Uncertainty in aquatic greenhouse gas flux estimates arises from subjective processing of floating chamber time series

Camille Minaudo[1,2,*], Miguel Cabrera-Brufau[1], Jorge Juan Montes-Pérez[1], Katrin Attermeyer[3,4], Antonio Camacho[5], Alba Camacho-Santamans[1,6], Constantin Cazacu[7], Relu Giuca[7], Ana Lillebø[8], Benjamin Misteli[3], Daniel Morant[5], Biel Obrador[1,2], Bruna R.F. Oliveira[8], Jolita Petkuviené[9], Antonio Picazo Mozo[5], Carlos Rochera[5], Daniel von Schiller[1,6].

[1] Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Universitat de Barcelona (UB), Diagonal 643, 08028 Barcelona, Spain

[2] Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona (UB), Diagonal 643, 08028 Barcelona, Spain

[3] WasserCluster Lunz - Biological Station, Dr. Carl Kupelwieser-Prom. 5, 3293 Lunz am See, Austria

[4] Department of Functional and Evolutionary Ecology, Unit Limnology, University of Vienna, Djerassiplatz 1, 1030 Vienna, Austria

[5] Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, C/ Catedrático José Beltrán 2, Valencia, Paterna E-46980, Spain

[6] Institut de Recerca de l'Aigua (IdRA), Universitat de Barcelona (UB), Martí Franquès 1, 08028 Barcelona, Spain

[7] University of Bucharest, Research Center in Systems Ecology and Sustainability, Spl. Independentei 91-95, 050095 Bucharest, Romania

[8] ECOMARE, CESAM - Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, Aveiro, Portugal

[9] Marine Research Institute, Klaipeda University, Universitetas av. 17, Klaipeda, 92294, Lithuania

*Corresponding author: camille.minaudo@ub.edu

Running head: Subjective processing of chamber data

Keywords: Greenhouse gas fluxes, Aquatic ecosystems, Data processing uncertainty, Floating chambers, Reproducibility

This manuscript is a non-peer reviewed preprint submitted to EarthArXiv. It was submitted to journal *Limnology and Oceanography: Methods* for peer review on 09/12/2025.

Abstract

Accurate quantification of greenhouse gas (GHG) fluxes from aquatic systems is essential for constraining regional and global carbon budgets. Closed floating chambers are widely used to measure carbon dioxide (CO_2) and methane (CH_4) fluxes at the water–air interface, yet large uncertainties persist due to subjective processing of chamber time series. In particular, the treatment of non-linear patterns and abrupt events such as ebullition often relies on expert judgement, which may strongly influence flux estimates. We present the first quantitative assessment of bias arising from expert subjectivity in the processing of floating chamber measurements. Seventeen researchers, all of whom participated in field sampling, independently evaluated a common dataset of 794 incubations from 36 European coastal wetlands. Each expert visually selected valid data segments and flagged abnormal time series prior to flux calculation. In total, 2,679 manual inspections were compared with fully automated flux estimates based on untrimmed time series. Experts showed substantial variability in handling non-linear or irregular concentration patterns. While flux variability was generally low for most CO_2 and CH_4 incubations, disagreements exceeded 100% for curved or abrupt time series. For CH_4, ebullition was a major source of divergence, but marked variability also occurred when ebullition contributed only marginally to total fluxes. This methodological experiment demonstrates that expert judgement introduces significant, previously unquantified uncertainty into aquatic GHG flux estimates. We advocate for transparent, standardised, and reproducible data-processing workflows, including automated tools for objective identification and treatment of non-linearities.

Introduction

Coastal and inland water habitats are collectively an important source of greenhouse gases (GHGs) to the atmosphere (Lauerwald et al. 2023). In the context of climate change and the urgent need to reduce GHG emissions, accurate quantification of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) fluxes is therefore essential for constraining regional and global carbon budgets, informing climate models, and supporting mitigation strategies. The accuracy and reliability of these flux estimates strongly depend on the measurement techniques employed in the field and subsequently used to inform upscaling models (Lauerwald et al. 2023).

Aquatic GHG fluxes are measured using a wide range of techniques and protocols, each with distinct advantages and limitations. Common approaches include floating chambers, which provide direct flux estimates at high temporal resolution; eddy covariance systems, which integrate fluxes over larger spatial scales; and dissolved-gas methods such as headspace equilibrations, mass balance approaches, or membrane-based sensors that infer air–water gas exchange indirectly. While this methodological diversity offers flexibility, it also results in substantial heterogeneity in sampling designs, data-processing choices, and reporting standards. Consequently, there remains a clear need for consistent, standardised protocols that enable more reliable comparison among studies.

Among available approaches, the closed floating chamber technique is widely used to estimate in situ short-term GHG fluxes at the water–atmosphere interface (e.g., Mannich et al., 2019; Martinsen et al., 2018; Vachon et al., 2010; Xiao et al., 2016). This method isolates a defined air volume directly above the water surface (the chamber headspace) for a limited incubation period, typically lasting 5 to 20 minutes. During this time, changes in GHG concentrations within the chamber headspace are monitored and subsequently used to infer fluxes across the boundary layer. Experts generally assume constant diffusive fluxes during short-term incubations (<15 minutes) and therefore commonly fit chamber concentration time series with linear regressions. While this assumption has been strongly debated within the soil science community for the processing of static chamber measurements (Hutchinson and Mosier 1981; Pirk et al. 2016; Hüppi et al. 2018; Maier et al. 2022), aquatic scientists often overlook non-linear patterns (Silva et al. 2015), which can lead to severe underestimation of actual fluxes (Kutzbach et al. 2007). Indeed, several studies report non-linear or abrupt patterns in short chamber time series (e.g., Hoffmann et al., 2017; Sø et al., 2024; Villa et al., 2021; Żygadłowska et al., 2024). Such behaviour may reflect the non-steady-state conditions inherent to closed chambers (Mannich et al. 2019), as well as short-term variability in the processes controlling GHG fluxes. For example, turbulence at the air–water interface is inherently unsteady, resulting in fluctuating gas-exchange velocities over short time scales (Zappa et al. 2007; Wang and Liao 2016; Perolo et al. 2021).

For CH_4, which is poorly soluble in water, emissions can occur not only through molecular diffusion (although a significant fraction of diffusing CH_4 gets oxidised in the water column, depending on stratification and mixing patterns; Vachon et al., 2019), but also via non-diffusive pathways such as

ebullition, whereby gas bubbles formed in water-saturated sediments escape to the surface. This additional transport mechanism adds further complexity to CH_4 flux dynamics and frequently produces abrupt jumps in concentration time series of floating chamber measurements (e.g., Gerardo-Nieto et al., 2019; Sø et al., 2024; Varadharajan and Hemond, 2012). Several studies have attempted to separate the diffusive and ebullitive components of CH_4 fluxes (Hoffmann et al. 2017; Sø et al. 2024), yet methodological inconsistencies persist.

Despite these well-documented challenges, there is no clear consensus on how floating chamber incubation time series should be processed. In practice, GHG flux calculations often rely on expert visual inspection and subjective selection of observations deemed suitable for regression analysis. This subjectivity raises several important questions:

i) How do experts treat non-linear patterns in CO_2 and CH_4 chamber time series?

ii) To what extent do experts agree on how such patterns should be handled?

iii) Under which conditions is consensus among experts stronger or weaker, and does this differ between CO_2 and CH_4?

In this study, we explore these questions by assessing the degree of agreement among experts in GHG data processing. By comparing expert decisions and the resulting flux estimates, we quantify the extent to which subjective choices influence results. Finally, we provide guidance on implementing more objective and transparent methodologies for processing GHG chamber time series.

Materials and procedures

A database of in-situ floating chamber incubations

A total of 794 floating chamber incubations were performed seasonally across 36 coastal wetland sites (Minaudo et al. 2025), including a large freshwater lagoon (Curonian Lagoon, Lithuania), freshwater ponds and marshes (Camargue, France), freshwater ponds and lakes (Danube Delta, Romania), brackish marshes (Marjal dels Moros, Spain), intertidal salt marshes (Oosterschelde and Grevelingen Deltas, the Netherlands), and seagrass beds (Ria de Aveiro, Portugal). These sites represent a wide range of ecological conditions, from natural and well-preserved systems to heavily impacted or restored environments.

All in situ measurements followed a standardized protocol (Minaudo et al. 2023). Incubations lasted up to 12 minutes and were conducted at water depths >10 cm without emergent vegetation inside the chamber. Chambers were flushed before and after each incubation, and trained operators were instructed to minimize any disturbance of the chamber. Opaque floating chambers (Fig. S1) were used to prevent overheating; all had identical dimensions (half-sphere, radius 19 cm, area 1134 cm^2, volume 14.37 L). Three portable gas analyzers were employed for direct CO_2 and CH_4 flux measurements: a Picarro GasScouter G4301, a LiCOR LI-7810, and a Los Gatos GLA132-GGA. Each incubation provided high-frequency (every second) simultaneous measurements of CO_2, CH_4, and H_2O concentrations in the chamber headspace. All CO_2 and CH_4 time series were corrected for water vapour, expressed on a dry-gas basis, and compiled into a single database.

Tracking experts' selection of valid data

Among the team members who participated in the sampling campaigns, a total of 17 researchers considered themselves sufficiently experienced to process the chamber incubation time series. Hereafter, we refer to this group as "experts." All experts received the same R scripts (available at https://github.com/camilleminaudo/ghg-flux-expert). After installing the necessary dependencies, experts were instructed to run the main script, "*expert_vs_automatic_processing.R*". This script loaded the dataset of incubation time series and randomly selected 10 incubations for each program run. All participant names were anonymised, and a unique ID was assigned to each expert to prevent potential bias during analysis.

Experts were asked to:

i) visually inspect CO_2 and CH_4 incubation time series;
ii) flag time series exhibiting abnormal patterns that prevented safe CO_2 and CH_4 flux calculation (independently for each gas);
iii) select data segments considered valid for flux calculation;
iv) for CH_4, specifically identify the segment of the time series suitable for estimating the diffusive flux component only.

To proceed with data selection, plots of raw CO_2 or CH_4 concentrations were shown (Fig. S2), and experts clicked on the start and end points of the selected segments. Each expert was asked to repeat this process until at least 100 incubations had been visually inspected. During the random draw of incubations, the script first prioritised incubations not yet evaluated by any expert and then favoured those with the fewest prior assessments, ensuring that most incubations were reviewed by at least three experts. At the end of each program run, selected start and end times were stored in a dedicated table.

Flux calculations

For each manual selection, CO_2 and CH_4 fluxes were calculated using the *goFlux* package (Rheault et al. 2024) with both linear and non-linear Hutchinson–Mosier models (Hutchinson and Mosier 1981). Selection of the best model followed the default objective criteria implemented in *goFlux.* The non-linear model was favoured unless:

- the ratio between non-linear and linear estimates (*g.factor*) was ≥ 2,
- curvature thresholds (*k.ratio* = 1) were exceeded,
- sensor-based minimum detectable flux criteria were violated
- the model with the smallest mean absolute error (MAE) was selected. If both models had MAE values smaller than the instrument precision, the non-linear flux was chosen by default. If the MAE exceeded instrument precision, a warning indicated that the measurement was likely noise-dominated.

The total CH_4 flux ($F_{CH4\ tot}$) was calculated by first smoothing the full time series using in R the function *stats::smooth.spline* (spar = 0.8). The minimum and maximum values of the smoothed signal were then extracted from the first and last 30 data points, respectively. Their difference was divided by the incubation duration ($t_{incubation}$) and multiplied by a correction factor ($flux.term$) to account for atmospheric pressure, air temperature, water vapour, chamber volume, and chamber surface area, as specified in *goFlux*:

$$F_{CH4\ tot} = \frac{\max\left(C_{last\ 30\ secs}^{smoothed}\right) - \min(C_{first\ 30\ secs}^{smoothed})}{t_{incubation}} \cdot flux.term$$

The diffusive CH_4 flux ($F_{CH4\ diff}$) was estimated by applying the linear model from *goFlux* to the expert-selected diffusive segment. The ebullitive flux ($F_{CH4\ ebull}$) was calculated as

$$F_{CH4\ ebull} = F_{CH4\ tot} - F_{CH4\ diff}$$

Fluxes derived from expert data selection were compared with a fully automated data processing, here considered as a benchmark model, in which no trimming of the time series was applied—that is, all observations from the measurements were retained and processed into flux estimates as described above. This fully automated calculation processed the entire dataset uniformly across all incubations. Hereafter, in both the text and figures, flux estimates based on expert data selection are denoted as $F^{expert}_{variable}$, and those derived from automatic processing as $F^{auto}_{variable}$ where $variable$ refers to CO_2, $CH_{4\ tot}$, $CH_{4\ diff}$, or $CH_{4\ ebull}$.

In the specific case of F^{auto}_{CH4} calculations, additional functions were developed to calculate the total flux and separate diffusive and ebullitive components. This was achieved using a running-variance model to identify bubbling events and an iterative linear model to detect diffusion patterns, building on previous approaches (Hoffmann et al. 2017; Sø et al. 2024). First, CH_4 concentration within the chamber headspace was normalised by the mean concentration over the incubation period. Then, for a moving window of 10 data points, the running variance of the normalised concentration was computed. All observations corresponding to a running variance exceeding the 75th percentile of the variance time series (or an arbitrary minimum threshold of 10^{-4}) were flagged as part of a probable ebullition event. Flagged data segments separated by fewer than ten data points were merged. Flagged segments shorter than five data points were discarded.

It was considered possible for an incubation to begin with an ebullition event. In such cases, the sudden increase in CH_4 concentration within the chamber headspace may induce back-diffusion of CH_4 into the water, thereby increasing the uncertainty of diffusion estimates once the rate of change in concentration stabilises. For this reason, when a probable ebullition event was detected within the first 20 seconds of an incubation, diffusion and ebullition were not separated, and only the total flux was retained. Otherwise, when no bubbling event was detected during the first 20 seconds of an incubation, the first linear segment of the time series was used to estimate $F^{auto}_{CH4diff}$. Fully automated F^{auto}_{CH4tot} was computed

using the smoothed full time series without any trimming of the time series. The ebullitive flux $F_{CH4ebull}^{auto}$ was calculated by subtracting $F_{CH4diff}^{auto}$ from F_{CH4tot}^{auto}.

Flux comparisons across expert and automated estimates

For each incubation assessed by at least three different experts, the mean, standard deviation (SD), and coefficient of variation (CV) of flux estimates were computed. This enabled an objective quantification of the variability in flux estimates across expert assessments. Mean expert fluxes were also compared with the fully automated flux estimates for CO_2, $CH_{4\ tot}$, $CH_{4\ diff}$ and $CH_{4\ ebull}$.

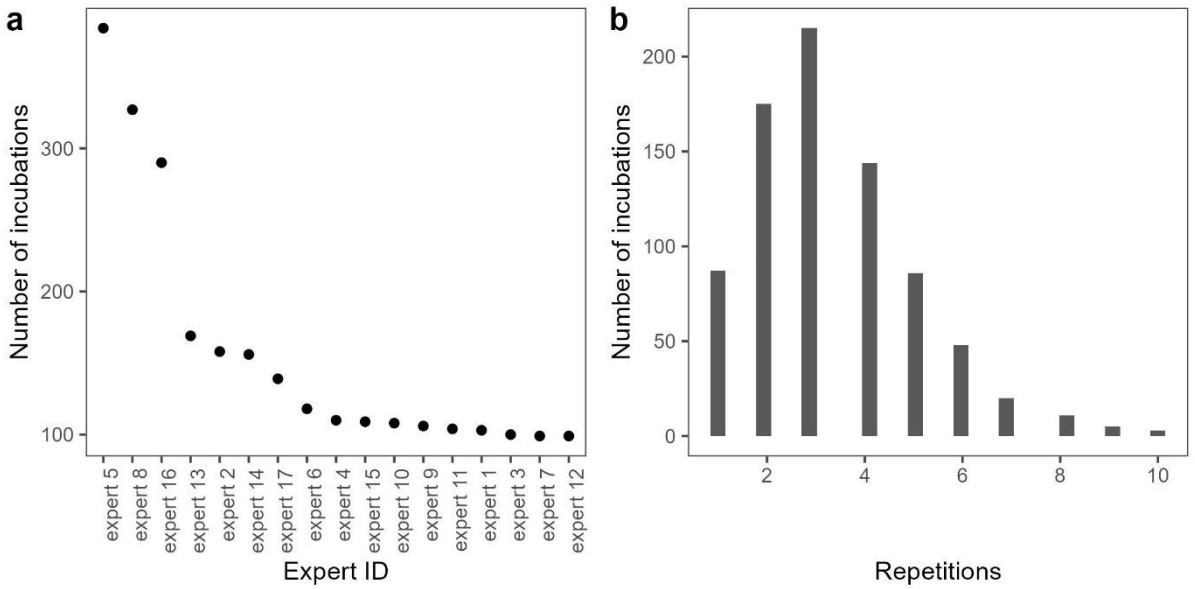
Non-linearity in the incubation time series was assessed by calculating the mean absolute error (LM.MAE) of a linear regression using all available observations (i.e. without trimming). Large LM.MAE values indicate poor model fits with the linear model, reflecting either non-linear patterns or abrupt concentration changes during the measurement period.

All data preparation, processing and analyses were performed in R 4.5.2 (R Core Team 2025).

Assessment

Overview of experts' contributions

A total of 764 incubations were included in the dataset, which collectively underwent 2,679 manual inspections by the 17 experts (Fig. 1). On average, each expert evaluated 157 incubations, with individual contributions ranging from 100 to 384. Of all incubations, 532 were inspected multiple times by different experts, receiving between 3 and 10 independent assessments; only 3 incubations reached the maximum of 10 assessments. Some incubations were also assessed multiple times by the same expert; this occurred 221 times, of which 209 involved two assessments by the same expert and only 12 involved three or more assessments.



212

213 **Figure 1.** Overview of experts' contributions. Number of incubations inspected for each of the 17 different experts (a) and count
214 of incubations inspected from once to the highest number of independent inspections (b).

215 During these inspections, 158 time series were flagged as abnormal by at least one expert, including
216 73 for CO_2 and 85 for CH_4. Overall, 23 incubations were identified as abnormal for both gases. none of
217 the incubations inspected at least three times were flagged as abnormal by all experts. Thus, in every
218 case, at least one expert considered part of the time series suitable for flux calculation. To ensure
219 consistency, we adopted a conservative approach in subsequent analyses: any incubation flagged as
220 abnormal by at least one expert was discarded. This yielded a working dataset of 629 incubations.

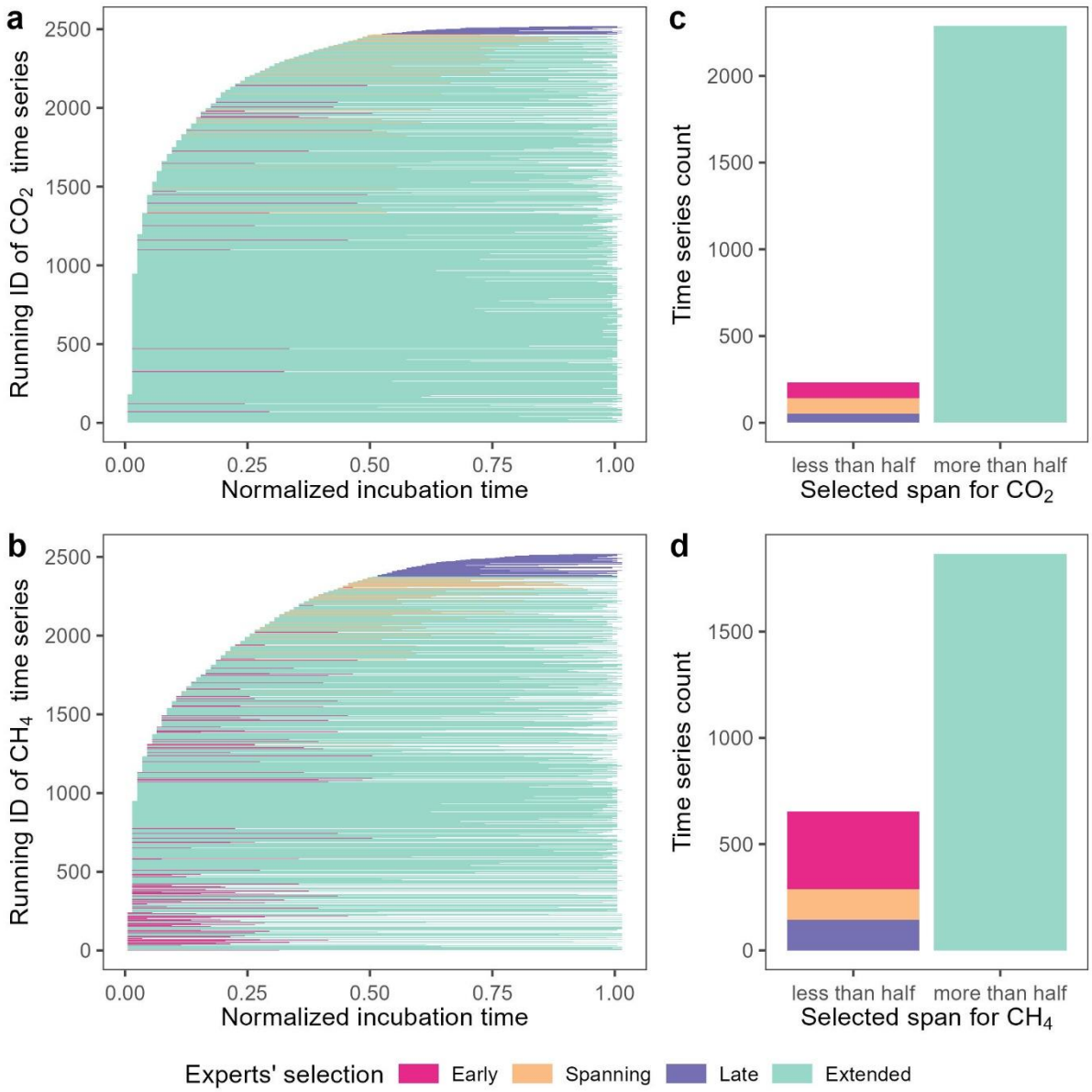


Figure 2. Experts' selection of time series segments for all CO_2 (a) and CH_4 (b) incubations, ordered by the starting time of the selected data segments, normalised from 0 (start) to 1 (end). Colours indicate whether >50% ("Extended") or <50% of the original measurement was retained. Subcategories for <50% indicate whether selections occurred in the early, late, or spanning portions of the time series. Panels (c–d) show counts for each selection category.

On average, experts retained 84% of the original CO_2 measurements and 71% of CH_4 measurements (Fig. 2). Experts preserved at least 90% of the original measurements in 57% of CO_2 and 41% of CH_4 assessments. Segments shorter than half of the original record were selected in 9% of CO_2 assessments and 26% of CH_4 assessments. In the most heavily trimmed cases, experts generally retained data from the first half of the incubation, discarding later observations. Conversely, only 2% of CO_2 and 6% of CH_4 assessments involved discarding the entire first half of the record.

The data segments selected by different experts for flux calculation revealed substantial subjectivity and contrasting methodological strategies. For example, for a highly fluctuating CO_2 time series (Fig. 3a), three experts selected an extended segment of the record, whereas the other three restricted their selection to a later, more linear segment. For a curved CO_2 time series (Fig. 3b), one expert selected only the first linear segment (first 85 seconds), while six others retained the second half of the record. These examples highlight differing tolerance for curvature, with most experts excluding the visibly non-linear portion.

Corresponding CH_4 time series revealed similar variability (Fig. 3c–d), with incubation 34 showing typical ebullition-related patterns (Fig. 3c) and incubation 140 displaying strong curvature similar to its CO_2 counterpart (Fig. 3d). In both cases, experts either selected the earliest linear portion as a conservative estimate of CH_4 diffusion or favoured a longer linear segment later in the record. These pronounced discrepancies underscore the high subjectivity in data selection and highlight the resulting uncertainty in flux estimates.

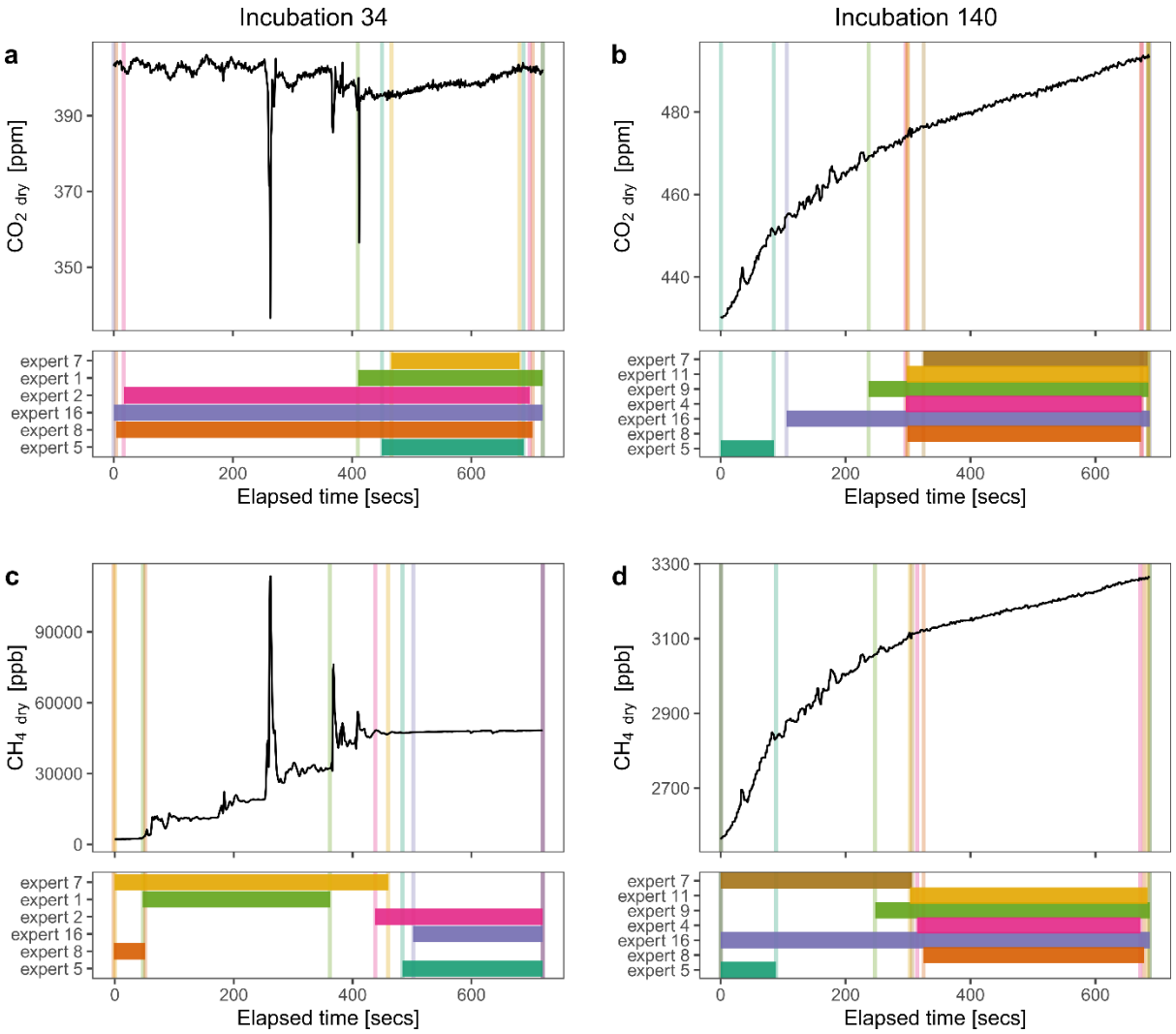


Figure 3. Example of experts' data selection for two incubations (given IDs are 34 and 140). Panels (a–b) show CO_2 selections; (c–d) show selections for estimating CH_4 diffusion. Vertical lines and horizontal bands denote each expert's start and end points.

Impact of subjective data selection on CO_2 and CH_4 flux estimates

After retaining the 498 CO_2 and CH_4 incubations that were assessed at least three times and never flagged as abnormal, we evaluated how agreement among experts influenced flux estimates.

Flux estimates ranged from –0.3 to 1.0 µmol m^{-2} s^{-1} for CO_2 and from -15 to 860 nmol m^{-2} s^{-1} for CH_4 $_{tot}$ (Fig. 4). Across all expert assessments, absolute flux variability increased with flux magnitude (Fig. 4a, d). When variability was expressed relative to the mean flux estimates (Fig. 4b), F_{CO2} generally exhibited low variability, with the CV below 0.1 in 74% of the CO_2 time series and exceeding 0.5 in only 5% of the time series. For F_{CH4tot}, variability was slightly higher, with CV < 0.1 in 68% of the time series and CV > 0.5 in 5% (Fig. 4e).

In some cases, experts strongly disagreed on data selection, resulting in markedly different flux estimates. Eleven F_{CO2} and eight F_{CH4tot} estimates exhibited CV > 1, indicating major discrepancies among expert-derived fluxes. These disagreements were not associated with flux magnitude, but rather with time series characterized by high LM.MAE values, i.e. poor linear model fits (Fig. 4). This suggests that the highest uncertainty arose from non-linear behaviours, where interpreting concentration patterns was less straightforward. Despite these cases, there was overall good agreement between fluxes derived from expert data selection and those obtained using fully automated calculations that included all data points in the regression (Fig. 4c, f). This consistency indicates that, for most cases, expert-driven data selection and fully automated full-series approaches yielded comparable CO_2 and CH_4 flux estimates, with only a limited subset of non-linear time series leading to substantial divergence. For both gases, the difference between experts' fluxes and the fully automated flux estimates was minimal when the simple fitting of incubation time series with a linear model produced low errors (Fig. S3). Conversely, when high values of LM.MAE indicated non-linearity, discrepancies between experts' flux estimates and the fully automated flux estimates were the highest.

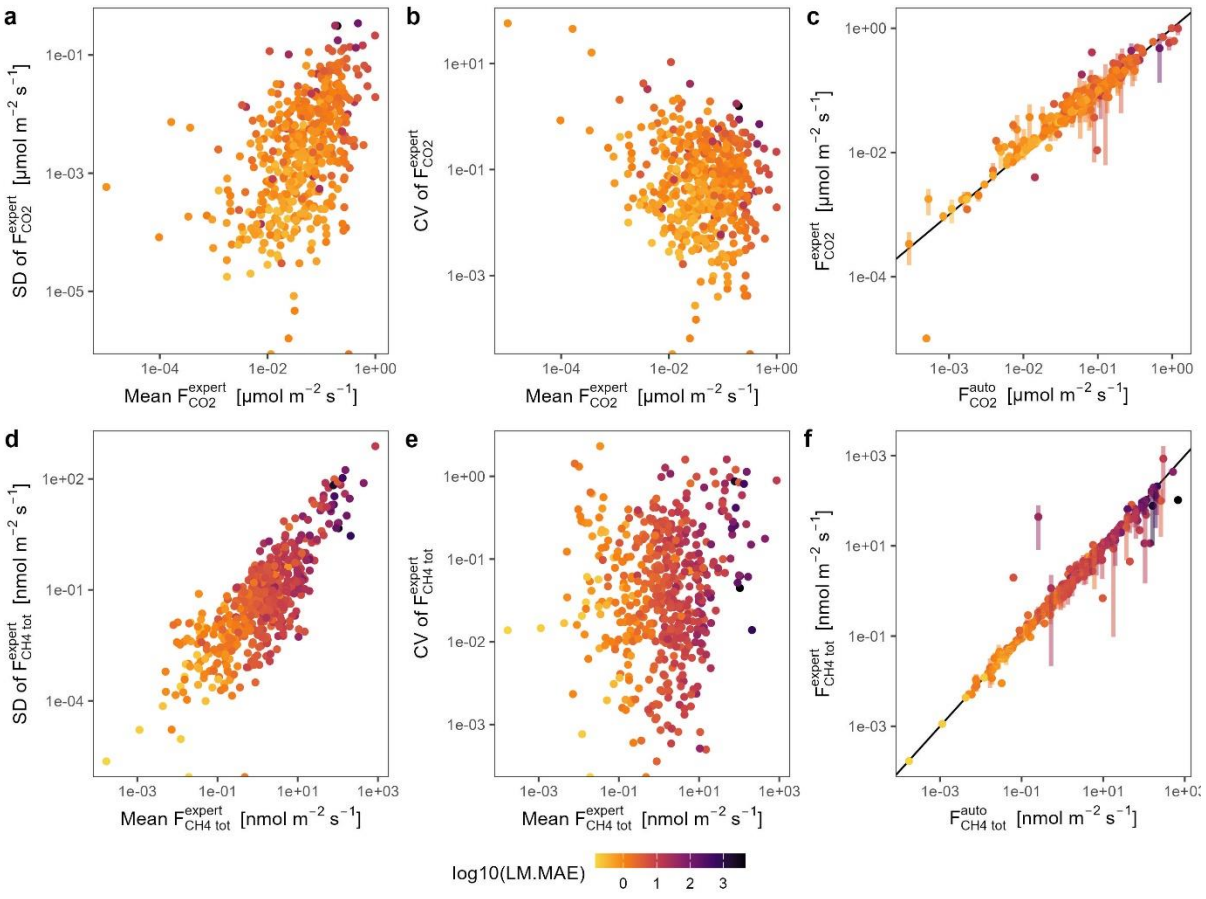


Figure 4. Variability in expert-derived CO_2 and $CH_{4\ tot}$ flux estimates. Panels (a,d): SD vs mean; (b, e): CV vs mean; (c, f): expert vs fully automated estimates with 1:1 line indicated in solid black. Colour scale indicates LM.MAE of the untrimmed incubation time series.

Influence of ebullition on expert variability

In the dataset, ebullition contributed more than 10% of the total CH_4 flux in 81 incubations and exceeded 50% in 46 of them. Variability across $F_{CH4\ ebull}$ estimates was not clearly related to the relative contribution of ebullition to the total flux (Fig. 5a) and remained consistently high, with a CV of 0.8 ± 0.65 (mean ± standard deviation). For both diffusive and total CH_4 fluxes, the variability among expert assessments increased with the magnitude of the ebullition contribution (Fig. 5b and c). When ebullition accounted for 10–50% of the total CH_4 flux, the CV across experts' flux estimates was 0.24 ± 0.30 for $F_{CH4\ diff}$ and 0.13 ± 0.24 for $F_{CH4\ tot}$. In contrast, when ebullition contributed more than 50% of the total CH_4 flux, variability rose markedly, with CV values of 0.57 ± 0.79 and 0.35 ± 0.43 for $F_{CH4\ diff}$ and $F_{CH4\ tot}$, respectively. Notably, many CH_4 time series showed minor ebullition contributions to total fluxes yet still exhibited substantial variability among experts' flux estimates.

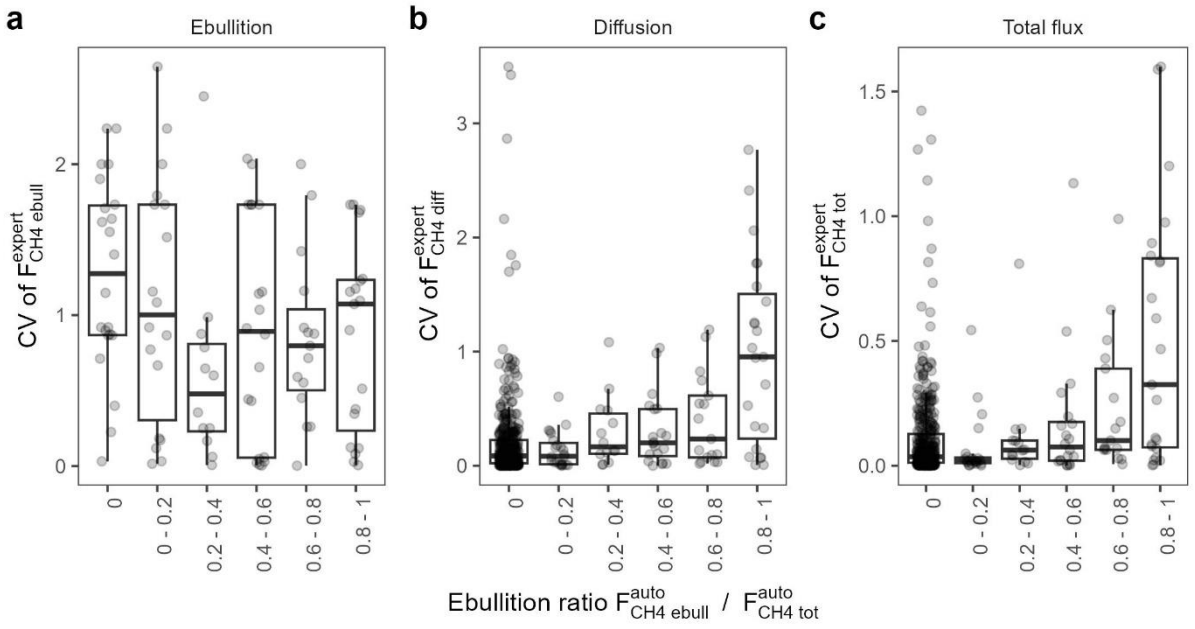


Figure 5. CV of expert-derived CH_4 fluxes for different levels of ebullition contribution to the total CH_4 flux. Ebullition ratio was calculated according to the fully automated data processing.

Across the dataset, the CV of expert-derived F_{CO2} increased with those for $F_{CH4\ tot}$, indicating that variability within a given incubation was often mirrored across both gases (Fig. 6a). A total of 39

incubations showed a CV > 0.4 for $F_{CH4\ tot}$. These incubations were all associated with moderate to high variability in F_{CO2}, with a mean CV of 0.67. We identified that 15 of these incubations (i.e. 38%) corresponded to cases where ebullition contributed most to the total CH_4 flux. This pattern likely reflects rapid pressure changes inside the chamber headspace or spectral interference in cavity ring-down spectrometry during strong CH_4 ebullition events, which can distort CO_2 concentration readings (Zhao et al. 2022) and thereby increase variability in derived CO_2 flux estimates, as illustrated by incubation A in Figures 6b and 6c. To our knowledge, no previous studies have reported this technical issue in CO_2 flux measurements at the air–water interface of aquatic ecosystems. High variability in flux estimates for both gases could not always be attributed to ebullition, as shown by incubation B in Figures 6b and 6c, which exhibit a clear non-linear pattern for both gases, leading to highly variable experts' flux estimates.

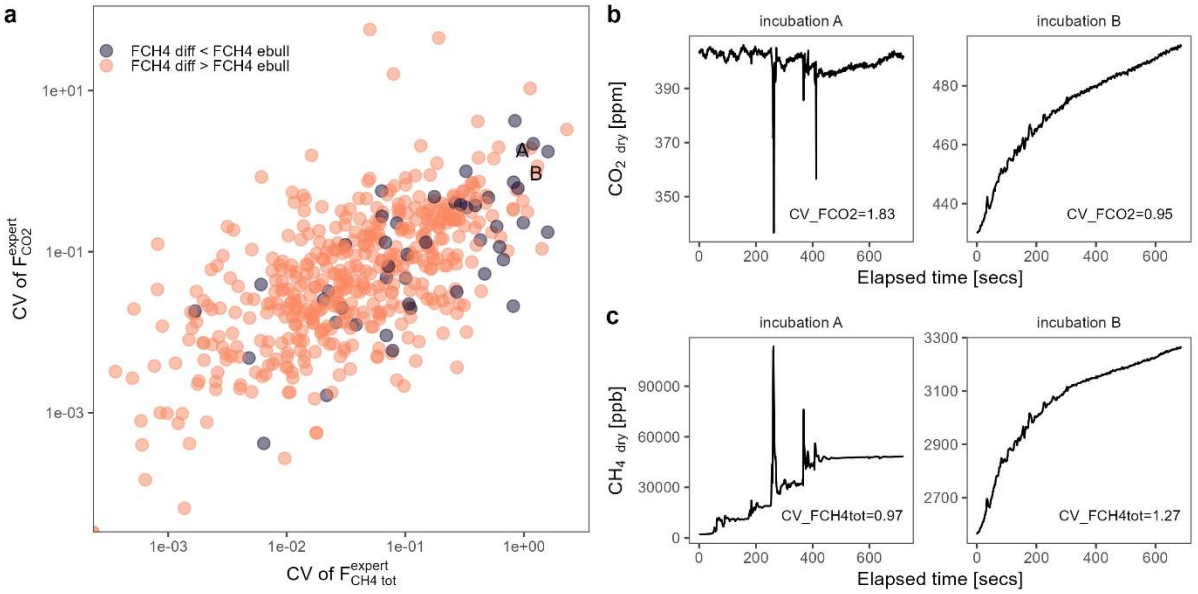


Figure 6. a) CV of expert-derived F_{CO2} vs CV of $F_{CH4\ tot}$ estimates. Colours indicate diffusion- (orange) or ebullition- (purple) dominated incubations. (b - c) Examples of CO_2 and CH_4 measurements corresponding to high variability in experts' flux estimates for both gases: incubation A (ebullition-dominated, non-linear pattern) and B (diffusion-dominated, curved).

Discussion

Prevalent non-linearities lead to high flux uncertainties

This methodological experiment provides a quantitative demonstration that expert judgement introduces substantial uncertainty in GHG flux estimates, particularly when incubation time series exhibit non-linear patterns. When faced with such complexities, experts adopt markedly different strategies for

data selection, leading to high variability in final flux estimates. This variability highlights a critical gap in standardized guidelines and training for the processing of floating – and, by extension, static chamber data. These findings underscore the need for transparent, reproducible, and harmonized procedures to minimize subjectivity in GHG flux estimation.

Experts showed heterogeneous criteria for flagging abnormal time series. Some applied highly conservative approaches, fully discarding incubations that displayed irregular or erratic concentration changes. Others adopted more permissive criteria, retaining data segments of the time series they considered sufficiently reliable for flux calculation. Such discrepancies reveal the subjective nature of expert-driven quality control, which, in the absence of formal criteria, can substantially affect the reproducibility of flux datasets.

Once CO_2 or CH_4 incubation time series deviated from a strictly linear trend, expert strategies diverged further. Some relied on shortened linear fits, others manually trimmed suspect data points, while a few accepted non-linear curve-fitting approaches. These differing practices produced flux estimate variabilities ranging from 10% to more than 100% across identical measurements. For CO_2, disagreements were mostly related to gradual non-linearities, which were previously reported in several static chamber studies for both soil and aquatic measurements (e.g., Hüppi et al., 2018; Kutzbach et al., 2007; Silva et al., 2015; Xiao et al., 2016) but for which no consensus treatment currently exists for aquatic ecosystems. The lack of agreement on how to interpret curvature in concentration–time relationships remains a major source of uncertainty in CO_2 flux estimation in aquatic ecosystems (Silva et al. 2015). Unlike soil chambers, where gas transport is primarily diffusion-controlled and fluxes tend to stabilise over time (Maier et al. 2022), aquatic chambers are subject to additional sources of short-term variability, including fluctuating turbulence, surface renewal processes, and rapid changes in photosynthetic and respiratory activity. These hydrodynamic and biogeochemical dynamics make the assumption of steady-state conditions—and thus linear behaviour—far less tenable in aquatic systems.

For CH_4, the situation is further complicated by ebullition, a dominant driver of abrupt, non-linear changes in concentration time series. Differences in expert judgement when isolating diffusive patterns led to large uncertainties in both diffusive and ebullitive flux estimates. Nonetheless, even when ebullition played only a minor role, substantial variability persisted among expert-derived total and

diffusive CH_4 fluxes, indicating that non-ebullitive processes (e.g. fluctuating turbulence, short-term variability in gas exchange) also contributed to interpretative divergence.

Towards an automated processing pipeline for more objectivity and reproducibility

Overall, the results of this study demonstrate that expert subjectivity can substantially influence GHG flux estimates, particularly when incubation data deviate from ideal linear behaviour. These discrepancies highlight the urgent need to establish standardised, transparent data processing protocols that reduce interpretative bias. The development and adoption of automated or semi-automated processing pipelines capable of objectively identifying and addressing non-linearities would represent a major step forward in ensuring the comparability, reproducibility, and robustness of GHG flux data across studies and ecosystems.

Several existing R packages, such as *goFlux* (Rheault et al. 2024), *fluxfinder* (Wilson et al. 2024), and *fluxible* (Gaudard et al. 2025), already incorporate algorithms designed to handle both linear and non-linear patterns in GHG concentration time series from closed chamber measurements. These tools follow clear and reproducible workflows to automatically load and process raw measurements and derive flux estimates. They typically include features for detecting abnormal measurements or outliers, fitting multiple candidate models, and comparing statistical performance metrics to select the most appropriate model for flux estimation. Such structured approaches substantially reduce operator influence, ensuring that results can be consistently reproduced and objectively compared across datasets.

While some degree of subjectivity remains—particularly in the selection of models or the criteria used to determine the best fit—the reproducibility of the computational workflow itself is guaranteed (Gaudard et al. 2025). This reproducibility is essential to building confidence in flux estimates, especially when results are used to upscale site-level observations to ecosystem, regional, national, or global assessments. Since international regulations request the reporting of complete national inventories of GHG fluxes from all sources, including ecosystems (e.g., European Union Regulation 2021/1119, 2021), transparent and standardised pipelines therefore play a key role in improving both the credibility and traceability of aquatic GHG budgets.

It is important to note, however, that all these tools were originally developed for terrestrial ecosystem measurements, and none are yet fully tailored to the specific characteristics of aquatic environments. Differences in turbulence dynamics, boundary-layer processes, and the prevalence of phenomena such as ebullition require adaptation and careful parameterisation. As such, there is a clear need for the aquatic research community to reach consensus on best practices for implementing, validating, and potentially extending these automated frameworks. Doing so would facilitate the development of a harmonised, community-endorsed pipeline for flux data processing, enhancing both scientific rigour and cross-study comparability in aquatic GHG research.

Conclusions and recommendations

In the context of global change, effective climate action relies on robust and transparent data to inform policy and guide mitigation strategies. Inland waters contribute significantly to GHG emissions, yet their role remains under-constrained, in part due to methodological inconsistencies. This study demonstrates that expert judgement in the processing of floating-chamber incubations can introduce substantial and previously unquantified uncertainty.

Building on the insights from this methodological experiment, we propose the following practical guidelines to enhance consistency, transparency, and reproducibility in the processing of aquatic GHG incubation time series:

1. Understand the theoretical foundations of chamber-based flux estimation.

Researchers should ensure a sound understanding of the physical and mathematical principles underlying static and floating chamber techniques. A comprehensive overview is provided by Xiao et al. (2016), who detail the assumptions and limitations associated with different modelling approaches. Such theoretical grounding is essential for correctly interpreting concentration–time relationships and identifying cases where standard assumptions (e.g. steady-state diffusion) may be violated.

2. Tolerate curved patterns and explicitly justify model selection.

Non-linear concentration changes are common in chamber measurements, and linear regressions can substantially underestimate fluxes when curvature is present (Silva et al., 2015; Xiao et al., 2016). Rather than automatically discarding non-linear series, researchers should explore multiple models (e.g.

exponential, quadratic) and document the criteria used to justify the final model choice. Reporting such criteria enhances transparency and allows others to reproduce or reassess flux estimates.

3. Recognise and appropriately treat complex measurement patterns.

Portable gas analysers can produce noisy or unstable readings, especially under fluctuating field conditions, typical of aquatic environments. Abrupt variations in concentration data do not necessarily invalidate the entire incubation. For example, in CH_4 measurements, ebullition events can generate short-lived spikes, yet total fluxes can still be computed and, under reasonable conditions (see guideline 4 below), it may remain possible to separate fluxes into diffusive and ebullitive components. Researchers should therefore carefully assess whether noise reflects instrument artefacts or real environmental dynamics before rejecting data.

4. Use the early linear segment to estimate diffusive CH_4 fluxes.

When ebullition occurs, back-diffusion of CH_4 in the chamber headspace can bias diffusion flux estimates if a linear pattern later in the time series is used. To avoid this, researchers should rely on the first linear segment of the time series (i.e. prior to back-diffusion) to estimate the diffusive flux component. We do not recommend any attempt for diffusion/ebullition separation if an incubation time series starts immediately with an ebullition event.

5. Consider cross-gas interference from ebullition events.

Ebullition can also affect CO_2 time series, particularly as highly elevated CH_4 concentration and rapidly changing pressure and moisture during ebullition events interferes with CO_2 readings of cavity ring-down spectrometers (Zhao et al. 2022). In such cases, the underlying diffusive CO_2 signal often remains recoverable between ebullition events, and identifying these stable intervals can preserve valuable information otherwise lost through complete data exclusion.

6. Apply established, standardised data processing tools.

Several R packages now provide frameworks for data harmonisation, quality control, and flux estimation, including *goFlux* (Rheault et al., 2024), *fluxfinder* (Wilson et al., 2024), and *fluxible* (Gaudard et al., 2025). These tools implement reproducible workflows for reading raw data, identifying outliers, and fitting

models. However, none yet offer a dedicated framework for distinguishing diffusion from ebullition, which remains an area for further development. The scripts developed for the purpose of the present study can serve as a basis for this (see Data and code availability section below).

7. Prefer harmonised workflows over ad hoc treatments.

Whenever possible, use a consistent data processing pipeline for all incubations rather than relying on subjective, case-by-case decisions. Harmonised workflows not only improve comparability between datasets but also reduce the risk of hidden biases introduced by individual interpretation.

8. Ensure transparency and reproducibility to improve accuracy.

Finally, all steps in data selection, model fitting, and flux estimation should be fully documented and reproducible, ideally through shared code and raw and processed data. Transparent methodologies are essential for building confidence in flux estimates and for enabling robust data synthesis across research groups and ecosystems.

Although this study focuses on variability arising from data processing, it is important to recognise that uncertainty in GHG flux estimates also originates from earlier stages of the measurement process. Standardised operating procedures are needed throughout all steps preceding and during field measurements, including the selection of chamber dimensions and materials (Mannich et al. 2019), the deployment setup, and the duration and timing of incubations. Variability in these parameters can influence chamber temperature, mixing dynamics, and gas accumulation rates, ultimately affecting flux estimates. Establishing clear methodological standards alongside automated data-processing pipelines would therefore ensure greater comparability and reproducibility of GHG flux measurements across studies and ecosystems.

Funding

This research has received funding from the project RESTORE4Cs - Modelling RESTORation of wEtlands for Carbon pathways, Climate Change mitigation and adaptation, ecosystem services, and biodiversity, Co-benefits (DOI: 10.3030/101056782), co-funded by the European Union under the Horizon Europe research and innovation programme (Grant Agreement ID: 101056782). The views and opinions expressed are those of the author(s) only and do not necessarily reflect those of the European

Union or the granting authority. Neither the European Union nor the granting authority can be held responsible for them. Additional funding was provided by Grants CNS2022-135283 and TED2021-130330B-I00 funded by MCIN/AEI/10.13039/501100011033 and by the European Union NextGenerationEU/PRT. Camille Minaudo has received funding from the postdoctoral fellowship programme Beatriu de Pinós funded by the Secretary of Universities and Research (Government of Catalonia) and by the Horizon 2020 programme of research and innovation of the European Union under the Marie Skłodowska-Curie grant agreement no. 801370.

Acknowledgements

We thank all field team members of the RESTORE4Cs project and the local institutions and teams who supported and facilitated the sampling campaigns. We also acknowledge the support of CESAM-Centro de Estudos do Ambiente e do Mar, funded by national funds through FCT – Fundação para a Ciência e a Tecnologia I.P., under the project references UID/50017/2025 (doi.org/10.54499/UID/50017/2025) and LA/P/0094/2020 (doi.org/10.54499/LA/P/0094/2020). The authors have used ChatGPT to correct grammatical errors and improve textual clarity.

Data and code availability

All the raw incubation time series are made available in a dedicated Zenodo repository (https://doi.org/10.5281/zenodo.17828220). All R scripts to proceed with the data preparation, the interactive data selection, and the data processing for this manuscript are accessible in a dedicated GitHub repository: https://github.com/camilleminaudo/ghg-flux-expert.git.

Authors contribution

CM: Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing – original draft. **MCB and JJMP:** Methodology, Validation. **All authors:** Resources, Data curation, Writing - Review & Editing. **DvS:** Supervision.

References

Gaudard, J. and others. 2025. fluxible: An R package to process ecosystem gas fluxes from closed-loop chambers in an automated and reproducible way. Methods in Ecology and Evolution **00**: 1–9. doi:10.1111/2041-210X.70161

Gerardo-Nieto, O., A. Vega-Peñaranda, R. Gonzalez-Valencia, Y. Alfano-Ojeda, and F. Thalasso. 2019. Continuous Measurement of Diffusive and Ebullitive Fluxes of Methane in Aquatic Ecosystems by an Open Dynamic Chamber Method. Environ. Sci. Technol. **53**: 5159–5167. doi:10.1021/acs.est.9b00425

Hoffmann, M., M. Schulz-Hanke, J. Garcia Alba, N. Jurisch, U. Hagemann, T. Sachs, M. Sommer, and J. Augustin. 2017. A simple calculation algorithm to separate high-resolution CH_4 flux measurements into ebullition- and diffusion-derived components. Atmospheric Measurement Techniques **10**: 109–118. doi:10.5194/amt-10-109-2017

Hüppi, R., R. Felber, M. Krauss, J. Six, J. Leifeld, and R. Fuß. 2018. Restricting the nonlinearity parameter in soil greenhouse gas flux calculation for more reliable flux estimates. PLOS ONE **13**: e0200876. doi:10.1371/journal.pone.0200876

Hutchinson, G. L., and A. R. Mosier. 1981. Improved Soil Cover Method for Field Measurement of Nitrous Oxide Fluxes. Soil Science Society of America Journal **45**: 311–316. doi:10.2136/sssaj1981.03615995004500020017x

Kutzbach, L. and others. 2007. CO_2 flux determination by closed-chamber methods can be seriously biased by inappropriate application of linear regression. Biogeosciences **4**: 1005–1025. doi:10.5194/bg-4-1005-2007

Lauerwald, R. and others. 2023. Inland Water Greenhouse Gas Budgets for RECCAP2: 1. State-Of-The-Art of Global Scale Assessments. Global Biogeochemical Cycles **37**: e2022GB007657. doi:10.1029/2022GB007657

Maier, M. and others. 2022. Introduction of a guideline for measurements of greenhouse gas fluxes from soils using non-steady-state chambers. Journal of Plant Nutrition and Soil Science **185**: 447–461. doi:10.1002/jpln.202200199

Mannich, M., C. V. S. Fernandes, and T. B. Bleninger. 2019. Uncertainty analysis of gas flux measurements at air–water interface using floating chambers. Ecohydrology & Hydrobiology **19**: 475–486. doi:10.1016/j.ecohyd.2017.09.002

Martinsen, K. T., T. Kragh, and K. Sand-Jensen. 2018. Technical note: A simple and cost-efficient automated floating chamber for continuous measurements of carbon dioxide gas flux on lakes. Biogeosciences **15**: 5565–5573. doi:10.5194/bg-15-5565-2018

Minaudo, C. and others. 2023. Methodological manual, Deliverable D4.2, EU Horizon Project RESTORE4Cs, Project GA ID: 101056782. Methodological Manual D4.2. D4.2.

Minaudo, C. and others. 2025. CO2 and CH4 floating chamber incubation time series from EU Horizon project RESTORE4Cs and results from methodological experiment. doi:10.5281/zenodo.17828220

Perolo, P., B. Fernández Castro, N. Escoffier, T. Lambert, D. Bouffard, and M.-E. Perga. 2021. Accounting for surface waves improves gas flux estimation at high wind speed in a large lake. Earth System Dynamics **12**: 1169–1189. doi:10.5194/esd-12-1169-2021

Pirk, N., M. Mastepanov, F.-J. W. Parmentier, M. Lund, P. Crill, and T. R. Christensen. 2016. Calculations of automatic chamber flux measurements of methane and carbon dioxide using short time series of concentrations. Biogeosciences **13**: 903–912. doi:10.5194/bg-13-903-2016

R Core Team. 2025. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Regulation (EU) 2021/1119. 2021. Regulation (EU) 2021/1119 of the European Parliament and of the Council of 30 June 2021 establishing the framework for achieving climate neutrality and amending Regulations (EC) No 401/2009 and (EU) 2018/1999 ('European Climate Law'). OJ L **243**.

Rheault, K., J. R. Christiansen, and K. S. Larsen. 2024. goFlux: A user-friendly way to calculate GHG fluxes yourself, regardless of user experience. Journal of Open Source Software **9**: 6393. doi:10.21105/joss.06393

Silva, J. P., A. Lasso, H. J. Lubberding, M. R. Peña, and H. J. Gijzen. 2015. Biases in greenhouse gases static chambers measurements in stabilization ponds: Comparison of flux estimation using

linear and non-linear models. Atmospheric Environment **109**: 130–138. doi:10.1016/j.atmosenv.2015.02.068

Sø, J. S., K. Sand-Jensen, and T. Kragh. 2024. Self-Made Equipment for Automatic Methane Diffusion and Ebullition Measurements From Aquatic Environments. Journal of Geophysical Research: Biogeosciences **129**: e2024JG008035. doi:10.1029/2024JG008035

Vachon, D., T. Langenegger, D. Donis, and D. F. McGinnis. 2019. Influence of water column stratification and mixing patterns on the fate of methane produced in deep sediments of a small eutrophic lake. Limnology and Oceanography **64**: 2114–2128. doi:10.1002/lno.11172

Vachon, D., Y. T. Prairie, and J. J. Cole. 2010. The relationship between near-surface turbulence and gas transfer velocity in freshwater systems and its implications for floating chamber measurements of gas exchange. Limnology & Oceanography **55**: 1723–1732. doi:10.4319/lo.2010.55.4.1723

Varadharajan, C., and H. F. Hemond. 2012. Time-series analysis of high-resolution ebullition fluxes from a stratified, freshwater lake. Journal of Geophysical Research: Biogeosciences **117**. doi:10.1029/2011JG001866

Villa, J. A., Y. Ju, T. Yazbeck, S. Waldo, K. C. Wrighton, and G. Bohrer. 2021. Ebullition dominates methane fluxes from the water surface across different ecohydrological patches in a temperate freshwater marsh at the end of the growing season. Science of The Total Environment **767**: 144498. doi:10.1016/j.scitotenv.2020.144498

Wang, B., and Q. Liao. 2016. Field observations of turbulent dissipation rate profiles immediately below the air-water interface. Journal of Geophysical Research: Oceans **121**: 4377–4391. doi:10.1002/2015JC011512

Wilson, S. J., B. Bond-Lamberty, G. Noyce, R. Bittencourt Peixoto, and J. P. Megonigal. 2024. fluxfinder: An R Package for Reproducible Calculation and Initial Processing of Greenhouse Gas Fluxes From Static Chamber Measurements. Journal of Geophysical Research: Biogeosciences **129**: e2024JG008208. doi:10.1029/2024JG008208

Xiao, S. and others. 2016. Theoretical model for diffusive greenhouse gas fluxes estimation across water-air interfaces measured with the static floating chamber method. Atmospheric Environment **137**: 45–52. doi:10.1016/j.atmosenv.2016.04.036

554 Zappa, C. J., W. R. McGillis, P. A. Raymond, J. B. Edson, E. J. Hintsa, H. J. Zemmelink, J. W. H. Dacey,
555 and D. T. Ho. 2007. Environmental turbulent mixing controls on air-water gas exchange in
556 marine and aquatic systems. Geophysical Research Letters **34**: 2006GL028790.
557 doi:10.1029/2006GL028790
558 Zhao, X. and others. 2022. Method for demodulating the overlapping absorption spectra of CO and CH_4.
559 Opt. Express, OE **30**: 43464–43479. doi:10.1364/OE.474833
560 Żygadłowska, O. M. and others. 2024. Ebullition drives high methane emissions from a eutrophic coastal
561 basin. Geochimica et Cosmochimica Acta **384**: 1–13. doi:10.1016/j.gca.2024.08.028
562

Figure Legends

Figure 1. Overview of experts' contributions. Number of incubations inspected for each of the 17 different experts (a) and count of incubations inspected from once to the highest number of independent inspections (b).

Figure 2. Experts' selection of time series segments for all CO_2 (a) and CH_4 (b) incubations, ordered by the starting time of the selected data segments, normalised from 0 (start) to 1 (end). Colours indicate whether >50% ("Extended") or <50% of the original measurement was retained. Subcategories for <50% indicate whether selections occurred in the early, late, or spanning portions of the time series. Panels (c–d) show counts for each selection category.

Figure 3. Example of experts' data selection for two incubations (given IDs are 34 and 140). Panels (a–b) show CO_2 selections; (c–d) show selections for estimating CH_4 diffusion. Vertical lines and horizontal bands denote each expert's start and end points.

Figure 4. Variability in expert-derived CO_2 and $CH_{4\ tot}$ flux estimates. Panels (a,d): SD vs mean; (b, e): CV vs mean; (c, f): expert vs fully automated estimates with 1:1 line indicated in solid black. Colour scale indicates LM.MAE of the untrimmed incubation time series.

Figure 5. CV of expert-derived CH_4 fluxes for different levels of ebullition contribution to the total CH_4 flux. Ebullition ratio was calculated according to the fully automated data processing.

Figure 6. a) CV of expert-derived F_{CO2} vs CV of $F_{CH4\ tot}$ estimates. Colours indicate diffusion- (orange) or ebullition- (purple) dominated incubations. (b - c) Examples of CO_2 and CH_4 measurements

corresponding to high variability in experts' flux estimates for both gases: incubation A (ebullition-dominated, non-linear pattern) and B (diffusion-dominated, curved).

Supplement

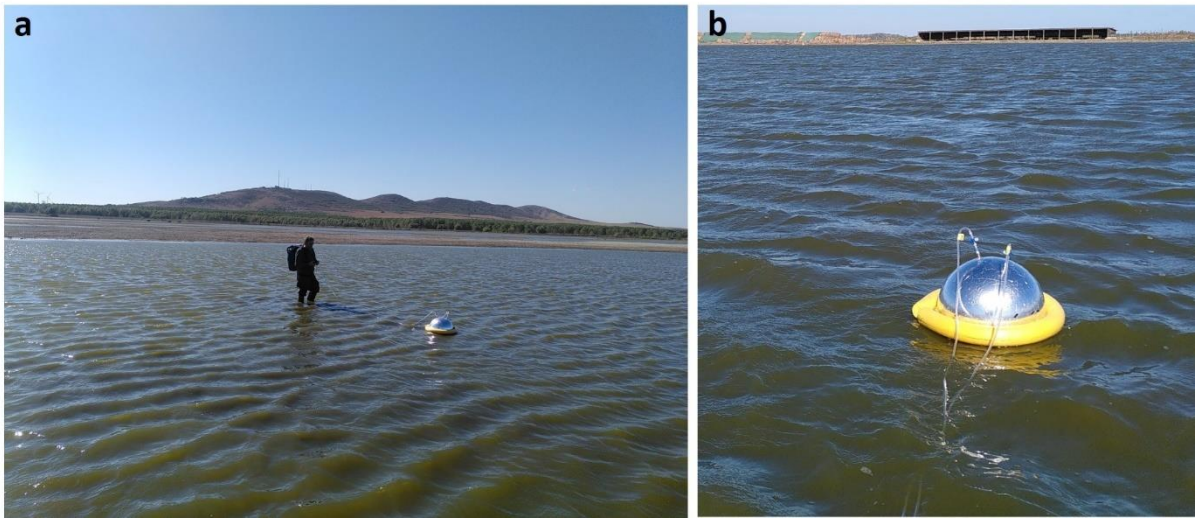


Figure S1. Floating chamber deployment during one of the project's sampling campaign, either on foot in shallow areas (a) or by boat (b), minimizing perturbation of the measurement as much as possible. The opaque floating chamber is connected to a portable gas analyser which measures GHG partial pressure in the chamber headspace every second by cavity ring-down spectrometry.

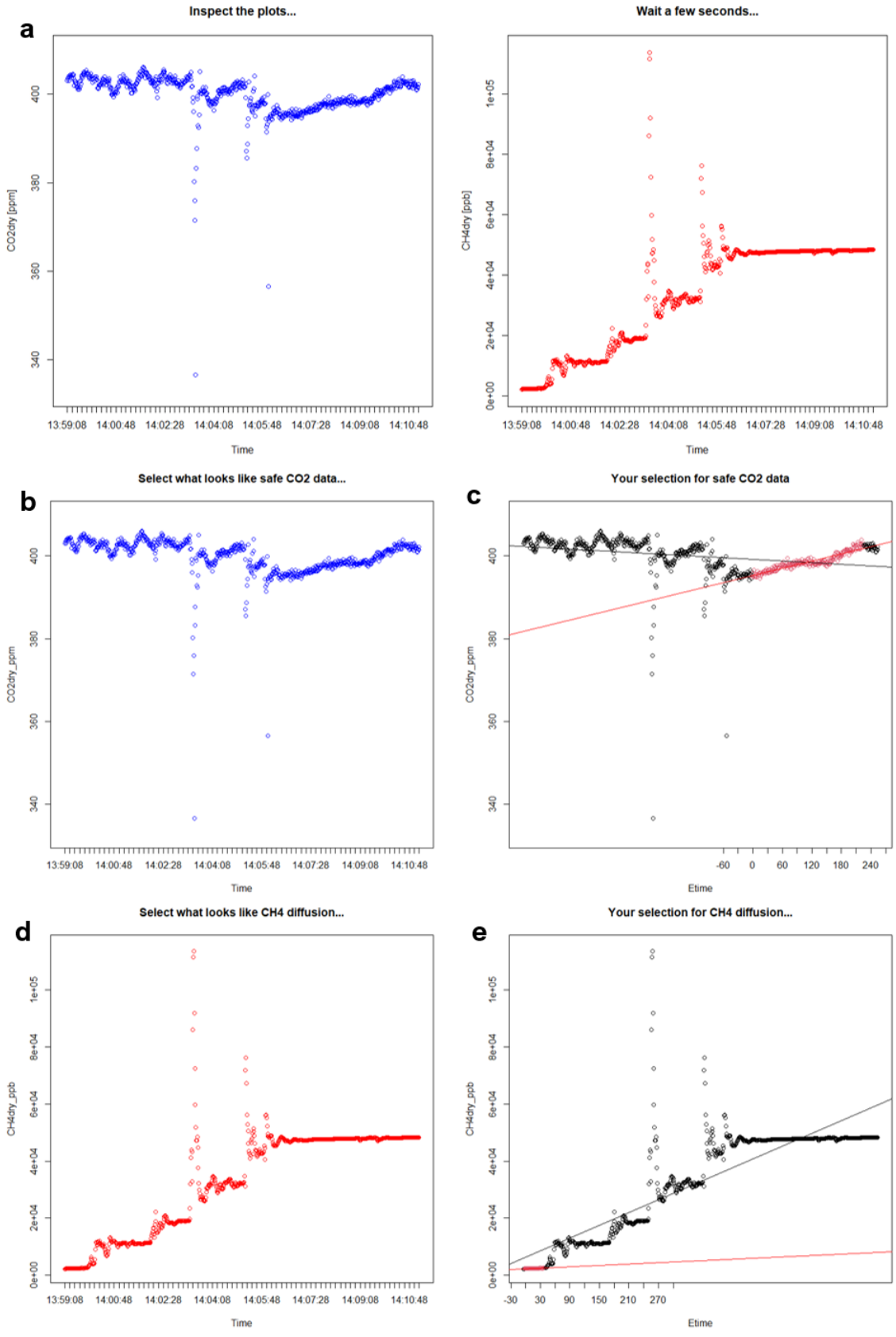


Figure S2. Expert's experience when running the script to perform manual selection of an incubation time series. First, both CO_2 and CH_4 time series were shown (a). Then experts had to manually select the starting and ending point of the data segment considered as valid CO_2 measurements (b). A plot was displayed with a linear regression applied in black to the entire time series

or to the expert's selection in red (c). Then, the CH_4 measurements were shown (d) and the expert had to select the starting and ending points of the time series considered valid for further calculation of diffusive patterns. Again, a plot was displayed with a linear regression applied in black to the entire CH_4 time series or to the expert's selection in red (e). Note that this particular incubation corresponds to incubation A in Figure 5.

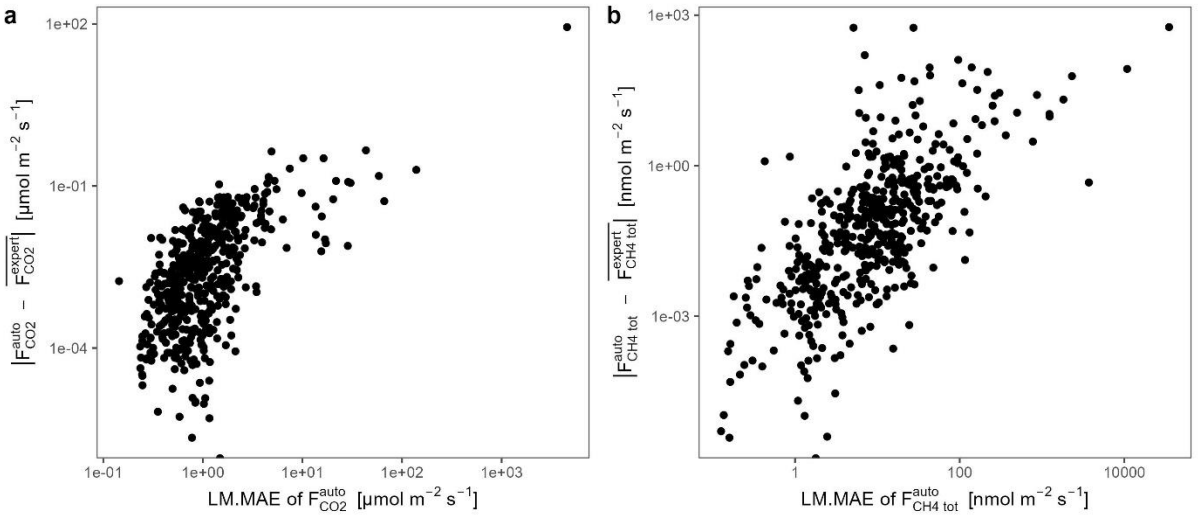


Figure S3. Absolute flux difference between the mean experts' flux and fully automated flux processing as a function of Mean Absolute Error of a linear model (LM.MAE) for CO_2 (a) and CH_4 (b) across all incubation time series.