Section 5: Project Description

1. **Project Objectives:**

(1) Quantify baseline GHG (carbon dioxide, methane, and nitrous oxide) fluxes and carbon storage in a partially-denuded Cascade Range mountain meadow (Rock Creek Meadow) before the meadow is restored.

(2) Evaluate the potential effects of climatic warming on meadow function by studying how soil moisture affects GHG fluxes and carbon storage in six diverse biocommunities (non-native herb/grassland, riparian scrub wetland, riparian wetland, seasonal wetland, spring/seep wetland, and ponderosa pine/mixed oak woodlands) within Rock Creek Meadow.

(3) Restore approximately 650 feet of Rock Creek stream channel to pre-disturbance conditions, and improve the efficiency of the water supply for CDFW's Crystal Lake Hatchery.

(4) Compare GHG flux rates and total soil carbon in the restored Rock Creek Meadow to baseline data and determine if the meadow restoration results in a net change in GHG fluxes and/or carbon storage.

Co-benefits:
The restored stream channel will provide approximately 13,550 square feet (1259 square meters) of habitat for the federally and state-listed endangered Shasta crayfish (*Pacifastacus fortis*), while maintaining the quality and improving the efficiency of the water supply for CDFW's Crystal Lake Hatchery.

2. ***Background and Conceptual Models:***

Existing Conditions

The upper Rock Creek Meadow is located in northeastern California in the southern Cascade Range Mountains near Lassen Volcanic National Park, at the southwestern edge of the Modoc Plateau. The region is dominated by volcanic landforms and spring-dominated waterways. The stream channel that flows through the meadow (Rock Creek) is a natural spring-fed creek 0.9 miles (1.4 kilometers) long that flows subsurface at various locations between its headwaters and its confluence with Baum Lake, which is an impounded reach of Hat Creek (Figure 3). Although it is not currently gaged, measurements indicate that Rock Creek discharge is approximately 19 cubic feet per second (cfs). Most of the flow originates from Rock Spring and two spring-fed pools (Kerns Pond and Mancuso's Pond) near Cassel-Fall River Road (see Figure 3), as well as other smaller springs in the upper, steeper portion of the drainage.

Where the drainage basin flattens and the upper meadow begins (roughly 0.4 miles downslope from Rock Spring), the Crystal Lake Hatchery diverts all the flow from Rock Creek through a 0.6-mile long pipeline to the hatchery. Substantial leaks (totaling approximately 0.5 cfs) at various locations along the pipeline and additional groundwater accretion saturate the soil between the stream channel and the pipeline. Due to these and other water inputs, the mostly dewatered channel of Rock Creek (below the hatchery diversion) still maintains a summer base flow of approximately 0.5 cfs. Marshy islands and fine organic substrates are more prevalent in the remnant, low-flow channel below the hatchery diversion than in the full-flow channel above the diversion.

A Wetlands Delineation for Rock Creek Meadow was completed during August 2011. The Wetlands Delineation Report, included as Attachment A, describes existing water resource and vegetation conditions within the Rock Creek Meadow Restoration Area. The Rock Creek Diversion Hydrologic Assessment, included as Attachment B, describes the Hydrogeologic Setting.

Rock Creek Restoration for Reintroduction of Shasta Crayfish

Rock Creek is within the native range of Shasta crayfish (*Pacifastacus fortis*), a species listed as endangered under the federal and state Endangered Species Acts (ESA). Shasta crayfish inhabited Rock Creek prior to its diversion in 1950 to supply water to CDFW's Crystal Lake Hatchery. The Rock Creek Meadow Restoration and Reintroduction Plan (Plan), which is provided in Attachment C, was developed to restore the Rock Creek stream channel and surrounding meadow to pre-diversion conditions and reintroduce Shasta crayfish to the restored channel.

Engineering design alternatives for the restoration work were reviewed by the Shasta Crayfish Technical Review Committee (which includes Spring Rivers Foundation, PG&E, USFWS, CDFW, and others) during 2013, and a preferred design alternative was selected. The Plan, which was finalized in October 2014, includes the 20% design of the preferred alternative. The USFWS, in consultation with the Shasta Crayfish Technical Review Committee (TRC), will be the project lead for implementation of the Restoration Plan. USFWS will be the lead for National Environmental Protection Agency (NEPA) compliance and consultation with Army Corps of Engineers (ACOE). CDFW will be the lead for

California Environmental Quality Act (CEQA) compliance. As the landowner, PG&E will submit the applications for change in point of diversion and the water quality certification to the State Water Resources Control Board

Cost estimates and potential funding for the restoration work is summarized in Section 9 of the attached Plan. The Hydrologic Assessment, Wetlands Delineation, Work Plan Development, and 20% Design have been completed. Additional monies are needed to bring the engineering design to 100%, conduct final botanical surveys, complete permitting, complete hatchery modifications including relocation of hatchery diversion structure with self-cleaning intake screen, and to complete Rock Creek channel restoration.

GHG and Carbon Storage Research

To address greenhouse gas (GHG) emissions, it is important not only to quantify the current magnitude of natural sources, but also to understand how human activities and climate change affect emissions from these sources (EPA 2010). The planned Rock Creek Meadow Restoration offers a unique opportunity to quantify GHG fluxes and carbon storage in a small mountain meadow, and to determine if modifications to the meadow habitat (stream channel restoration) result in a net change in GHG fluxes and/or carbon storage.

Changes in land use and environmental variables, such as soil moisture, can affect mountain meadow function and ecosystem services. In Rock Creek Meadow, there may be a greater amount of saturated organic soils than existed under pre-diversion conditions, because of the substantial amount of encroachment of organic material between the previously wetted channels edge and the present-day low-flow channel edge. Wetter meadow habitats can emit more carbon dioxide than drier upland habitats (Riveros-Iregui and McGlynn, 2009), and can be significant sources of methane and nitrous oxide due to high organic matter content (Hörtnagl and Wohlfahrt 2014). Some studies have also found that methane uptake is greater in drier meadows or drier portions of meadows (Blankenship and Hart 2014, Koch et al. 2007). While wetter meadows may produce more GHG emissions than drier meadows under certain environmental conditions, they can also sequester substantially more carbon than drier upland habitats (Norton et al. 2014). Understanding the relative importance of soil moisture and other environmental variables on GHG fluxes and carbon storage is crucial for determining how climatic warming may affect mountain meadow function and ecosystem services.

Under the Rock Creek Meadow Restoration and Reintroduction Plan for Shasta Crayfish (Attachment C), 650 feet of the stream channel and surrounding meadow will be restored to pre-diversion conditions. The restoration work may affect the overall volume of saturated organic soils and/or the volumetric water content (VWC) in the riparian wetlands in upper Rock Creek Meadow. The overall changes are not certain, however, as the width and depth of the channel will increase and shift the area of saturation to the new channel margins. Additionally, the geomorphic channel restoration design includes the removal of organic sod material that encroached between the historic and the low-flow channel edges and repositioning the sod along the restored channel margins. Although soil moisture is only one of many environmental variables known to affect GHG production/uptake in mountain meadows, this study of Rock Creek Meadow will investigate if and how changes in meadow habitat, including changes in VWC, affect GHG fluxes and carbon storage.

3. ***Detailed project description, including all tasks to be performed:***

Baseline Data Collection (Project Objectives 1 and 2)

The upper portion of Rock Creek Meadow is approximately 250 feet wide and includes six terrestrial biocommunities (Non-Native Herb/Grassland, Riparian Scrub Wetland, Wetland, Seasonal Wetland, Spring/Seep Wetland, and Ponderosa Pine/Mixed Oak Woodland).

We propose to measure field fluxes of carbon dioxide, methane, and nitrous oxide, as well as soil total carbon and nitrogen within each biocommunity by establishing sampling transects perpendicular to Rock Creek that will be strategically placed to encompass all six biocommunities. We will measure field gas fluxes, plant species richness, and soil variables (total carbon, total nitrogen, volumetric water content (VWC), and temperature) at 60 locations (10 locations across 6 transects) during July or August 2015.

Gas flux measurements will be made using the Static Chamber Technique (Livingston and Hutchinson 1995), following methods described in Blankenship and Hart (2014). Gas flux chambers will have a volume greater than 0.015 m^3 (Pihlatie et al. 2013), and will be made from PVC or stainless steel. Four headspace gas samples (18 ml) will be collected 0, 20, 40, and 60 minutes after the chamber top is sealed using a polyethylene syringe and 20-gauge needle. A vent tube will be used to reduce air disturbance during sampling. The gas samples will be injected into 12-ml evacuated glass vials with rubber septa until laboratory analysis on a gas chromatograph. A simultaneous analysis method will be used to determine the concentrations of carbon dioxide, methane, and nitrous oxide in each sample. We plan on sending gas headspace samples to UC Davis or a private analytical lab for analysis.

Soil samples will be collected near each gas sample location immediately after gas sampling using a field soil probe. Soil samples will be sent to the UC Davis Analytical Lab for analysis of total carbon, total nitrogen, and chemical analysis.

Spring Rivers Foundation, working with of Spring Rivers Ecological Sciences LLC, will be responsible for data collection, data analysis, and report preparation. Personnel will include Maria Ellis, Lorrie Haley, Jeff Cook, Lauren Bridgeman, Koen Breedveld, Mark Stalcup, and Steve Breth.

Rock Creek Meadow Restoration (Project Objective 3)

The restoration phase of the Plan will restore and rewater the approximately 650-foot-long Upper Rock Creek Meadow channel to create continuous Shasta crayfish habitat throughout the Upper Rock Creek Meadow upstream of the new hatchery intake structure. In addition to ensuring that both the quality and quantity of CDFW’s water needs for the Crystal Lake Hatchery continue to be met, implementation of the Plan will also provide the hatchery with an improved water diversion and supply system. The restoration of the Upper Rock Creek Meadow as shown in the 20% design drawings (Figure 10 of Attachment C) involves the following steps.

1) Reconstruct the Upper Rock Creek Meadow by removing encroached vegetation and sod along the banks.
2) Construct a new hatchery diversion structure and water supply intake structure at the lower end of Upper Rock Creek Meadow approximately 650 feet downstream of the current diversion.

3) Remove the old hatchery diversion structure and water supply line from the restoration area and rewater of the Upper Rock Creek Meadow.

An Engineering firm, which will be chosen by CDFW and partially funded with Section 6 Funding ($90,000), will bring the engineering design from 20% to 100%. John Dittes of Dittes and Guardino Consulting will conduct the final botanical surveys in spring 2015. Spring Rivers personnel (Maria Ellis, Ph.D. and Jeffrey Cook) will assist USFWS and PG&E in completing necessary permitting. A contract engineer will oversee the hatchery modifications including construction of a new hatchery diversion structure with self-cleaning intake screen. Rick Poore of Streamwise, a restoration construction specialist, will complete the Rock Creek channel restoration and construction of the runoff channel. In addition to the Section 6 Funding, there is $245,000 in USFWS Recovery funding and $25,000 in USFWS Partners for Fish and Wildlife Funding that will be used as Partners Cost Share for the Project, including the Final Engineering Design.

After construction and habitat restoration has been completed, and the stream channel has been rewatered, adequate time will be allowed for the restored and rewatered channel to equilibrate to the new conditions. The riparian corridor will be revegetated during the restoration phase by sod matting and willow staking along the new/restored channel bank. The meadow channel will be monitored to ensure that revegetation (done during the restoration phase) has been effective, i.e., that plants, willow stakes, and sod-mats have survived. Monitoring will also ensure that newly created aquatic habitat is recolonized by native aquatic invertebrates and aquatic vegetation before Shasta crayfish reintroduction can begin.

Details of the Rock Creek Meadow restoration work, including a description of the agencies/personnel and contractors responsible for implementing the work, are described in the Rock Creek Meadow Restoration and Reintroduction Plan for Shasta Crayfish (Attachment C).

Post-restoration Data Collection (Project Objective 4)

During the first and second summers after the revegetation phase of the restoration work is completed, the 60 locations sampled during baseline data collection will be resampled to evaluate the effects of the restoration on GHG fluxes and carbon storage.

Means and standard errors of soil volumetric water content, soil temperature, carbon dioxide flux, methane flux, and nitrous oxide flux will be calculated for each location and sampling date. Differences between sampling dates (i.e., before and after restoration) will be statistically compared using one-way Analysis of Variance. The direction and rate of gas fluxes at each sample location will be determined using linear or exponential regression (Venterea et al. 2009).

Spring Rivers will be responsible for data collection, data analysis, and report preparation. Personnel will include Maria Ellis, Lorrie Haley, Jeff Cook, Lauren Bridgeman, Koen Breedveld, Mark Stalcup, Steve Breth, Allison Breedveld, and Andrew Mayancsik. A seasonal field assistant may be hired for the summer 2015 field work.

4. *Timeline:*
Baseline data collection will occur during July or August 2015. Stream/meadow restoration work is scheduled to begin during Fall 2015. Post-restoration data collection will occur

during the first and second summers after restoration is completed (presumably 2016 and 2017). A long-term GHG monitoring schedule may be developed that will coincide with the Shasta crayfish monitoring schedule.

5. ***Deliverables:***

Spring Rivers Foundation will submit to CDFW:

- Interim report summarizing baseline GHG data collected during 2015
- Copies of all documents generated for the Rock Creek Meadow Restoration Project and the Investigation of Greenhouse Gas (GHG) Fluxes and Carbon Sequestration in a Cascade Range Mountain Meadow Restored for Reintroduction of Endangered Shasta Crayfish (*Pacifastacus fortis*).
- Interim report summarizing GHG data collected the first year after the Rock Creek Meadow Restoration Project is completed
- Final Report summarizing baseline GHG data, first-year data, and second-year data.

Project results will be published in an open-access, peer-reviewed journal

6. ***Expected quantitative results (project summary):***

This mountain meadow restoration project is a pilot project, designed to advance scientific understanding of carbon sequestration in mountain meadow ecosystems in order to help inform future GHG mitigation projects. Our results will include standard values for soil total carbon, soil total nitrogen, and GHG fluxes that can be compared to past and future studies of carbon flux in mountain meadow habitats. Our results will also address the potential effects of climatic warming by including soil moisture as a variable in all statistical analyses.

Although we have not yet quantified baseline GHG fluxes and carbon storage in Rock Creek Meadow, we do expect that restoration of the meadow will enhance meadow ecosystem function. The restoration of the stream channel may result in drier riparian wetlands in some portions of the meadow and wetter soils in others. Some research has shown that GHG production is higher in wetter meadow habitats (Hörtnagl and Wohlfahrt, 2014, Riveros-Iregui and McGlynn, 2009), and methane uptake is higher in drier meadow habitats (Blankenship and Hart 2014, Koch et al. 2007). We expect our pre- and post-restoration monitoring to determine how the restoration has affected soil moisture conditions in specific locations in the meadow (i.e., wetter or drier conditions), and that GHG monitoring data will add to the knowledge base of how such changes affect carbon sequestration and GHG fluxes in mountain meadows.

In addition to improving meadow ecosystem function, the restoration of Rock Creek Meadow will create 13,550 square feet (1259 square meters) of habitat for the federally and state-listed endangered Shasta crayfish (*Pacifastacus fortis*). The restoration will also improve the efficiency of the water supply for CDFW's Crystal Lake Hatchery.

USFWS' desired conditions and management objectives for the Rock Creek Meadow Restoration and Reintroduction Project are:

1. Restore and rewater the Upper Rock Creek Meadow to provide approximately 13,550 square feet (1259 square meters) of Shasta crayfish habitat;
2. Ensure with a high level of confidence that a reintroduced Shasta crayfish population would be viable and protected from invasion by non-native crayfish.

CDFW's requirements for the Rock Creek Meadow Restoration and Reintroduction Project are:

1. Ensure that both the quality and quantity of CDFW's water needs for the Crystal Lake Hatchery continue to be met;
2. Ensure that no diseases may be hosted, incubated by, or transferred from Shasta crayfish to hatchery trout;
3. Ensure that no pathogens or non-native species are introduced to Rock Creek or Rock Creek water during the study, restoration, reintroduction, or monitoring phases of the project.

This mountain meadow restoration project proposal includes the GHG Research Component and the Rock Creek Meadow Restoration and Construction Engineering Components, which will create 13,550 square feet (1259 square meters) of habitat for the federally and state-listed endangered Shasta crayfish (*Pacifastacus fortis*), as well as improve the efficiency of the water supply for CDFW's Crystal Lake Hatchery. The reintroduction of Shasta crayfish into the restored Rock Creek Meadow channel (anticipated to occur in spring 2016), channel cross-section monitoring; photo point monitoring; and Shasta crayfish monitoring will be funded through other sources.

Project success will be evaluated by GHG monitoring (first two years after restoration is complete, then every 5 to 10 years depending on other monitoring schedules); channel cross-section monitoring (first two years after restoration is complete, then every 5 to 10 years); photo point monitoring (first year after restoration is complete, then every 5 to 10 years); and Shasta crayfish monitoring (annual for the first five years, then every 5 to 10 years).

The private ownership of the Rock Creek Meadow by PG&E, in conjunction with the nature of the land use—water supply for CDFW's Crystal Lake Hatchery, protected refuge for the endangered Shasta crayfish—ensures that the project benefits will be long-lasting with virtually no risk of reversal.

7. ***Protocols:***

To quantify baseline GHG (carbon dioxide, methane, and nitrous oxide) fluxes within Rock Creek Meadow, we will measure field gas fluxes at 60 locations (10 locations across each of 6 transects) during July or August 2015. Gas flux measurements will be made using the Static Chamber Technique (Livingston and Hutchinson 1995), following methods described in Blankenship and Hart (2014). Gas flux chambers will have a volume greater than 0.015 m^3 (Pihlatie et al. 2013), and will be made from PVC or stainless steel. Four headspace gas samples (18 ml) will be collected using a polyethylene syringe and 20-gauge needle at 0, 20, 40, and 60 minutes after the chamber top is sealed. A vent tube will be used to reduce air disturbance during sampling. The gas samples will be injected into 12-ml evacuated glass vials with rubber septa until laboratory analysis on a gas chromatograph. A simultaneous analysis method will be used to determine the concentrations of carbon dioxide, methane, and nitrous oxide in each sample. We anticipate sending the gas samples to the UC Davis Stable Isotope Lab for analysis.

To quantify soil total carbon and nitrogen, soil samples will be collected near each gas sample location immediately after gas sampling using a field soil probe. Soil samples will be sent to the UC Davis Analytical Lab for analysis of total carbon and total nitrogen.

Means and standard errors of soil volumetric water content, soil carbon, soil nitrogen, soil temperature, carbon dioxide flux, methane flux, and nitrous oxide flux will be calculated for each location and sampling date. Differences between sampling dates (i.e., before and after restoration) will be statically compared using one-way Analysis of Variance. The direction and rate of gas fluxes at each sample location will be determined using linear or exponential regression (Venterea et al. 2009).

The attached Rock Creek Meadow Restoration and Reintroduction Plan (Plan) describes methods and time sequences for design, restoration, and Shasta crayfish reintroduction. The Plan addresses the biological and ecological aspects of the proposed reintroduction. It provides an engineering-design-alternatives analysis for moving the Crystal Lake Hatchery intake, a 20% engineering design of the preferred alternative, and estimated Project costs. The USFWS will be the lead agency for implementation of the Plan; Spring Rivers will assist USFWS.

Spring Rivers will be responsible for implementation of GHG research and Shasta crayfish reintroduction and monitoring. The Technical Review Committee, which includes staff from Spring Rivers, PG&E, USFWS, CDFW, and other interested stakeholders will be responsible for project performance evaluations.

Guidelines used to develop the proposed GHG Research Protocols are summarized below. The guidelines are from “Nitrous Oxide Chamber Methodological Guidelines” published by Global Research Alliance on Agricultural Greenhouse Gases (see http://www.globalresearchalliance.org).

DESIGN PROTOCOL

Design feature	Minimum requirements
Materials	Inert to N_2O, such as stainless steel, aluminum, PVC, acrylic
Area	Recommendation is for chamber area: perimeter ratio to be ≥10 cm.
Height	Chamber height (cm) to deployment time (h), ratio should be ≥40 cm h^{-1}.
Base depth	Ratio of insertion depth: deployment time of ≥12 cm h^{-1}. Height above soil surface should be as close to the soil surface as practical (<5 cm).
Gas tight seal	A water trough or rubber/closed-cell foam gasket. Gaskets should have low internal cross-sectional area, and be compressible. Appropriate fasteners are required with rubber gaskets.
Sampling port	Inert rubber septa or syringe taps.
Vent while placing chamber on base	Opening a vent or sampling port while placing the chamber is recommended.
Vent during deployment	*No consensus on whether vents should be used or not – evolving issue.* However, if used, vents should be located close to the soil surface, or be designed to withstand wind. Appropriate vent dimensions are dependent on expected wind speeds during deployment, and should be adjusted accordingly (see references in text). Chambers and their vents should be bench-tested to ensure no Venturi effect occurs.
Insulation	Use reflective foil, foam, polystyrene. Test effectiveness by comparing surface soil temperatures inside and outside the chambers.

Deployment protocol

Chapter 3 of these guidelines discusses the deployment protocol. In addition to chamber *design*, good practice in *deployment* is also important to achieve the acquisition of best quality data for emission estimation. In addition to the individual chamber deployment, there are recommendations for designing plot experiments for group deployment, replication and for accompanying environmental measurements that should be made.

Deployment issue	Minimum requirements
Site disturbance	Chamber bases to be inserted at least 24 h prior to the first sampling – preferably longer, if logistics allow it. Avoid disturbance of the soil around the chambers. Relocate chambers when soil water content within the chamber differs from surroundings.
Chamber deployment	For chambers with a maximum height of 20 cm, use a deployment period ≤ 30 – 40 min.
Chamber sequence and grouping	Ensure that measurements are sampled per block, rather than per treatment, to ensure each block is sampled in the shortest possible period. Whenever possible, vary the block sampling sequence between sampling days, to avoid potential bias.
Number of samples per flux measurement	Three headspace samples per flux measurement, especially at times when high emissions are expected[1].
First air sample (T0)	Take T_0 sample immediately after chamber placement on the base[2].
Ancillary measurements	Measure soil texture, bulk density, pH, oranic C and total N content at least once for each campaign. Measure average soil and air temperature and total rainfall hourly or daily. Measure soil water content on each sampling day.
Time of day	Studies suggest that between 10 am and 12 noon reflects daily average, but whenever possible, researchers need to determine the diurnal pattern of N_2O emissions to assess time of day that best represents the average daily flux for their study.

[1]When high spatial variability of fluxes requires an increase of chamber replication and resources are limited, two or three headspace samples may be taken. However, researchers must quantify any bias that may be introduced by assuming a linear increase in headspace N_2O concentration.

[2]When ambient air samples are used as an estimate of T_0, researchers need to establish that the N_2O concentration in T_0 samples taken from within the chambers is not significantly different from ambient air.

Duration of experiment	Continue measurements until there is no significant difference in N_2O emissions, or driving variables of N_2O emissions between treated and control plots. For emission factor measurements for inventory, measurements should ideally cover 12 months.
Frequency of sampling	When N_2O peak fluxes are expected, sample at least twice per week, PLUS sample one to two days prior and one to two days after any event likely to induce peak emissions. During periods of low N_2O flux, sample at least once a week. When fluxes have returned to background levels, the sampling interval can be further increased.
Size and number of chambers	Chambers should cover an area as large as practical, while providing information at the smallest scale for which it is needed (see also Chapter 2).
Placement of chambers	Assess if spatial gradient in fluxes exists, divide area into relatively homogenous areas and stratify sampling accordingly. In absence of spatial structure, place chambers randomly.
Treatment replication	A minimum of three replicate plots is needed, preferably more.

Sample collection, storage and analysis requirements

Chapter 4 of these guidelines outlines best practice for analytical lab determination of N_2O gas samples, calibration requirements for optimal accuracy and how to assess adequate analytical precision.

Sampling issue	Minimum requirements
Sample collection and storage	Clean, non-reactive material that can be sealed; container evacuation recommended.
Sample analysis by GC	Commercially-made GC system; flow control and automated sample injection recommended.
Reference gases **GC system calibration**	Confidence in the N_2O concentration of all standards. Similar ranges of standards and samples, and many 'ambient checks' recommended.
Processing GC data	Determine repeatability standard deviation of standards and air samples.
Sample analysis and N_2O fluxes	Determine repeatability standard deviations for the air samples and associated N_2O flux.

Data analysis

Chapter 6 of these guidelines discusses data analysis considerations. Guidance is provided to allow selection of the most appropriate flux calculation method, how to best interpolate non-continuous measurements to obtain best estimates of emissions and emission factors.

Analysis topic	Minimum requirements
Selection and use of a flux calculation method	Method should be matched to the number of headspace samples taken (see Table 6.2, and also Chapter 3 - Chamber Deployment).
Estimation of emissions using non-continuous flux data	Daily fluxes can be integrated, using trapezoidal integration. To improve the accuracy of cumulative emissions estimates, maximise sampling frequencies and spatial replication given available resources. Repeat experiments over multiple years, and consider using spatial or temporal gap filling procedures.
Assessment of minimum detectable flux (MDF)	Determine random measurement error associated with sampling and analysis of replicate standards of known concentration, and use the resulting error rates to determine MDF.
Statistical considerations for analysing inherently heterogeneous flux data	If treatments are replicated (at least three blocks), the variability between replicates can be assessed by calculating means of chambers in each replicate. The variability within the replicate can also be determined by assessing the chamber variability.
Estimation of emission factor (EF)	Requires the inclusion of no-N control treatment, and the subtraction of cumulative emissions in control from cumulative emissions in experimental treatment(s) receiving N addition.

Chamber design

The use of a chamber vent during the flux measurement	*There remains a lack of consensus amongst researchers, with many opting not to vent during chamber deployment due to possible Venturi effects.Further data sets pertaining specifically to N_2O fluxes are needed to resolve this issue.*
Headspace mixing	*Effects of mixing should be tested and reported on. There has been very little work done on evaluating specific requirements for best approach to mixing.*

Deployment protocol

Ancillary measurements	*N_2O data sets are generated for many purposes, including the parameterisation and validation of models. Researchers should consult with modellers on any additional model input parameters that need to be measured, and at what frequency.*
Accounting for diurnal variability in fluxes	*Measure diurnal pattern of driving variables to assess if daily N_2O flux should be corrected.*
Sampling frequency	*Determination of sampling strategies that optimise temporal and spatial coverage of soil N_2O fluxes.*

Data analysis

Flux calculation method	*Criteria for site-specific selection of best non-linear scheme need to be developed.*
Estimation of emissions using non-continuous flux data	*Use of automated chamber systems (see chapter 5) can help minimise temporal uncertainties, but better estimates of spatial variability require a very large number of chambers, or the use of non-chamber (e.g. micro-meteorological) methods.*
Assessment of minimum detectable flux (MDF)	*Different flux calculation schemes can differ in their MDF, therefore the choice of flux calculation scheme can change MDF (see evolving issues for section 6.1).*
Statistical considerations for analysing inherently heterogeneous flux data	*To assist with comparison of heterogeneous chamber datasets, record the spatial coverage of observations (chamber area times the number of chambers, relative to the plot size: i.e., the plot area covered by the chambers). Advanced techniques are being developed to improve description of non-normal and spatially heterogeneous data-sets and use this to select the best method for mean estimation.*
Estimation of emission factor (EF)	*Non-linearity of N_2O response to N addition needs to be assessed in different systems (i.e., EFs may vary, depending on rate of N input).*

8. ***Literature Cited:***

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