Charteris, A., Chadwick, D. R., Thorman, R. E., Vallejo, A., De Klein, C. A. M., Rochette, P. and Cardenas, L. M. 2020. Global Research Alliance N2O chamber methodology guidelines: Recommendations for 2 deployment and accounting for sources of variability. *Journal of Environmental Quality.*

Core Ideas

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Core Idea 1: â€¢ Account for spatial variation in site selection & chamber placement & coverage

Core Idea 2: â€¢ Account for temporal variability with strategic sampling over a sufficient duration

Core Idea 3: â€¢ Allocate resources to minimise the overall uncertainty of N2O fluxes

Core Idea 4: CUST_CORE_IDEA_4 :No data available.

Core Idea 5: CUST_CORE_IDEA_5 :No data available.

Word count: 8350

Global Research Alliance N_2O chamber methodology guidelines: Recommendations for deployment and accounting for sources of variability

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Abbreviations

Chamber bias correction – CBC

Coefficient of variation – CV

Emission factor – EF

Non-steady state – NSS

Quantum cascade laser – QCL

Water filled pore space – WFPS

Core ideas

- Account for spatial variation in site selection & chamber placement & coverage
- Account for temporal variability with strategic sampling over a sufficient duration
- Allocate resources to minimise the overall uncertainty of N_2O fluxes

Abstract

Adequately estimating soil nitrous oxide (N_2O) emissions using static chambers is challenging due to the high spatial variability and episodic nature of these fluxes. This paper discusses how static chamber N_2O experiments can be designed, and protocols implemented, to better account for this variability and reduce the uncertainty of N_2O emission estimates. It is part of a series of papers in this special issue, each discussing a particular aspect of N_2O chamber methodology. Aspects of experimental design and sampling affected by spatial variability include site selection, and chamber layout, size and areal coverage. Where used, treatment application adds a further level of spatial variability. Time of day, frequency and duration of sampling (both in terms of individual chamber closures and overall experiment duration) affect the temporal variability captured. In addition, we present best practice recommendations for experimental chamber installation and sampling protocols to minimise the introduction of further uncertainty.

To obtain the best N_2O emission estimates, resources should be allocated to minimise the overall uncertainty in line with experiment objectives. In some cases, this will mean prioritising individual flux measurements and increasing their accuracy and precision by, for example, collecting ≥4 headspace samples during each chamber closure. However, where N_2O fluxes are exceptionally spatially variable, for example, in heterogeneous agricultural landscapes, such as uneven and woody grazed pastures, using available

resources to deploy more chambers with fewer headspace samples per chamber may be beneficial. Similarly, for particularly episodic N_2O fluxes, generated for example by irrigation or freeze-thaw cycles, increasing chamber sampling frequency will improve the accuracy and reduce the uncertainty of temporally interpolated N_2O fluxes.

Table 1. Summary of aspects of variability and recommendations discussed in this paper.

Aspect of spatial variability	*Recommendation to account for variability and reduce uncertainty*
Site selection	Identify representative area and assess whether spatial structure in N_2O fluxes exists.
Experiment spatial structure	Divide area into homogenous sections (blocks) and stratify sampling. If no spatial structure, select plots and place chambers randomly. Each plot must have at least one chamber. A minimum of three replicate plots is required. A statistical 'power' analysis to determine the required level of replication is recommended.
Spatial coverage	Chambers should cover an area as large as practical, while providing information at the smallest scale for which it is needed, and avoiding resource intensive large numbers of small chambers, to achieve good coverage at a small scale.
Background emissions and control plots	Pre-treatment N_2O flux measurements indicate underlying flux patterns and can be useful as covariates in statistical analyses. Replicated untreated control plots are recommended to estimate background emissions throughout and are required to calculate emission factors (de Klein et al., 2020b, this issue).
Chamber size	Chambers having larger areal coverage integrate spatial variability. Chambers should integrate N_2O fluxes at the desired scale and meet other requirements for good design with respect to area, height and other considerations (Clough et al., 2020 and Venterea et al., 2020, this issue).
Strategic chamber placement	Chamber placement must account for local features (e.g. crop row and inter-row gradients, irrigation-induced soil moisture gradients or urine and dung patches) by either spanning chambers across features to integrate them or locating individual chambers on all desired features and accounting for the feature area as a proportion of the total and sampled areas in total calculations.
Treatment application	Different approaches exist (e.g. including/excluding urine patch diffusional areas), options should be considered, and approach selected reported in detail (including calculation details) to facilitate comparison between studies (see also de Klein et al., 2020b, this issue).
Aspect of temporal variability	**Recommendation to account for variability and reduce uncertainty**
Chamber closure duration	Effect of closure time depends on flux-calculation method used and other factors (e.g. soil properties). Longer closures tend to increase

	uncertainty with linear regression and can have varying effects for non-linear methods (Venterea et al., 2020, this issue).
Daily mean emissions	Previously, sampling between 10:00 – 12:00 was recommended to capture the daily mean N_2O flux in temperate climates (Smith and Dobbie, 2001; Parkin, 2008; Alves et al., 2012). However, recent studies have suggested an earlier time period might be better for some sites. Whenever possible, researchers should determine local diurnal N_2O emission patterns to assess times which best represent the daily mean N_2O flux for their study. At a minimum, researchers should assess the time which best represents the mean daily soil temperature, at a depth appropriate to their experimental study.
Temporal coverage	A strategic sampling frequency in response to events is preferred, but the whole 'envelope' of an N_2O emission peak (pre and post event) must be included to avoid cumulative emission overestimation. Sampling frequency should be as high as resources allow. As a minimum, when higher soil N_2O emissions are occurring, chambers should be deployed at least twice per week and at higher intensities around events. When N_2O fluxes are low, deployment frequencies of once per week are appropriate. Deployment intervals may be increased only when near-zero or background fluxes are sustained (e.g. in dry or cold soils).
Duration of the experiment	Ideally, continue the experiment until there is no significant difference between pre/control and post-treatment N_2O emissions and/or driving soil properties (e.g. soil NH_4^+ and NO_3^- concentrations) are not statistically different from background/control. Recent work (Vangeli et al., submitted) provides guidance for shortening experiments while still capturing 90% of 365-day N_2O emissions. For emission factor measurements for inventories, measurements should ideally be continued for 12 months.
Practical/experimental aspects	**Recommendation to account for variability and reduce uncertainty**
Chamber installation and site disturbance	Chamber bases must be inserted at least 24 hours before the first sampling occasion and their GPS locations recorded. Minimise soil disturbance around chambers. Chamber relocation may be considered if within-chamber soil conditions become different from those externally, but there will be implications for data analysis.
Sequence and grouping of chamber measurements	Experiments should be sampled per block (rather than per treatment) to minimise within-block differences and the order of block sampling should be rotated. Multiple operators allow the experiment size to be increased, but training to ensure protocol standardisation is essential.
Headspace air sampling	Ideally, ≥4 headspace samples per flux measurement to determine accurate fluxes for individual chambers (Venterea et al., 2020, this issue). However, when spatial variability is high (e.g. when within-treatment variability is similar to between treatment variability), overall uncertainty may be reduced by deploying more chambers with fewer headspace samples. In such cases, (non-)linearity must be investigated and the potential bias introduced by assuming a linear increase in headspace N_2O concentrations stated.
t_0 sample	t_0 headspace air samples should be taken immediately after chamber closure. If ambient air samples are used to estimate t_0 N_2O concentrations, researchers need to establish that ambient air N_2O

	concentrations are not significantly different from within chamber t_0 samples.
Ancillary measurements	The need for additional measurements depends on the experiment objectives. Measurements of soil water content, bulk density and temperature allow for application of the chamber bias correction (CBC) method (Venterea et al., 2020, this issue). To interpret N_2O fluxes, soil and air temperature and rainfall should be measured on a daily or hourly basis; soil moisture as often as needed to provide a representative estimate of conditions on each gas sampling occasion; soil mineral N as often as resources allow and especially after N additions; and soil bulk density, pH, organic C and total N content at least once during the experiment. When possible, all ancillary measurements should be made in order to meet requirements for eventual flux calculations using mathematical models.

1. Introduction

Static (or non-steady state; NSS) chambers are widely used for measuring nitrous oxide (N_2O) emissions worldwide (Rochette, 2011). They are simple, inexpensive and versatile, but their (necessarily) small size (Clough et al., 2020, this issue) makes obtaining spatially representative/accurate field-scale N_2O fluxes challenging, and manual sampling imposes sampling frequency and duration constraints. Automated chamber methods that better account for temporal variability are becoming increasingly available (Grace et al., 2020, this issue), but manual sampling methods still represent the majority of measurements. Soil is not a homogeneous medium and most ecosystems (including agronomical plots) are a mosaic of N_2O sources of various intensities (Yanai et al., 2003; Matthews et al., 2010). Spatial variability in management practices (e.g. fertiliser or water inputs) exacerbates this soil heterogeneity. N_2O emissions from agricultural systems also vary over time, responding to nitrogen (N) additions (e.g. manufactured fertiliser, manure, crop residues or grazing returns) and rainfall (or irrigation) induced changes in soil moisture, for example (Parkin, 2008). Capturing spatial and temporal variability and reducing the uncertainty of N_2O emission estimates requires careful experimental design and chamber

deployment. Moreover, resource limitations restrict chamber numbers and sampling frequencies, necessitating design and sampling strategy optimisation to generate accurate and comprehensive flux datasets which, in conjunction with ancillary data, achieve experiment aims.

Optimisation of data collection must consider all sources of uncertainty relating to chamber deployment and N_2O measurement protocols. The relative importance of different sources of uncertainty depends on the specific experiment aims and site characteristics. The flux calculation method used (Venterea et al., 2020, this issue) has been found to be the single largest source of uncertainty in hourly flux estimates from individual chambers (Levy et al., 2011). More refined flux calculation methods require a greater number of headspace samples to be taken during chamber closure. This approach may give the best overall results if the aim is to calculate accurate N_2O fluxes from individual chambers but becomes resource intensive as a larger number of chambers and/or sampling dates are required to adequately capture the spatial and temporal variability of N_2O emissions. McDaniel et al. (2017) recorded a mean temporal coefficient of variation (CV) of over 1200% and a mean spatial CV of nearly 400% for automated chambers sampling at a high frequency compared with a static chamber array. However, a wider range (and standard deviation) of N_2O fluxes was recorded from the static chambers (-19 – 476 μg N_2O m^{-2} h^{-1}, cf. -129 – 63 μg N_2O m^{-2} h^{-1} for the autochambers). The uncertainties associated with the spatial and temporal variability of N_2O fluxes vary with experimental site and could sometimes be larger than those relating to individual chambers or the flux calculation method.

The 2015 Nitrous Oxide Chamber Methodology Guidelines (de Klein et al. 2020a, this issue) provided guidance on chamber methodologies for sampling N_2O emitted from soils. The papers presented in this special issue provide updates on the 2015 guidelines. Here, we

focus on updating recommendations for chamber deployment to reduce the uncertainty associated with the spatial, temporal and experimental variability in N_2O fluxes. Our recommendations centre on NSS chamber use to assess emissions from treatments and determine emission factors (EFs) but are applicable to any N_2O emission study using static chambers (e.g. using chamber arrays to assess the spatial variability of N_2O emissions and/or determine representative emissions in a particular environment; Charteris et al., in prep.).

2. Factors responsible for the variability of N_2O fluxes

Soil N_2O fluxes are spatially and temporally extremely variable. Large ranges in N_2O fluxes have been measured in 'snapshot' spatial variability studies. For example, Turner et al. (2008) recorded fluxes of 45 – 765 ng N_2O-N m^{-2} s^{-1} (average: 165 ng N_2O-N m^{-2} s^{-1}) and 20 – 953 ng N_2O-N m^{-2} s^{-1} (average: 138 ng N_2O-N m^{-2} s^{-1}) for two experiments in summer and autumn, respectively, on an Australian irrigated dairy pasture, while Cowan et al. (2015) recorded fluxes varying from 2 – 79 000 µg N_2O-N m^{-2} h^{-1} from 100 sampling points across an intensively-managed, grazed 7 ha grassland in central Scotland. Temporal monitoring studies have similarly recorded large ranges, with episodic behaviour in N_2O fluxes, even when spatial variations are excluded (e.g. 6.5 – 39.7 mg N_2O-N m^{-2} d^{-1} from cropland in the USA measured using eddy covariance; Molodovskaya et al., 2012).

Soil-derived N_2O is produced largely via microbial processing, usually mainly by incomplete denitrification or during nitrification (Butterbach-Bahl et al., 2013). Denitrification is an anaerobic process which is favoured by higher soil moisture contents (percentage water filled pore space [% WFPS] >70%), while nitrification is an oxidative process favoured by lower % WFPS (Bateman and Baggs, 2005). In addition, both processes

are subject to other important controls, such as N substrate, carbon (C) availability and pH. N_2O fluxes therefore differ spatially with the variation of these processes in soil (depending on edaphic conditions, which in turn can depend on slope, aspect, larger scale features, management, weather etc.) and temporally with changes in these conditions (due to weather and management). Soil N_2O fluxes are typically low and commonly the emissions contributing to spatial integrations or annual budgets are observed from hotspots (Cowan et al., 2015) or during peaks which can last from a few hours to several weeks after events, e.g. soil disturbance, rainfall, irrigation, spring thaw or N addition (Chadwick et al., 2011; Molodovskaya et al., 2012; Schelde et al., 2012; Loick et al., 2017). In both cases, uncertainties in measured fluxes result from uncertainties associated with properly capturing the underlying spatial and temporal heterogeneity of N_2O fluxes and those relating to NSS chamber protocols.

3. Improved sampling protocols to account for the spatial variability in N_2O fluxes and reduce uncertainty in N_2O emission estimates measured by static chambers

3.1. Site selection

Experimental site locations are often determined by a combination of practicalities and overall project/experiment goals. Where some choice remains, site selection should be considered in the context of wider local, regional and national ecosystems, land uses, soil types and climatic conditions and whether the site and management are representative.

In experiments aiming to determine emissions from a treatment (and often then calculate EFs), fluxes occurring prior to/without treatment are considered 'background' or control emissions (Pennock et al., 2006). Selecting relatively uniform areas helps to minimise interference from spatial heterogeneity in background emissions, although care

needs to be taken to ensure site selection is still representative. Identification of homogeneous areas, in terms of N_2O fluxes, within a landscape (e.g. a grazed paddock or cropped field) can be achieved through exploratory flux sampling. Where this is not possible, the selection of areas within which landscape characteristics (e.g. aspect, slope/topography, distance from field features), management (both recent and historic, e.g. N application or irrigation), vegetation and soil type (or preferably properties, determined by basic soil sampling and analysis, e.g. pH, electrical conductivity, C/total organic C, N/extractable ammonium and nitrate) are consistent should reduce spatial variability in background emissions. Note however, fluxes may vary according to different factors at different sites (Charteris et al., in prep.) and it may be difficult to estimate the spatial structure in N_2O fluxes. In addition, some soil properties are dynamic, so for maximum utility, soil sampling for baseline soil variables would need to be conducted shortly before the gas sampling experiment. For grazed pastures, the distribution of animals within the field, additional heterogeneity of grazing returns and persistence time of these effects should be considered (Supplementary Information Section 1).

3.2. Experiment spatial structure and spatial coverage

A plot is a discrete area to which a single treatment is applied. Plots should be kept as small as possible for improved homogeneity but must be large enough to allow for all sampling (N_2O and other ancillary measurements) required for the duration of the experiment. However, trade-offs often exist between keeping plots as small as possible (while ensuring a large enough area for all sampling activities), and ensuring the chambers cover as much of the plot as possible for accurate plot N_2O flux estimates (while leaving space for other sampling activities), without exceeding a chamber size that meets the

requirements for good chamber design (Clough et al., 2020, this issue), or using many small chambers which would be resource intensive. The size of the experimental plots (and number of chambers required per plot) can also be minimised by sampling some ancillary variables, such as soil pH and soil moisture content, at a lower spatial resolution than N_2O fluxes. Alternatively, pooling of pseudo-replicate soil samples prior to analysis to integrate plot-scale spatial variability and reduce resource demand is a common practice. Recently, this approach has been extended to gas samples (Arias-Navarro et al., 2013).

Each plot should have at least one NSS chamber on it. Where larger plots are required (e.g. for yield assessments), such that a single chamber can no longer provide an acceptable plot-scale estimate of N_2O fluxes, multiple static chambers are recommended to account for within-plot spatial variability and improve plot N_2O emission estimate accuracy. Chadwick et al. (2014) assessed the reliability of the standard deviation of the N_2O flux calculated from two, three, four and five out the five chambers deployed on each experimental plot and found that there was a 10-fold reduction in the error as replication increased from two chambers to five. These multiple chambers per plot are pseudo-replicates but can be used to assess the within-plot spatial variability in N_2O fluxes. Only the average fluxes from each plot can be used in statistical analysis of treatment effects (e.g. Cardenas et al., 2019).

Statistical analysis of treatment effects also requires a minimum of three replicate plots of each treatment. More replicates will increase the ability of the experiment to identify treatment differences. This is the statistical power of the experiment, i.e. the probability (expressed as a percentage) a difference of a specified size will be detected as significant at a specified significance level (such as 5%, which equates to accepting a 5% probability of a false positive). The power is the probability of a true positive being detected

and is commonly set at 80%. Given a required power, statistical software packages can calculate the ideal number of replicates required for the experiment. However, this may exceed available resources, necessitating compromise.

Fully replicated untreated control plots are recommended to assess background emissions, which will vary spatially and temporally and are required for the calculation of EFs (de Klein et al., 2020b, this issue). In addition, pre-treatment N_2O flux measurements for treated plots provides information on pre-existing spatial patterns of emissions, which can be used as covariates in statistical analyses.

Within the identified experimental area, and in the absence of any flux spatial structure, plots and chambers should be located randomly. Where differences or a trend in background emissions or conditions across the site are present, replicate plots should be divided between areas that are uniform in themselves but differ from one another (blocks). Blocking enables variability between these areas to be isolated from the overall background variability and treatment effects. In a study exploring within and between-block variability, Giltrap et al. (2014) found spatial variability at both scales, highlighting the need for multiple replicates (and if plot size requires it, multiple chambers per plot) to obtain representative N_2O emission estimates.

Good plot spatial coverage by chambers is essential to obtain representative plot N_2O fluxes. If this cannot be achieved within the available resources, consider reducing: i) the number of plots (via fewer sites, treatments or replicates); or ii) the number of headspace samples per chamber deployment. Due to the high spatial variability of N_2O fluxes, care must be taken when reducing treatment replicates below four for sufficient statistical power. Additionally, reducing the number of plots may reduce the overall experiment spatial coverage, leading to measured N_2O fluxes which are not representative

221 at the site/experiment scale. The number of headspace samples can be reduced by
222 replacing individual initial closure (t_0) headspace samples with average ambient air samples
223 (Section 5.3.1.), and/or reducing the number of headspace samples taken during chamber
224 closure (Section 5.3.). This approach reduces both sampling and analytical workloads and
225 costs, leaving more resources to increase plot and site spatial coverage. However, a
226 reduction in the number of headspace samples increases the uncertainty in individual
227 chamber flux calculations (Venterea et al., 2020, this issue) and could affect the choice of
228 calculation method, which has previously been shown to be a large contributor to
229 uncertainty (Levy et al., 2011). Reduced headspace sampling must therefore not offset the
230 benefits of increased spatial coverage.
231
232 **3.3. Chamber size**
233 Dimension requirements for good chamber design are discussed in Clough et al.
234 (2020, this issue). The effect of chamber height on flux-calculation accuracy and precision is
235 discussed in Venterea et al. (2020, this issue). In this paper we consider chamber area and
236 height in terms of capturing spatial variability and minimising the uncertainty in measured
237 N_2O fluxes. The interaction between chamber height and closure duration is discussed in
238 Section 4.1.
239
240 **3.3.1. Chamber area**
241 Chamber areal coverage affects the spatial variability captured (Giltrap et al., 2014)
242 and uncertainty in N_2O fluxes. The greater the plot area covered by static chamber(s), the
243 more accurate the plot-scale N_2O flux will be (although note again, accurate plot-scale N_2O
244 fluxes do not equate to representative field/landscape-scale fluxes, which depend on larger

scales of spatial variability being captured by the overall experiment design). Larger chambers integrate fluxes over a larger area, averaging spatial variability at that scale. In studies seeking to understand spatial variability, multiple small chambers (ideally at variable spacings) can be used to determine its magnitude. However, it is recommended the chamber area/perimeter ratio be ≥10 cm to minimise the relative error associated with a poor chamber seal, which decreases as chamber area increases (Rochette and Eriksen-Hamel, 2008).

Chambers covering an area up to 2 m^2 have been used, but most common designs have an area smaller than 0.5 m^2. There have been few studies investigating the impact of differences in chamber area on the CV determined for a study area. Ambus et al. (1993) compared N_2O emissions from 15 × 0.0078 m^2 cylindrical chambers with 4 × 0.49 m^2 chambers along a transect. Emission patterns from the small chambers along the transect, and a higher than anticipated CV for the large chambers (40%) compared with the small chambers (77%), showed mesoscale variation in N_2O emissions was present alongside small-scale N_2O hotspots. A statistically indistinguishable mean N_2O flux, but higher variability from 20 smaller cylindrical chambers (0.049 m^2) compared with eight larger rectangular chambers (0.5 m^2) was similarly observed in another study (Saggar et al., 2008). The extent to which chamber shape (cylindrical versus square) might also have affected variation (e.g. if one shape has a greater propensity for leaks) was not discussed in either paper. Smith et al. (1994) found the CV for 24 small (0.13 and 0.49 m^2) chambers was 75% across an ungrazed field but estimated (by geostatistical analysis) the CV for 51 simulations of a much larger (62 m^2) chamber would be much lower (25%), indicating spatial heterogeneity at the 10 – 100 m scale was present in the field. Thus, chamber size can impact the variability measured due to

the scale of spatial N_2O variability (Section 2) captured by the chamber size (as well as layout – see Sections 3.2. and 3.4.).

3.3.2. Chamber height

Increasing chamber height (and hence headspace volume) reduces the physical impacts of enclosure but increases the minimum detectable flux (requiring longer chamber closures). It also affects the relative performance of different flux-calculation schemes depending on measurement precision (Venterea et al., 2020, this issue). (Note, however, chambers with higher *permanent* bases can cause greater within-chamber differences via e.g. sun and rain shadows). Venterea et al. (2020, this issue) describe methods for quantitatively assessing the impacts of varying chamber height on flux-calculation accuracy and precision in the context of other important factors. These methods are recommended for site-specific evaluation, including evaluating the use of larger chamber heights to accommodate growing crops or for paddy crops (e.g. Olfs et al., 2018; Bertora et al., 2018; Section 3.4.). (Note that for paddy crops, the headspace volume above the water level affects the uncertainty of N_2O flux measurements and should be recorded.). Similar to Venterea et al. (2020, this issue), along with raising the minimum detectable flux, Lammirato et al. (2018) found the uncertainty of individual N_2O flux estimates (calculated by linear regression over five headspace sampling points) increased with increasing chamber volume (perhaps indicating headspace mixing is required; Clough et al., 2020, this issue).

3.4. Strategic chamber placement

In many instances, management practices or cropping characteristics can create additional spatial variability (e.g. crop rows, irrigation patterns, grazed pasture etc.).

Adequately capturing field-scale N_2O emissions in these environments requires special consideration. Row crops may produce inter-row gradients in soil water and nitrogen content, which can be accounted for by an adequate sampling pattern, e.g. by placing chambers to include both row and inter-row areas (Cai et al., 2012). Indeed, Olfs et al. (2018) describe a new chamber design to account for both row and inter-row areas (Clough et al., 2020, this issue). On irrigated crops, different irrigation systems can lead to different patterns of water distribution and, accordingly, soil moisture (Supplementary Information Section 2). This needs to be considered for chamber location (e.g. by selecting wetter and drier areas and ensuring some chambers are located on each). As does the N application method (e.g. band spreading, broadcast, drip fertigation), which affects N distribution and thereby appropriate chamber orientation (e.g. on-bands and between-bands, or encompassing a full band and half the space between bands on either side to obtain emissions from the full N gradient). N_2O emission calculations per hectare need to include the area of each sampled component (e.g. bands and between-bands). This is also the case for animal urine patches (Supplementary Information Section 3).

3.5. Treatment application

As discussed above, background spatial variability can be separated from treatment induced effects via good experimental design. However, spatial variability is also associated with treatment application. For example, an experiment designed to measure the effects of adding manure, animal urine, crop residues, manufactured N fertiliser etc. can be conducted in three ways: i) prescribed amounts of N can be manually applied within the chambers *in situ* within their sub-plot (e.g. Krol et al., 2016, for urine and dung to pasture), ii) N can be applied to a larger area than the chamber, e.g. to a small plot before placing the chamber

316 (e.g. Nicholson et al., 2017), or iii) N can be applied via farm-scale spreading equipment and
317 chambers placed over the amended soil (e.g. Thorman et al., 2007). For all methods, there
318 will be variability between plots of the same treatment due to underlying differences in the
319 potential to produce N_2O emissions (i.e. spatial variability in the soil environment).
320 However, methods (i) and (ii) usually reduce spatial variability, compared to (iii), as uneven
321 amendment distribution by farm machinery will contribute further to the spatial variability
322 of N_2O emissions. For (iii), the heterogeneity of the application method may require more
323 chambers to be used. The treatment application method depends on the experiment
324 objective(s) and whether typical agricultural practices need to be represented.
325 Moreover, N amendments to the soil affect both an immediate soil area/volume, as
326 well as a greater diffusional area/volume which develops over time. Buckthought et al.
327 (2016) recovered 21.5% of the ^{15}N applied to a central urine patch in diffusional zones
328 outside the central patch. Furthermore, Marsden et al. (2016a) showed the N_2O EF including
329 the diffusional area of a simulated urine patch applied to a moist soil (70% WFPS) was larger
330 than the EF measured from the wetted area only. Different vegetation cover and soil
331 types/textures (and even soil moisture content) affect urine patch diffusion. The relative
332 importance of emissions from the wetted and diffusional treatment areas therefore varies
333 with patch size, site and season.
334 Several different approaches to treatment application (e.g. urine patches) for NSS
335 chambers exist in the literature. Chadwick et al. (2018), for example, applied urine to a 60 ×
336 60 cm area, and then placed a 40 × 40 cm area chamber within this, excluding N_2O
337 emissions from the diffusive area. Other researchers have taken the opposite approach and
338 applied a single small urine patch within a chamber allowing for patch diffusion within the
339 chamber area and thereby accounting for all diffusive area N_2O emissions. For example,

Marsden et al. (2017, 2018) used 150 – 385 ml sheep urine with wetted areas of 113 – 300 cm^2 within 50 × 50 cm chambers. Depending on treatment concentration, however, for smaller patches this can lead to low/ more difficult to detect treatment N_2O emissions. Accordingly, Marsden et al. (2019) used three sheep urine patches (each 195 ml with wetted areas of ca. 100 cm^2) in 50 × 50 cm chambers, where the sum of the areas of the three patches represented 12% (by wetted area) of the chamber area.

Recent work has indicated the total amount of N applied, rather than the concentration of N determines N_2O losses from urine patches (i.e. N_2O emissions from a small, high concentration patch are similar to those from a large low concentration patch; Orwin et al., 2009; Marsden et al., 2016b; Loick et al., 2017; Hoogendoorn et al., 2018). However, the spatial distribution of equal amounts of urine N to several small areas or one large area may affect N_2O emissions (Orwin et al., 2009; Marsden et al., 2016b).

Different approaches also exist in calculating treatment EFs from static chambers with partial treatment coverage, with some researchers using only the wetted area and others the whole chamber area in calculations (e.g. Mori and Hojito, 2015; López-Aizpún et al., 2020). Care must therefore be taken when comparing EFs between studies. In addition, the delivery methods of treatments with additives (e.g. N amendments with nitrification inhibitors or ^{15}N-labelled tracers) can be a source of variability. Both pre-mixing of amendments and inhibitors/tracers (e.g. Chadwick et al., 2018; Guardia et al., 2018) or spray-application of inhibitors after the N source has been applied (e.g. Misselbrook et al., 2014) are common approaches. Repeated applications of treatments and inhibitors (e.g. additional urine patches to represent patch overlap) have also been used (e.g. Di et al., 2007) and may further complicate EF calculations. Furthermore, inhibitors often add further N to treatment plots (e.g. DCD contains 67% N) and not all studies account for this in EF

364 calculations. Greater standardisation in experimental protocols and EF calculations are
365 required to facilitate the use of EFs as comparable indicators (de Klein et al., 2020b, this
366 issue).

367 To minimise the uncertainty in N_2O estimates and EF calculations due to treatment
368 application, researchers should consider: i) the treatment application method; ii)
369 appropriate application rates for the treatment being investigated, but ensuring sufficient
370 treatment/N to induce a discernible effect; iii) the treatment area (and potential diffusive
371 area) and distribution within the plot (and the necessary chamber size; Section 3.3.); iv) the
372 delivery method of treatments with additives; v) how repeated or overlapping treatments
373 will be accounted for; and vi) the EF calculation (de Klein et al., 2020b, this issue), to ensure
374 the chosen approach is appropriate to the study aim(s) and site(s).

375

376 **4. Improved sampling protocols to account for the temporal variability in N_2O fluxes and**
377 **reduce uncertainty in N_2O emission estimates measured by static chambers**

378 Given the episodic nature of N_2O fluxes, high frequency or continuous measurement
379 techniques such as automated chamber systems (Grace et al., 2020, this issue) or
380 micrometeorological methods (e.g. eddy covariance; Cowan et al., 2020) can provide better
381 estimates of integrated N_2O emissions (Jones et al., 2011). However, these approaches
382 require expensive equipment and experienced operators, beyond the scope of many project
383 budgets. Additionally, measurement techniques which integrate fluxes over large areas are
384 not suited for exploring statistical differences between typical replicated treatment plots,
385 and eddy covariance systems are ill-suited to some environments (e.g. steep slopes/short
386 fetches). Thus, most cumulative N_2O emission estimates, such as the amendment induced
387 EFs used for national soil N_2O inventories, are determined using data obtained from manual

NSS chambers (Bell et al., 2015; Chadwick et al., 2018; Cardenas et al., 2019). These chambers are typically deployed for short durations, sampled daily at best, and used for experiments of up to approximately twelve months. Sufficiently capturing N_2O fluxes for accurate temporal integration can therefore be challenging.

4.1. Chamber closure duration

Changes in the within-chamber physical environment, the risk of leaks, and potential for diffusional feedbacks due to accumulating headspace concentrations (Rochette and Eriksen-Hamel, 2008), increase with deployment time (Clough et al., 2020 and Venterea et al., 2020, this issue). Long closure times have been found to significantly increase N_2O flux uncertainties when linear regression is used to calculate the N_2O flux (Cowan et al., 2014a). Although short deployment periods can lead to low chamber N_2O concentrations, 30 min closures for 0.2 m-high chambers should produce headspace N_2O concentrations (>3 μg N m^{-2} h^{-1}) detectable by gas chromatographs (Rochette and Eriksen-Hamel, 2008). However, when using non-linear flux calculation methods for estimating the flux at t_0 (Venterea et al., 2020, this issue) the flux estimate is independent of deployment time, and a longer closure duration allows researchers to take more gas samples per chamber. This in turn provides more options in choice of flux calculation method (Venterea et al., 2020, this issue). More recently, technological advances have enabled infrared quantum cascade lasers (QCLs) to be employed with NSS chambers (e.g. Cowan et al., 2014b; Cowan et al., 2015) providing lower detection limits (<2 μg N_2O-N m^{-2} h^{-1}) with shorter (5 min) closure times (Cowan et al., 2014a). In addition, there is a greater chance the assumption of a linear increase in chamber headspace N_2O concentrations is satisfied over a shorter closure period. However, the guidance provided by Venterea et al. (2020, this issue) for the selection of a flux calculation

412 method should still be considered. The disadvantages of QCL systems are their relatively
413 high purchase costs and power supply requirements, which can limit mobility/reach (Cowan
414 et al., 2014a).

415 Where higher chambers are required (e.g. over growing crops), duration may be
416 increased. Additionally, a longer closure duration (60 min) with smaller chambers (35.6 cm
417 diameter × 11 cm high) is required in ^{15}N tracer experiments to obtain detectable $^{15}N_2O$
418 headspace concentrations (Guardia et al., 2018). For logistical reasons, the chamber
419 deployment duration employed in experimental protocols may also depend on: i) the
420 number of headspace samples taken during the enclosure period (Section 5.3.); ii) the
421 number and spacing of simultaneously deployed chambers; and iii) the number of field
422 operators.

423

424 **4.2. Approximating daily mean emissions**

425 Soil N_2O fluxes vary diurnally (Cardenas et al., submitted), but manual static
426 chambers can usually only be deployed once per day at best (both for practical reasons and
427 to avoid excessive disturbance; Sections 4.4. and 5.1.). Daily deployments therefore aim to
428 capture N_2O fluxes approximately equal to the daily mean. In the absence of transient fluxes
429 following a disturbance of soil N_2O producing processes (e.g. N application, soil tillage or
430 rainfall), fluxes are largely controlled by soil temperature (Livesley et al., 2008). Thus, NSS
431 chamber deployment at the time of the daily mean soil temperature (e.g. measured in the
432 plough layer at arable sites) will often capture the daily mean N_2O flux (Laville et al., 2011;
433 Supplementary Information Section 4). Alternatively, periodic measurements of the diurnal
434 pattern in soil N_2O emissions during an experiment are an adequate way to determine the

435 deployment time representative of daily mean N_2O fluxes. However, such measurements
436 have resource implications.
437 Smith and Dobbie (2001) reported deployments at 03:00, 11:00 and 19:00 yielded
438 fluxes similar to mean daily values, while estimates by Parkin (2008) at 06:00 and 12:00
439 were 14% lower and 8% greater, respectively, than daily means. Measurements by Alves et
440 al. (2012) in Scotland and Brazil suggested in both countries, despite the contrasting climatic
441 conditions, the times which best represented daily mean N_2O fluxes were 09:00 – 10:00 and
442 21:00 – 22:00. In a New Zealand study using near-continuous measurements of N_2O
443 emissions from urine patches, van der Weerden et al. (2013) found mean daily fluxes
444 occurred between 10:00 – 12:00 and 18:00 – 21:00. Recent work by Cardenas et al.
445 (submitted) based on the N_2O fluxes measured in three pastures over six years using
446 automated chambers, has indicated the mean time of the daily mean N_2O flux (across all
447 years, months and pasture types) was 09:00 or 21:00. A sampling time of 09:00 is earlier
448 than previously suggested (10:00 – 12:00) for N_2O sampling in temperate climates (Smith
449 and Dobbie, 2001; Parkin, 2008; Alves et al., 2012).
450 Most experimental designs and measurement protocols assume diurnal emissions
451 patterns are the same for all treatments and throughout the year, which may not be the
452 case. If treatments alter soil surface albedo or insulation, for example, the time of daily
453 minimum and maximum soil temperature near the surface soil will likely differ. Similarly,
454 placing N fertilisers at different depths can also produce different temporal patterns in
455 surface fluxes. Corrections can be made using 'flux vs. temperature' relationships but fully
456 accounting for these biases is difficult (Parkin and Kaspar, 2006).
457
458 **4.3. Temporal coverage**

459 Static chambers are deployed for short periods (<1 h) and typically sampled at
460 relatively long intervals (from 1 – 14 days). Therefore, they provide direct estimates of soil
461 N_2O fluxes for a very small fraction of the time over which they are intended to estimate the
462 cumulative emissions (month, season, year). Using 28 year-long autochamber datasets
463 spanning three continents (Europe, Asia and Australia), Barton et al. (2015) found daily
464 sampling was required to generate an estimate of annual N_2O emissions within 10% of the
465 best estimate for each dataset. As N_2O flux peak duration and chamber sampling frequency
466 decrease, the error associated with time-integrated emissions of a soil N_2O emission peak
467 will increase (Parkin, 2008). Maximum errors are observed when an emission peak occurs
468 between two consecutive deployments, and when infrequent measurements coincide with
469 short-lived peaks. Consequently, it is crucial to select an adequate number and time of
470 sampling events when linear interpolation is used to integrate emissions between sampling
471 points.

472 The maximum number of sampling dates during an experiment is finite, and depends
473 on available resources, number of chambers and the site characteristics (distance from the
474 laboratory, spatial arrangement of plots). Therefore, sampling frequency can vary from
475 daily, for simple experiments located at nearby sites, to weekly or longer for those at
476 remote locations. However, as the weighting of individual measurements increases as
477 sampling frequency lessens, intervals greater than 7 days are usually only appropriate when
478 conditions are conducive to near-zero fluxes (Parkin, 2008). This is most likely when soils
479 remain dry for long periods (e.g. during the summer in rainfed Mediterranean regions;
480 Sanchez-Martin et al., 2010), or cold for extended periods.

481 A fixed sampling interval is often used, but a better option is usually to vary the
482 frequency based on whether emission peaks are expected, e.g. due to triggers such as

rainfall or fertiliser application (Barton et al., 2015; Saha et al., 2017). If this approach is used, the whole 'envelope' of an N_2O emission peak (pre and post the event) should be captured to prevent overestimating cumulative fluxes. For example, where soils are irrigated in summer and evaporation and evapotranspiration rates are high, soil moisture in the top layers can fluctuate from dry to very wet to dry again and high N_2O sampling frequencies (depending on moisture loss rates but ideally daily until dry conditions are restored) are required to reduce bias in the total calculated emissions (e.g. Guardia et al., 2018; Supplementary Information Section 5). Similarly, despite cold conditions, freeze-thaw cycles can increase N_2O emissions and should be monitored (Ruan and Robertson, 2017). Rapid gas sample analysis allows responsive monitoring and helps determine when the sampling frequency can be reduced.

Finally, consideration should be given to whether conditions during the studied period were representative (e.g. of the season), and the number of replicate experiments over time/ different years required to accurately assess seasonal or annual emissions at that site. Differences in weather between years can affect N_2O emissions considerably, so EFs based on one year of measurements only may misrepresent emissions. Accordingly, journals are increasingly requiring more than one site year of N_2O flux data. Researchers should consider this in grant applications, experiment planning and overall use of the resulting emissions data, as single year measurements are still useful for model validation and in future meta-analyses (especially if appropriate meta-data are included in the study; de Klein et al., 2020b, this issue).

4.4. Duration of the experiment

In studies intended to quantify the emissions induced by a climatic event, agricultural practice (e.g. N fertiliser application) or experimental treatment (e.g. nitrification inhibitor or fertiliser form and application method), measurements should continue for as long as soil properties impacting on the N_2O emission are changed by the event/practice (to capture the entire treatment-induced 'emission envelope'). This can be achieved by continuing emission measurements until soil ammonium and nitrate concentrations in the treated soil are not statistically different from the control. Alternatively, Vangeli et al. (submitted) provides guidance on experiment duration by determining the minimum duration of measurements required to capture 90% of 365-day N_2O emissions from different excretal-N sources, using a database of spring, summer and autumn UK and Irish studies. On average, periods of 3, 5, 7 and 9 months were sufficient for urine, farmyard manure, dung and slurry treatments, respectively. The season of application did affect this average, however, with spring applications requiring the shortest duration of measurements and summer applications the longest.

If the measurements are to be used to determine EFs for soil N_2O inventories, they must ideally be taken over a year to comply with IPCC recommendations. There can be challenges in measuring fluxes over long periods, however. Soil compaction from repeated foot traffic next to the sampling sites can bias flux measurements by modifying gas production and vertical transfer (Section 5.1.). Additionally, sometimes soil conditions are not suited to NSS chamber use, such as during flooding or when covered by thick snow. The resulting gaps in the coverage of annual emissions must then be estimated by other means, for example, by using a gap filling approach (Dorich et al., 2020, this issue).

5. Practical recommendations for experiment design and chamber deployment

530 **5.1. Chamber installation and site disturbance**

531 Static chamber base installation causes soil disturbance, which may impact gas
532 emissions (Matthias et al., 1978; Norman et al., 1992). Bases should be installed long
533 enough before chamber deployments to allow for soil and crop conditions to return to a
534 steady state approximating undisturbed conditions. On bare soil, this might take as little as
535 one hour for coarse-textured soils, or a few days for clay soils (Rochette et al., 2012).
536 Pavelka et al. (2018) recommend installation at least 24 hours prior to the first N_2O flux
537 measurement.

538 Base installation in vegetated areas often damages roots, so several days, perhaps
539 weeks (even months) will be required to allow root regrowth (Rochette and Hutchinson,
540 2005). This will avoid any potential impact of root death, which will disrupt C and N-cycling
541 and affect N_2O production in the soil profile. This is important if the study aims to assess the
542 effects of root C leakage on N_2O emissions (e.g. Luo et al., 2018). Otherwise, shallower wall
543 insertions may be needed, (such as in forest ecosystems; Pavelka et al., 2018) but only if
544 other criteria for good design and deployment are used (Clough et al., 2020, this issue).
545 Alternatively, control treatments experiencing the same root damage effects can be used to
546 exclude this factor from the assessment of treatment effects.

547 For annual crops, bases should ideally be installed either shortly after sowing, to
548 allow roots to grow within the inner area, or between the rows, depending on the research
549 question. Chamber extensions are usually used to keep the crop within the chamber height,
550 but this can reduce sensitivity in detecting N_2O emissions and chamber closure periods
551 often need to be extended, which has some disadvantages (Section 4.1.). Additionally, farm
552 activities (e.g. cultivation, drilling, reseeding, fertiliser application etc.) may require
553 temporary chamber/base removal. Accordingly, it is recommended exact chamber locations

554 are recorded (e.g. using a GPS) to enable same-location re-installation post activity for
555 consistency. Even if chambers are unlikely to be removed and replaced, recording exact
556 locations is good practice and may later be useful for comparisons between years at that
557 site.

558 Soil water content can impact chamber performance in several ways. Researchers
559 walking around the chambers, especially in very wet conditions, can displace soil gases as
560 well as compact the soil. For this reason, when chambers are located on a slope, it is
561 advisable chambers are accessed from the downslope position to minimise the impact of
562 sampling on the chamber soil conditions. Sampling in wet conditions can disturb the soil and
563 modify N_2O production and vertical transport. Walking boards reduce this but sampling NSS
564 chambers in saturated soil often causes site deterioration that requires bases must be
565 relocated. The implications of this for subsequent data analysis must be considered. Bases
566 may also affect lateral surface water flow, and they should be relocated when soil water
567 content differs from surroundings (Rochette and Bertrand, 2008). In paddy fields, where
568 saturated conditions are the norm, wooden access bridges have been used (Bertora et al.,
569 2018). Finally, under very dry conditions, clay soils may shrink away from the edge of the
570 chamber base. In such circumstances, researchers should loosen and tamp down the soil at
571 the outer edge of the base prior to measurement to fill the gap and improve the seal
572 between the soil and the base.

573

574 **5.2. Sequence and grouping of chamber measurements**

575 Grouping and sequence of chamber measurements vary depending on deployment
576 duration, experimental design and human resources. The number of chambers which can be
577 handled by one operator increases with deployment duration but decreases with the

number of headspace samples and distance between bases. Chamber size and height, or stacking requirement (tall crops), may also impact on the number of chambers an operator can handle safely and competently. The time interval between sampling two chambers varies, depending on their location, but it is usually ≥60 s. Where an operator samples a different chamber every minute, the four air samples (at 0, 8, 16, 24 min) for eight chambers will be completed in 32 min.

For experiments with treatment replicates (or blocks), a full set of each of the different treatments (i.e. replicate one of treatments A, B and C, or one whole block) should be sampled as a group in as short a period as possible, before moving on to sample the second replicate of each treatment (or the next block). This reduces differences between treatments or within blocks due to sampling time and facilitates statistical analysis. The sampling sequence should also vary between sampling dates (e.g. the next day start with replicate two of treatments A, B and C, or block two), to avoid any potential bias from always sampling in a particular order. This is also avoided through multiple operators for chamber sampling (e.g. one per block), as they can each measure a different block at the same time. Increasing the number of operators is also useful for larger experiments. In both cases, however, training is required to ensure the same sampling protocol is used by all operators.

5.3. Headspace air sampling

When deploying chambers for measuring N_2O emissions, it is important to determine the requisite number of headspace samples to provide the least biased flux estimate (Venterea et al., 2020, this issue). The more headspace samples taken, the better the characterisation of N_2O accumulation and thus, the less biased each individual flux

estimate. However, resources are finite and excessive headspace samplings from a small chamber may induce unwanted effects.

Rochette (2011) proposed ≥4 air samples should be taken during static chamber deployment, to adequately assess the quality of the calculated flux (detection of outliers and technical problems during handling and analysis of samples), and to account for the increased likelihood of a non-linear N_2O flux with increasing deployment time. Venterea et al. (2020, this issue) similarly advocate for the collection of ≥4 headspace samples *alongside soil data*. In this paper, we reinforce this recommendation, but also acknowledge a less intensive chamber headspace sampling protocol may be acceptable for certain situations. An analysis by Levy et al. (2011) suggested prioritising the number of headspace samples per chamber, rather than the number of chambers, improved estimation of the mean flux at that point in time. In addition, Lammirato et al. (2018) suggested since reducing the number of headspace samples increases the uncertainty of the estimated flux and the detection limit, it may not be appropriate to reduce the number of headspace samples when very low (near baseline) fluxes are expected. Subsequently, Jungkunst et al. (2018) concluded while the above holds for shorter term studies, longer term studies (e.g. annual budgets) or those with high spatial heterogeneity (e.g. within-treatment variability is similar to between treatment variability) may benefit from better spatial coverage (Section 3.2.) with fewer headspace sampling points. Moreover, very low fluxes do not contribute greatly to annual budgets, so the additional uncertainty associated with them may not be important. Any consideration regarding reducing headspace sampling intensity should be based on minimising the overall uncertainty of the N_2O emission estimate.

Venterea et al. (2020, this issue) provide guidance on the selection of flux-calculation method depending on the number of headspace samples available, and the relative

626 favourability of sampling options where ≥4 headspace samples, plus soil data, cannot be
627 achieved. If fewer (2 – 3) headspace samples are taken, it is essential to quantify any
628 potential bias introduced. This can be done by taking a random subset of chambers on each
629 sampling occasion and conducting ≥4 headspace samples during the two- or three-point
630 sampling strategy (e.g. Cardenas et al., 2010). Treatment effects (e.g. different application
631 methods or high N application rates) do not seem to alter the tendency for linearity
632 (Pedersen et al., 2010; Chadwick et al., 2014) so a random subset of chambers should be
633 used for this assessment. Each dataset of ≥4 headspace samples should be statistically
634 analysed to determine (non-) linearity. Researchers should summarise this information,
635 provide a percentage of cases when linearity was observed and cite this alongside their
636 calculated flux (Chadwick et al., 2014; Thorman et al., 2020). This provides an indication of
637 the bias in the results which may have been introduced by assuming linearity in the flux
638 calculations. In the analysis of 1970 chamber measurements with ≥4 headspace samples
639 over a 40 – 60-min closure period from nine UK studies (27 experimental treatments),
640 Chadwick et al. (2014) found on average, only 8% increased non-linearly (varying from 0 –
641 22% of measurements by site, or 0 – 14% where measurements with no net flux due to dry
642 soil conditions were excluded). The level of bias can be quantified as in Venterea et al.
643 (2020, this issue) by calculating the N_2O fluxes of the subset of chambers where ≥4
644 headspace samples were taken using the most appropriate non-linear scheme and
645 comparing them with fluxes calculated from the same chambers using only three headspace
646 sampling points and linear regressions.
647

648 **5.3.1. First air sample (t_0)**

649 Estimation of unbiased fluxes requires the change in chamber headspace N_2O
650 concentrations over time (dC/dt) to be determined within the chamber, so the initial (t_0)
651 chamber headspace N_2O concentration should be sampled immediately after deployment.
652 There is some evidence, however, for typical field flux measurements, individual chamber t_0
653 N_2O concentrations are indistinguishable from ambient air concentrations (or indeed one
654 another), and ambient air samples taken at mid-chamber height can be used instead of
655 individual t_0 samples (Chadwick et al., 2014). In addition, Chadwick et al. (2014) found that
656 across eight sites, where t_0 and ambient N_2O concentrations where significantly different,
657 this strongly affected resulting fluxes (calculated by linear regression) at only two of the
658 eight sites (with three sites showing small but significant differences and the final three, no
659 significant differences). Underlying reasons for the different effects at these sites was not
660 investigated.
661 Indeed, further investigation is required to better ascertain why (and therefore
662 when) ambient N_2O concentrations will be significantly different from t_0 concentrations.
663 Consistency may be challenged by weather conditions that prevent N_2O produced in the soil
664 from mixing with the atmosphere. In the absence of wind to remove N_2O accumulating at or
665 immediately below the soil surface, the t_0 headspace sample may be above ambient N_2O
666 concentrations, especially if the chamber contains a fan promoting headspace mixing. An
667 example of such accumulation during night-time is shown in Fig. 1 for a 24-hour
668 measurement period with automated chambers (data from Petersen et al., 2020). According
669 to Fig. 1, the t_0 samples were in fact near ambient level around mid-morning, when manual
670 static chamber gas sampling typically takes place (Section 4.2.). Interestingly, wind velocities
671 at 2 m height remained at 0 – 2 m s^{-1} also during the day, whereas air temperature
672 fluctuated between 3 and 16.6 °C. It suggests that cooling can contribute to the

673 development of a layer of (heavier) stagnant air at the soil surface where N_2O may be
674 trapped.

675 Re-prioritisation of resources to better capture spatial and temporal variability may
676 be effective in reducing the overall uncertainty of N_2O emission estimates. However, several
677 precautions are necessary: i) the N_2O concentration above the soil may be influenced by the
678 soil N_2O fluxes, so ambient air samples from above each plot should only be used as t_0
679 estimates for chambers placed on that plot; ii) permanently-inserted bases should be low so
680 they do not restrict lateral air flow and mixing of air in the chamber area; iii) similarly,
681 growing vegetation may reduce ambient air mixing; iv) sampling time of day to approximate
682 daily mean N_2O emissions should also consider the impact of time of day on t_0 cf. ambient
683 air N_2O concentrations; and v) ideally adequate testing should be conducted to show there
684 is no significant difference between individual chamber t_0 N_2O concentrations and ambient
685 air samples, noting that this difference may vary with weather conditions. If individual
686 chamber headspace t_0 concentrations are proportional to N_2O fluxes, however, using a
687 single ambient air N_2O concentration for a group of chambers will produce an
688 underestimate of lower fluxes, and an overestimate of higher fluxes.
689

690 **5.4. Ancillary measurements**

691 The need for additional measurements depends upon the experiment objective(s).
692 Recommended best practice for the calculation of N_2O fluxes from individual chambers
693 requires measurements of soil moisture, bulk density and temperature to allow for
694 application of the chamber bias correction (CBC) method (Venterea et al., 2020, this issue).
695 The CBC method has the potential to improve flux estimate accuracy and precision
696 depending on other factors, and its potential performance can be assessed using methods

described by Venterea et al. (2020, this issue). If the aim is to generate new N_2O EFs, soil mineral N contents are usually recorded, but may not be necessary (López-Aizpún et al., 2020). A recommended minimum set of ancillary measurements for N_2O EF studies would improve the potential for subsequent meta-analyses (de Klein et al., 2020b, this issue; López-Aizpún et al., 2020). If the goal is to understand temporal patterns in N_2O emissions, or for model development or verification, then a wider range of (frequent) ancillary measurements are necessary (Giltrap et al., 2020, Dorich et al., 2020 and de Klein et al., 2020b, this issue).

Soil N_2O production, reduction and transport depends on the availability of C and N substrates (Loick et al., 2017), gas diffusivity (Bateman and Baggs, 2005) and redox potential (Rubol et al., 2012). To understand and predict N_2O net production processes and emission rates, therefore, these controlling parameters should be monitored during soil N_2O flux studies. However, different ancillary measurements will be required at different frequencies. Soil bulk density, pH, organic C and total N content usually need to be measured only infrequently, e.g. once per experiment, once per season, or following an expected significant change, such as cultivation. Average soil and air temperature, and rainfall should be measured on a daily or hourly basis, and soil WFPS at daily or weekly intervals - as often as needed to provide a representative estimate of the chamber soil conditions on each gas sampling occasion. Automated sensors placed in each chamber are advantageous in providing high frequency and resolution data and the use of sensors for air and soil temperature and soil moisture are recommended (Pavelka et al., 2018). Soil mineral N measurements are needed as often as resources allow, especially during the first 30 days after fertiliser, manure or urine application (and will inevitably include soil moisture content determinations).

721 The spatial scale of variation of each ancillary variable will also differ and samples
722 representative of conditions for each chamber should ideally be collected (i.e. some
723 variables may be consistent across the block scale, while others may vary at the within-plot
724 scale). Care should be taken to ensure destructive sampling areas (often near chambers for
725 comparable data) are large enough for the required number of samples to be taken, without
726 the structure or hydraulic properties of the soil near the NSS chamber being altered (Section
727 4.4. and 5.1.). The use of small non-destructive soil moisture, temperature and nitrate
728 sensors/samplers inserted within chambers represents an advantage in this respect, as well
729 as providing chamber specific, high frequency ancillary data (Supplementary Information
730 Section 6). Intermittent spot-checking or validation of sensor data via established
731 destructive methods may be worthwhile.
732
733 **6. Conclusion**
734 Obtaining accurate and precise soil N_2O emission estimates using small static
735 chambers is challenging due to the high spatial variability and episodic nature of soil N_2O
736 fluxes. Experimental design and chamber deployment protocols must consider all sources of
737 uncertainty (spatial, temporal and experimental) associated with N_2O fluxes and prioritise
738 resources effectively to minimise overall uncertainty based on the experiment objectives
739 (Supplementary Information Section 7). For some small-scale experiments, this may mean
740 focusing resources on determining individual chamber N_2O emission estimates, while for
741 spatial variability assessments and integrations, a greater number of chambers, better
742 capturing spatial variability and sampled less intensively over a longer period with a simpler
743 individual chamber protocol (e.g. Chadwick et al., 2014) could be more appropriate.
744

Acknowledgments

This work was funded by Global Research Alliance (GRA) Secretariat SCF0105. AFC, DRC, RET and LMC thank Defra for supporting the UK contribution to this paper. The authors wish to thank Surinder Saggar for reviewing all the papers in this special issue. We are also thankful for support for the publishing costs from the New Zealand Government, in support of the objectives of the Livestock Research Group of the Global Research Alliance on Agricultural Greenhouse Gases.

Conflict of Interest Statement

The authors confirm that there are no conflicts of interest.

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1013

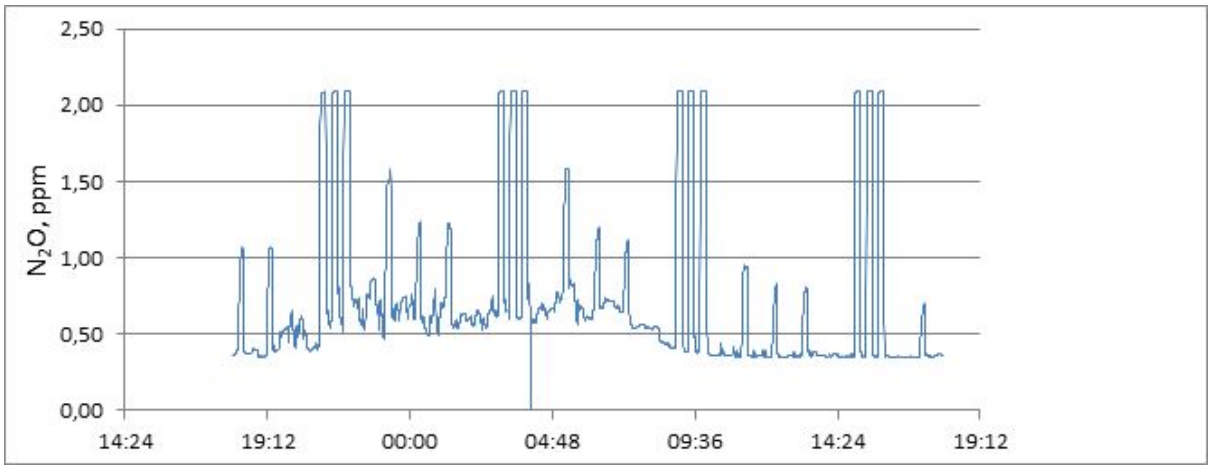
1014 **Figure Caption**

1015 **Figure 1:** Example of ambient N_2O concentrations over a 24-hour period from a field study
1016 of N_2O emissions at a raised bog in northern Denmark (Store Vildmose) drained for
1017 agriculture. The data show high background air concentrations of N_2O through the night-
1018 time, which interfered with flux measurements during that period, and which were
1019 subsequently discarded. The analytical setup included a LI-8100A automated soil gas flux
1020 system (LI-COR Ltd., Cambridge, UK) interfaced with a N_2O Isotope Analyzer (Los Gatos
1021 Research, Mountain View, CA). A reference gas was analysed between six-hourly cycles.
1022 Data from: S.O. Petersen (pers. comm.).

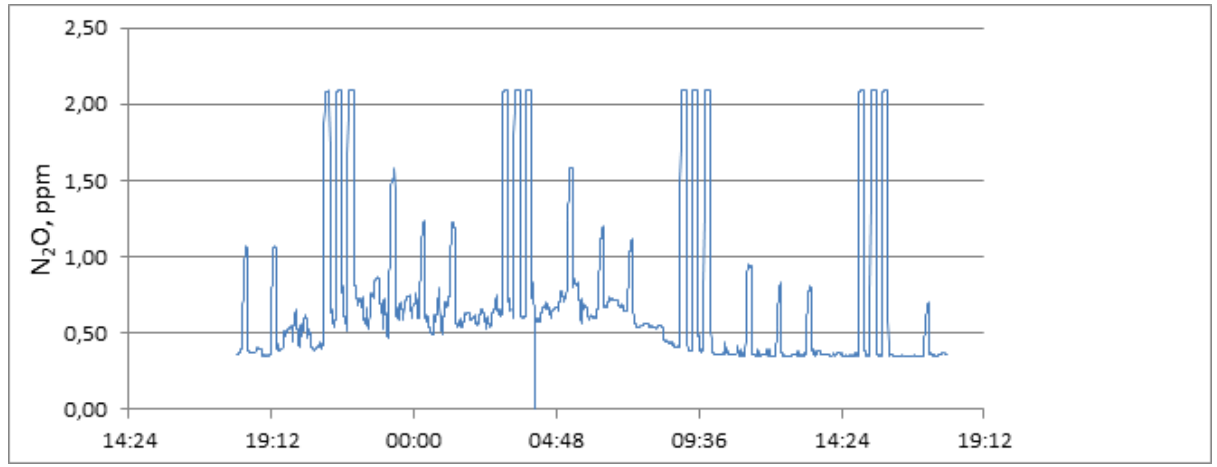
1023

1024

1025 **Figure 1**



1026


2,50
2,00
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N_2O, ppm
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Supplementary Information

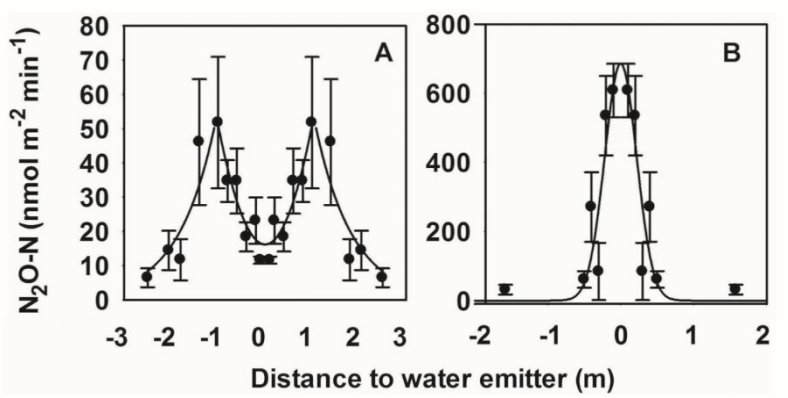
1. Additional site selection considerations for grazed pastures

For example, bovine urine has been found to alter the soil microbial community, significantly increasing the abundance of *amo*A 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 N_2O 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

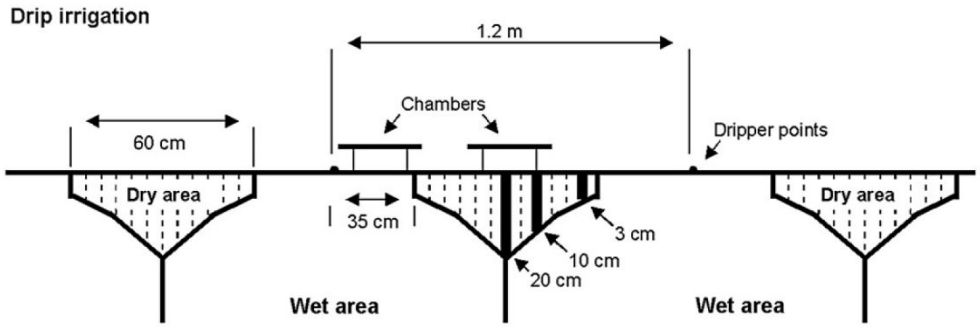
subsurface drip irrigators. The system used thus strongly affects the spatial and temporal variability of soil moisture contents, and consequently, of N_2O fluxes. This must be captured by the experimental design chamber layout (and sampling frequency; Section 4.3. in main text).



Supplementary Figure 1. N_2O 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 N_2O 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

N_2O 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 N_2O sampling. (Adapted from Sánchez-Martín et al., 2008).

Overall, a gradient in N_2O 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 N_2O fluxes were weighted by the relative proportions of each area.

3. Strategic chamber placement and calculating N_2O emissions from grazed pastures

In grazed pasture systems, where the majority of the N_2O 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:

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

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 N_2O within hours to days (Bergstermann et al., 2011), which are often quantitatively important in terms of cumulative annual N_2O 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 N_2O 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 N_2O emission experiment types: i) experiments to investigate representative N_2O 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 N_2O 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 N_2O 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).

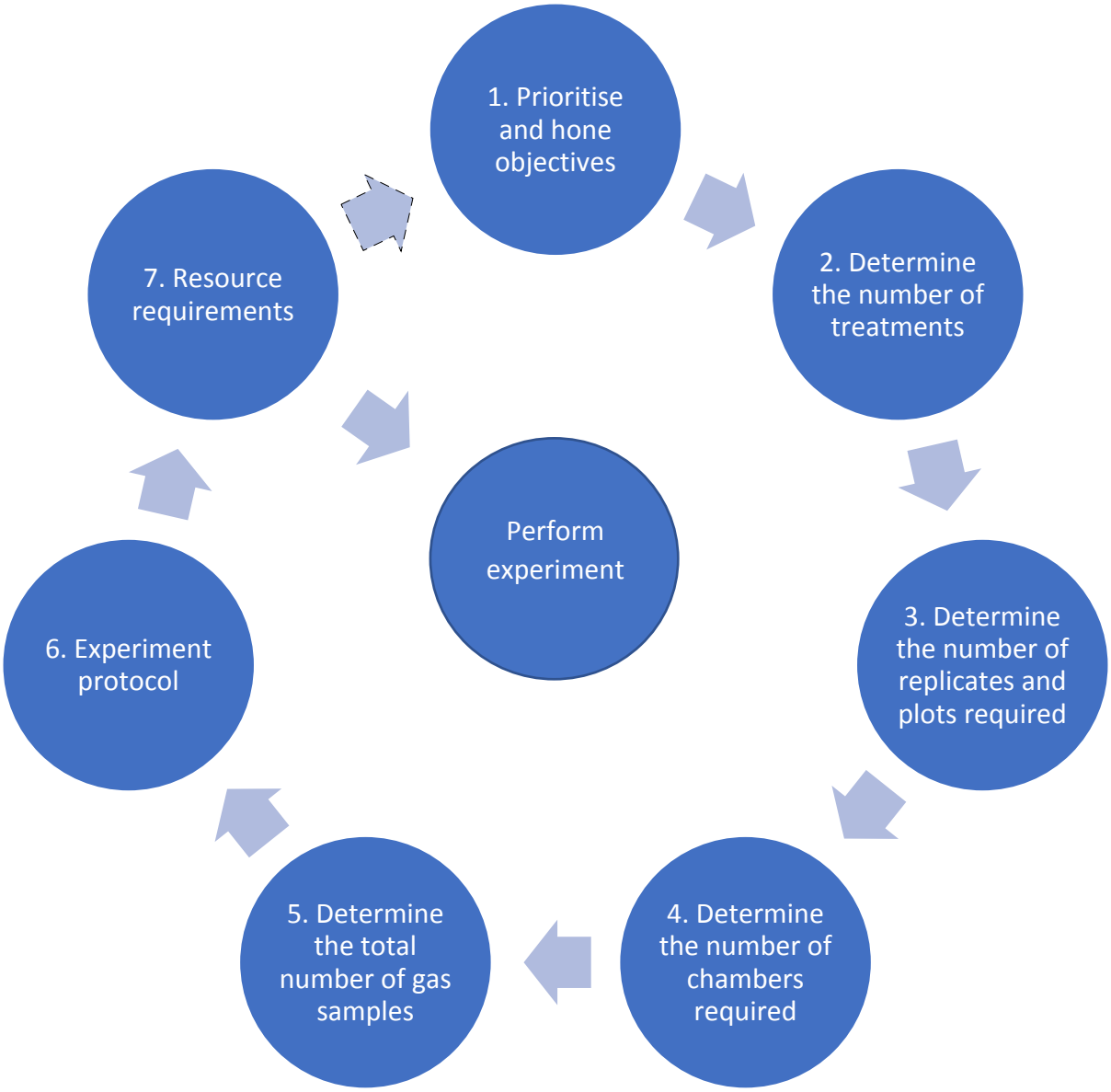
1. Prioritise and hone objectives:
    - Identify key amendment(s)/practice(s).
    - For what scale/situation does the experiment aim to generate representative EFs (e.g. national/regional/local; land use type/management [& historic]; soil type; topography/slope/aspect, etc.). Are appropriate sites 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 N_2O 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 N_2O 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 N_2O 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 N_2O 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 N_2O emissions at larger scales, measurement techniques that integrate N_2O emissions over larger scales micrometeorological methods [e.g. eddy covariance] are more appropriate). The temporal

variability of N_2O 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 N_2O 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 N_2O emissions from spatially distinct areas/ features? Generate a representative aggregated N_2O 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 N_2O at different scales across the field (Charteris et al., in prep.)

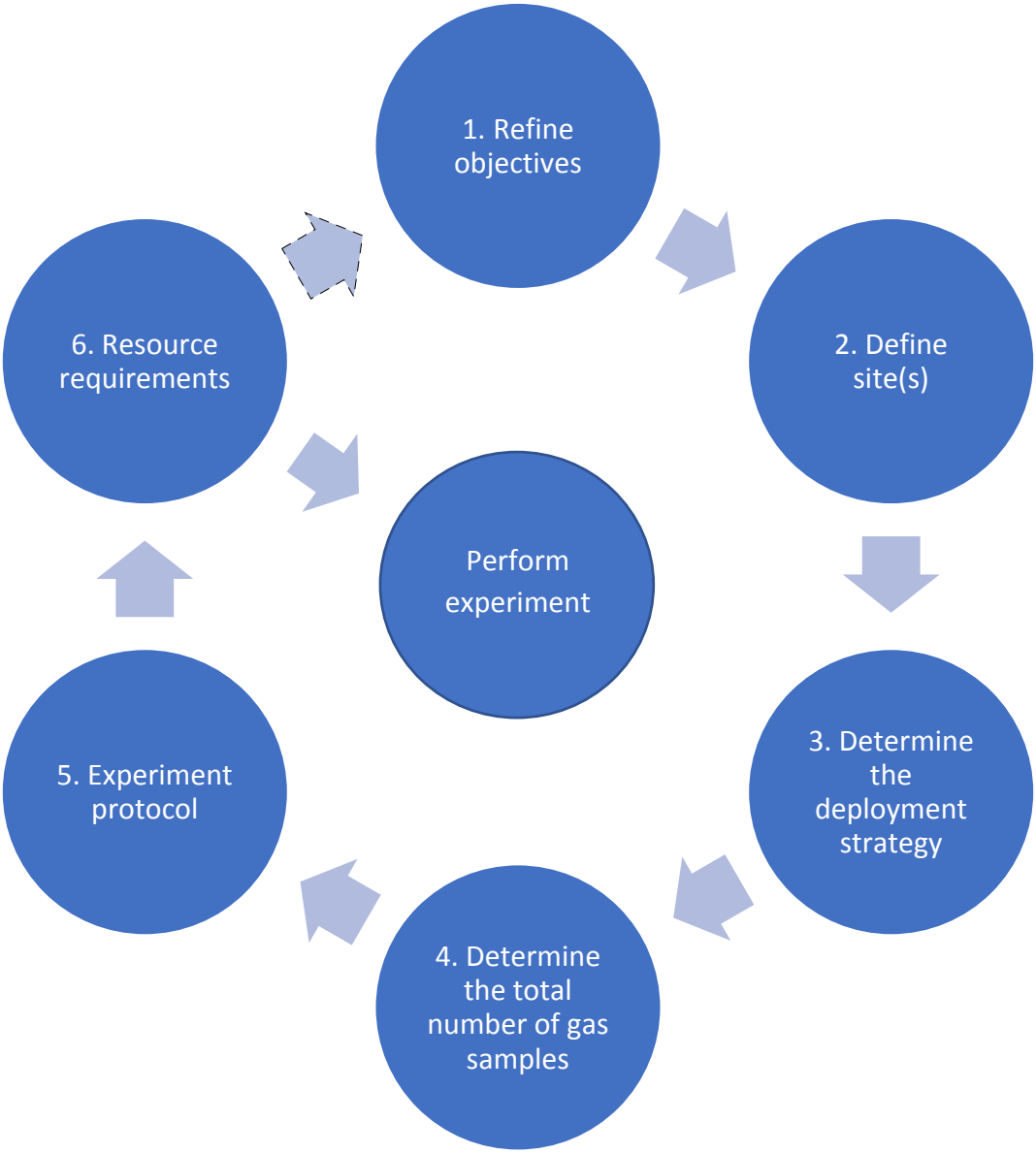
- What size and type of static chambers are available/ best (Section 3.3. in main text; Clough et al. this issue)?
- How many spatial sampling points/static chambers are needed to cover a sufficient proportion of the field to generate representative aggregated emissions (Section 3.2. in main text)?

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 N_2O 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 N_2O using static chambers.

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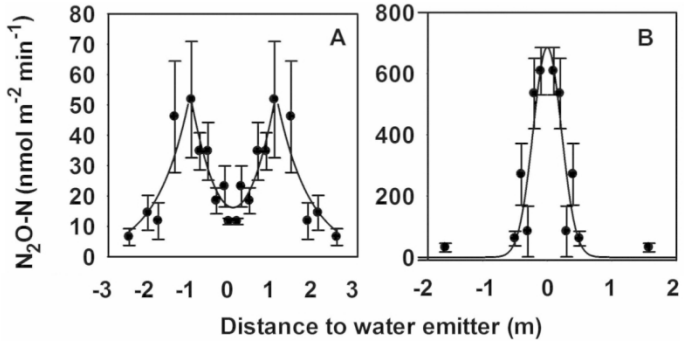

Supplementary Figure 1. N_2O fluxes with distance from the source on one sampling day for A) microsprinkler irrigation, and B) drip irrigation. Error bars represent the standard error of the mean of replicates in a randomized complete blocks design (SEM; n=3). (Adapted from Alsina et al., 2013).

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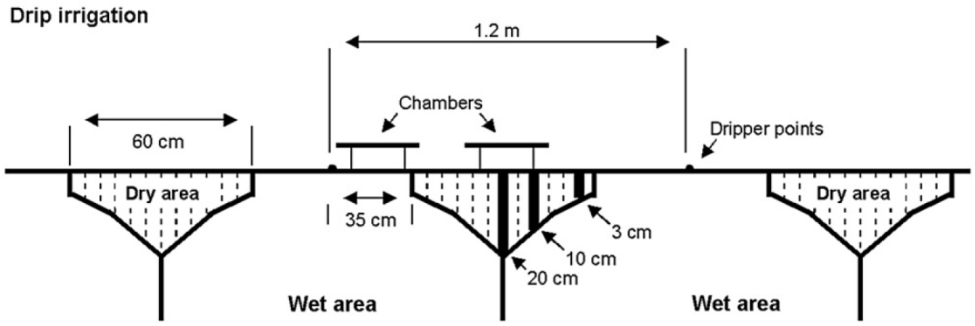

Supplementary Figure 2. Schematic distribution of wet and dry areas for drip irrigation systems showing the location of static chambers for soil N_2O sampling. (Adapted from Sánchez-Martín et al., 2008).

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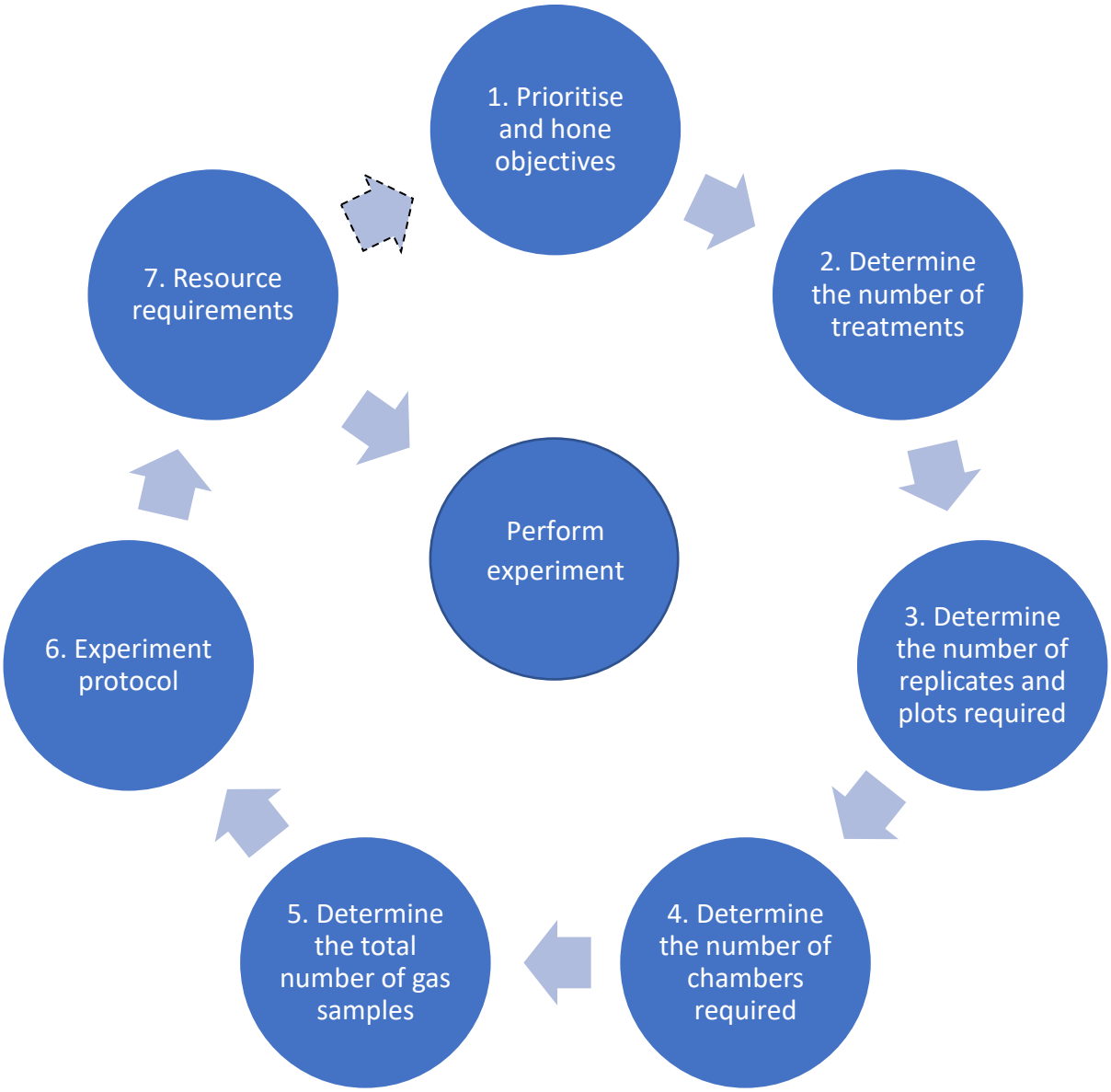
1. Prioritise and hone objectives
2. Determine the number of treatments
3. Determine the number of replicates and plots required
4. Determine the number of chambers required
5. Determine the total number of gas samples
6. Experiment protocol
7. Resource requirements
Perform experiment


1. Refine objectives
2. Define site(s)
3. Determine the deployment strategy
4. Determine the total number of gas samples
5. Experiment protocol
6. Resource requirements
Perform experiment