevant for those which do not fall into these two categories (e.g. mechanistic-type experiments that aim to study the controlling factors behind N₂O emissions etc.).

7.1. Experimental design process guiding questions for emission factor experiments

The starting point for this type of experiment is usually a wish to determine representative emission factors from a (number of) treatment(s) at a (number of) site(s).

1. Prioritise and hone objectives:

- Identify key amendment(s)/practice(s).
- For what scale/situation does the experiment aim to generate representative EFs (e.g. national/regional/local; land use type/management [& historic]; soil type; topography/slope/aspect, etc.). Are appropriate sites available, or do the objectives need to be revised? In some cases, the objectives will guide site selection, while in others, site availability will help to define the objectives.
- Identify appropriate site(s) (Section 3.1. in main text). Consider their historic and current use (e.g. legacy effects of recent grazing events) and likely response to changes in conditions (e.g. heavy rainfall) for suitability in accordance with the objectives. Given the variability between sites, how many are needed to provide representative EFs in terms of the objectives?

2. Determine the total number of treatments (sites or situations × amendments or practices):

- Consider the scale of the experiment and the spatial variability of each site. At each site, is more than one field needed/available (e.g. to create plots on different local soil types/crop types/management practices/management histories/aspects/slopes etc.)? (Depending on

the objectives, these could either be considered different treatments or a blocking factor; Section 3.2. in main text).

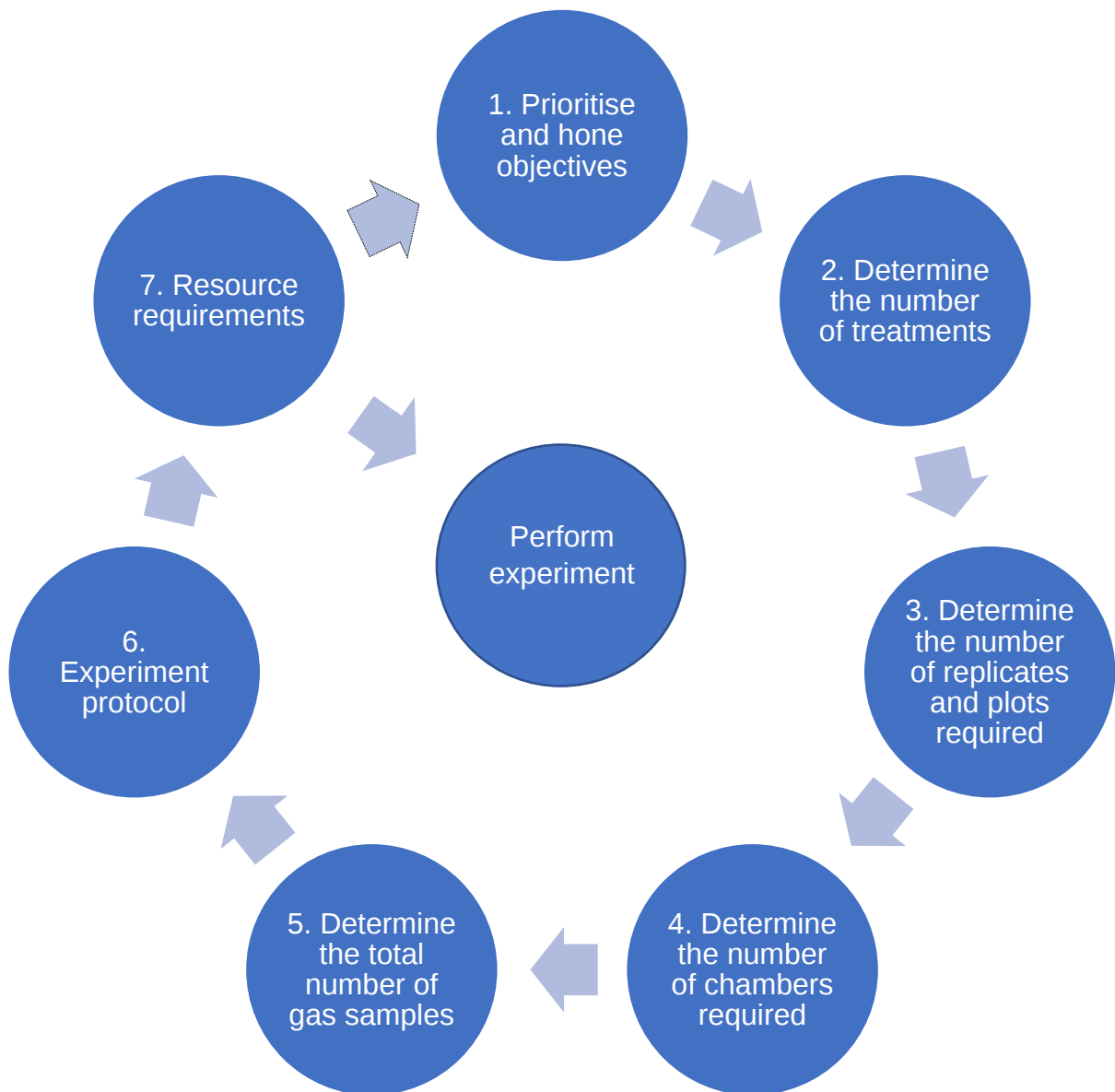
- Include no-amendment/change controls.

3. Determine the number of replicates and plots required (treatments × replicates = plots) and their layout:

- How many replicates of each treatment are required (e.g. one per field if using ≥ three replicate fields at the site and each field is spatially relatively homogenous in itself - in this case each field would be a block; or perhaps up to five or more treatment replicates per field if, for example, only one spatially variable field is being using at each site - in this case, there would be five blocks within the field)?
- Generally, at least three replicate plots of each treatment are required (but depends on experiment design, e.g. a factorial experiment design achieves replication by different combinations of treatments – no complete replicates, but many replicates of each treatment). Ensure adequate degrees of freedom for required statistical analyses.
- Experiment structures with uneven numbers of blocks/treatment replicates at each site (appropriate to the site)/for particular treatments/fields are likely to impact later statistical analysis approaches.
- How should the replicates be arranged in each field/block (e.g. randomly/in rows/columns to account for gradual changes across the field/block)? What are the most important changes across the field? This can be used to divide the field into blocks. Consider: aspect, slope angle, position on slope, topography, field features, proximity to field

features, shading, management variations, vegetation composition, soil type, soil physical and chemical properties. Are there any rules for e.g. the difference in slope angle known to result in different emissions? Or the distance from field features required to ensure independence (probably depends on field feature in question)?

- What is the total area and plot size available at each site? Is this sufficient for ancillary sampling requirements (especially crop yield measurements)? For arable experiments, does the plot size fit the farmer's tramline widths? What proportion of the field/site does the total plot area cover? Is the total plot area large enough to be representative of the site?



Supplementary Figure 3. Experiment design cycle for experiments to determine EFs.

4. Determine the number of chambers required and their location (plots × chambers per plot):

- How homogenous is each plot in terms of N₂O emissions (or underlying drivers)?
- What size and type of static chambers are available/ best (Section 3.3. in main text; Clough et al. this issue)?

- How many static chambers are needed to cover a sufficient proportion of the plot to capture representative plot-scale emissions (Section 3.2. in main text)? Multiple chambers per plot are pseudo-replicates, which improve the accuracy of individual plot N₂O emissions estimates but do not increase the statistical power of the experiment.
 - How should static chambers be placed on each plot (e.g. randomly/strategically; Section 3.4. in main text)?
5. Determine the total number of gas samples (samples per chamber × chambers × sampling occasions):
- What is the individual chamber sampling protocol? How many headspace samples will be taken on each occasion (Section 5.3. in main text)?
 - How often will the static chambers be sampled (Section 4.3. in main text)? Regularly/reactively? Fluxes are temporally heterogenous. Any variability over periods longer than the chamber closure will be important. Fluxes vary diurnally, seasonally and in response to weather and management events. Generally, a high (daily) sampling frequency is recommended following events, increasing to every other day, twice weekly, weekly and finally biweekly or even monthly if fluxes have stabilised to pre-treatment/control levels. Take care to consider events that might induce high transient fluxes during periods of otherwise low fluxes (e.g. freeze-thawing events during cold winter periods or sudden rainfall/irrigation events in dry summers) and increase sampling frequency accordingly. Include pre-treatment sampling. For EF experiments, 12 months of measurements post-treatment are required

(Section 4.4. in main text). What will be the total number of sampling days over this period?

6. Record and disseminate the experiment protocol:

- Plan to prepare the site and install chambers sufficiently in advance of the experiment (Section 5.1. in main text).
- Select and describe the treatment application approach (Section 3.5. in main text).
- Describe the individual chamber deployment protocol in detail. Select the chamber closure duration (depending on likely magnitude of N₂O fluxes vs. chamber volumes, and practicalities in terms of operator availability and the timings of headspace samplings; Section 4.1. in main text). Determine a sampling sequence (Section 5.2. in main text).
- Are any automated chambers /relevant data available to determine the best time of day for sampling? (Section 4.2. in main text).
- Determine the type and frequency of ancillary sampling (Section 5.4. in main text).
- When experiments include multiple sites, consideration must be given to local conditions and management and protocols for each site adjusted accordingly.

7. Estimate the total resources required and whether this is within the budget:

- Include operator availability (and costs), equipment purchases, consumables costs (e.g. gas vials), sample analysis costs (gas samples and ancillary) etc.
- Do the outputs (data/information) justify the resources?

- What is the minimum amount of information required for the experiment to achieve its objectives? Can the number of treatments be reduced?
- If necessary, revise the experiment design and scale-back accordingly.
- Weigh up whether uncertainties due to between/within plot spatial variation, temporal coverage, or the individual chamber sampling protocol will be greatest and scale back the experiment accordingly. Some decisions may be made for practical reasons (e.g. daily sampling protocol reduced as only one operator available).

7.2. Experimental design process guiding questions for experiments

investigating the spatial variation

Soil N₂O emissions are known to be highly spatially and temporally variable but detailed information regarding this variability at a particular site/in a particular environment can be valuable. Static chambers are well-suited for investigating spatial variability at the within site/field scale and below (for investigating the spatial variability of N₂O emissions at larger scales, measurement techniques that integrate N₂O emissions over larger scales micrometeorological methods [e.g. eddy covariance] are more appropriate). The temporal variability of N₂O emissions is, however, better captured by high frequency or continuous measurement techniques such as automated chamber systems or micrometeorological methods. Experiments that aim to capture the temporal variability of N₂O emissions at small spatial scales (i.e. using static chambers) are highly resource intensive and, as a result, are rare, or instead employ a variety of techniques simultaneously. The most common approach with static chambers is to capture a small number of spatially intensive ‘snap-shots’

in time, sometimes before and after treatments. For such experiments, resources may be prioritised as follows:

1. Refine objectives:

- Investigate the scale of spatial variation or compare N₂O emissions from spatially distinct areas/ features? Generate a representative aggregated N₂O emission for the site (note, however, that this may be better achieved using micrometeorological methods, if available and practical)?
- Measurements before and after treatment or after a certain period (e.g. monthly/seasonally/annually) or event (e.g. certain amount of rainfall)?

2. Site(s):

- Define the site(s).
- Identify key features? Potential hotspots (space and time) identified?
- Scale spatial autocorrelation known?

3. Determine the deployment strategy, number of chambers required and their location:

- Could deploy chambers in a transect across a particular feature, cluster chambers on and around important features or spread chambers evenly across the field in a grid. If a grid approach is used, the superimposition of two different sized grids is recommended to provide information regarding the variation of N₂O at different scales across the field (Charteris et al., in prep.)
- What size and type of static chambers are available/ best (Section 3.3. in main text; Clough et al. this issue)?

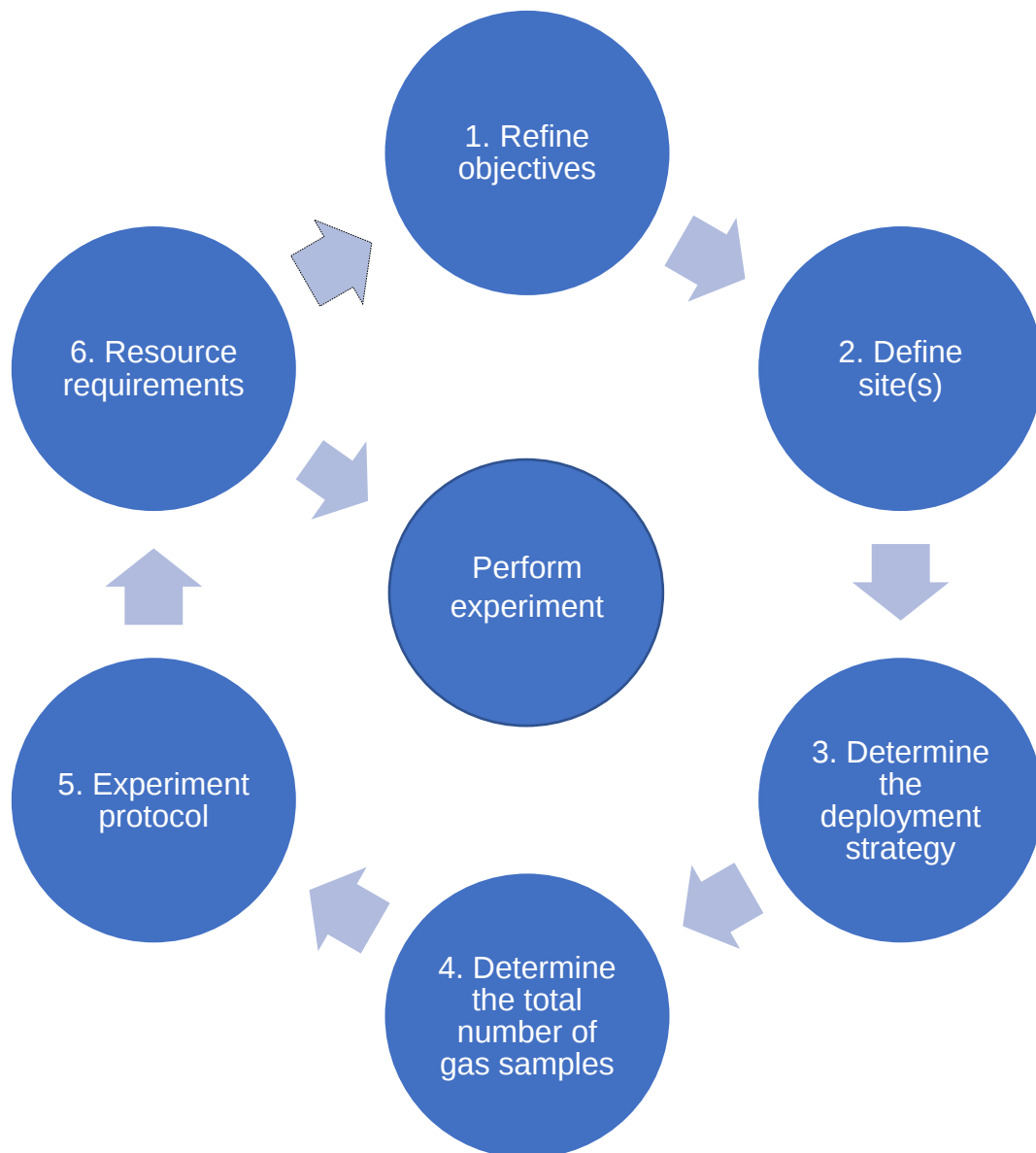
- How many spatial sampling points/static chambers are needed to cover a sufficient proportion of the field to generate representative aggregated emissions (Section 3.2. in main text)?
4. Determine the total number of gas samples (samples per chamber × chambers × sampling occasions):
- What is the individual chamber sampling protocol? How many headspace samples will be taken on each occasion (Section 5.3. in main text)? This is likely to be reduced, given the experiment objectives and large number of chambers.
 - How many times will the static chambers be sampled (Section 4.3. in main text)? Regularly/reactively? Have likely periods of higher fluxes been identified? Due to the large number of chambers, usually only a small number of deployments (e.g. 1-4) is manageable.
5. Record and disseminate the experiment protocol:
- Plan to prepare the site and install chambers sufficiently in advance of the experiment (Section 5.1. in main text). In such experiments, it is particularly important that the GPS locations of chambers are recorded.
 - Select and describe the treatment application approach (Section 3.5. in main text). Unless investigating the spatial variability of the field plus the treatment application (e.g. for investigation of effects of farm-scale equipment on variability of amendment application), treatments are usually applied to each chamber individually and each chamber is independent (and may be thought of as an individual plot).
 - Describe the individual chamber deployment protocol in detail. Select the chamber closure duration (depending on likely magnitude of N₂O

fluxes vs. chamber volumes, and practicalities in terms of operator availability and the timings of headspace samplings; Section 4.1. in main text). Determine a deployment sequence (Section 5.2. in main text).

- Are any automated chambers /relevant data available to determine the best time of day for sampling? (Section 4.2. in main text).
- Determine the type and frequency of ancillary sampling (Section 5.4. in main text).

6. Estimate the total resources required and whether this is within the budget:

- Include operator availability (and costs), equipment purchases, consumables costs (e.g. gas vials), sample analysis costs (gas samples and ancillary) etc.
- Do the outputs (data/information) justify the resources?
- What is the minimum amount of information required for the experiment to achieve its objectives? Can the number of spatial points/chambers be reduced? Or the number of sampling occasions?
- If necessary, revise the experiment design and scale-back accordingly.
- Some decisions may be made for practical reasons (e.g. daily sampling protocol reduced as only one operator available).



Supplementary Figure 4. Experiment design cycle for experiments to investigate the spatial variation of N₂O using static chambers.

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