Reviewer 1 - General comments

Keck et al. report methodological advances in monitoring isotope ratios of N2O emitted from waste water treatment processes. They conducted a detailed investigation of the dependence of N_2O isotope ratios on N_2O and oxygen concentrations and long-term stability of the measurements, and established an automated method for obtaining accurate analytical values using infrared absorption spectroscopy for sources with high variability.

The strength of this paper is that the developed system allows for frequent measurement of N_2O isotope ratios at the source site. This is particularly useful for assessing anthropogenic sources that fluctuate significantly over time. I hope that this method will provide information on the hour-by-hour processes of N_2O formation and dissipation, thereby contributing to proposals aimed at reducing N_2O emissions from various N2O sources including WWTP.

However, I am concerned that the authors have made little mention of the shortcomings and limitations of this method compared to conventional mass-spectroscopic or infrared-absorption-spectroscopic measurements using an intermittent gas sampling method. The following disadvantages can be cited. First, the system requires a large amount of dilution gases and calibration gases to maintain constant N2O concentration of the analyte gas fed to the laser spectrometer and to correct for temporal drift of the spectrometer responses, respectively. Although the cost of synthetic air to dilute raw sample gases may be low, it is necessary to install pure air cylinders or pure air generators at the site, and one has to consider logistics. Preparation of calibration standards will likely require both money and effort, as some of the authors have already reported. Second, as stated in conclusion, the developed system actually allows three 5-minute averaged measurements per hour. I expect the authors' further efforts to improve the measurement frequency, but at this stage, I think it would be an exaggeration to state that this method allows "continuous analysis" of N2O in source gases.

In summary, this paper is worth publishing in Atmospheric Measurement Techniques after revision regarding above points and minor comments below.

Reply: Dear Reviewer, first we would like to express our gratitude for your time and efforts to review our manuscript. Your thorough commentary is highly appreciated and helped significantly in improving the manuscript and making it more relevant for the AMT readers.

Reviewer 1 - Reply to the general comment

The reviewer rightly points out certain constraints of the method proposed here.

For the method proposed in this manuscript, we projected an annual gas consumption of approx. 25 m^3 yr^{-1} of dilution gas (< four 50 L high pressure cylinders at 150 bar) and 14.6 m^3 yr^{-1} of calibration gas (i.e. less than two 50 L high pressure cylinders of Cal1/drift gas and less than half a 30 L cylinder of Cal2 gas, both at 150 bar). This represents a significant effort and cost, but needs to be compared a) to alternatives for a comparable evaluation and b) to the potential benefits, i.e. reduction in GHG emission resulting from better understanding of the emission dynamics and according plant optimization (potentially worldwide).

Using an intermittent sampling protocol and analyzing the samples using a laser absorption spectroscopy (LAS) system installed in the lab (e.g., similar to Gruber et al. 2022), will lead to significant labor costs, as for a regular sampling interval bag samples need to be manually collected from the WWTP reactors. Costs will rise further if an assessment of the diurnal cycle is of interest. The collected bag samples need to be analyzed using a similar setup as introduced in our manuscript and therefore, dilution and calibration gases as well as expertise in calibration gas handling and analytical corrections of LAS systems would still be required. In addition, procedures for bag sampling and off-line analysis need to be carefully tested and validated to avoid data loss due to fractionation effects during storage etc.

Using an intermittent sampling protocol and IRMS analysis will have similar constraints as listed above. Applying a combined grab sampling / offline analysis procedure inevitably limits the sampling frequency, whereas the method proposed in this manuscript is scalable, i.e. the measurement frequency can be increased for sequential analysis of multiple reactors.

In the revised version of our manuscript, we addressed the shortcomings as well as advantages in more detail on page 19 L 376 (under Sect. 3.1, Performance of the measurement setup):

"*A further possible constraint is the necessity to use relatively large volumes of N_2O-free dilution air and the need for frequent drift corrections and calibrations, especially as necessary isotopic calibration standards at the required process concentrations and in the appropriate gas matrix are not yet commercially available (Mohn et al., 2022; Os-*

trom et al., 2018). However, the resulting efforts and costs need to be evaluated against the alternatives. Intermittent sampling protocols bear significant labour costs, as the samples need to be collected manually, the sample containers need to be cleaned and prepared beforehand, and the analytics setup adapted for the target application. In addition, procedures for sample storage need to be carefully tested to avoid data loss due to fractionation effects. Furthermore, off-line analytics requires a similar analytical setup, dilution gas and isotopic calibration standards Furthermore, the setup described in this study is scalable, i.e the measurement frequency can be increased significantly if higher temporal resolution is needed or for sequential analysis of multiple reactors. This enables high-frequency monitoring, providing high temporal resolution to capture short-term fluctuations and dynamic processes."

Reviewer 1 - Replies to the specific comments:

Comment #2: Title. "continuous" would be better replaced with "high-frequency" or "on-line".

Reply: Thank you, we changed the title accordingly.

Comment #3: L62. There is a paper (Toyoda et al., 2011) published earlier than Wunderlin et al. (2013) that reported isotope ratios of N2O emitted from WWTP.

Reply: We added the citation of Toyoda et al. (2011).

Comment#4: L74 & L80. "resN2O" and "redN2O" look very similar and are easy to confuse. I suggest the authors to use a single character (with sub/super scripts, if any) to represent a variable. Please also refer to this journal's submission guidelines.

Reply: We now substituted resN2O with "f_{N2O}" and redN2O with "f_{N2}".

Comment #5: L120. From this point onward, measurements have been conducted with the N_2O concentration set to 12 ppm. Please explain why this value was chosen —was it set to match the lowest expected N_2O concentration in WWTP emissions, or is there another reason?

Reply: The reason for choosing 12 ppm as the dilution target was a balance between choosing a low enough concentration to resolve significant emissions from the WWTP, as well as making sure that the analytic precision remains high as it reduces (gets worse) with lower concentrations.

We added the following changes to the text at page 12, line 222, section 2.3.1: "*The target N_2O mole fraction (12 ppm) represents as a compromise for analyser sensitivity and non-linearity, which both degrade data quality at low N_2O concentrations and temporal coverage, i.e. sufficiently low N_2O target concentration to cover relevant emissions.*"

Comment #6: L137. Although two-point calibration was conducted, neither the isotopic values of N2O in target gas nor sample gas fall within the range of the two standard gas values. This means the authors are extrapolating; please explain whether this is acceptable and justify the validity of this approach.

Reply: Thank you for this comment, we agree with the reviewer's observation that Cal1 and Cal2 do not cover the actual measurement range in WWTP. However, accuracy of results within uncertainty targets is confirmed by independent analysis of the target gas, which offers similar isotopic composition to the sample gas as indicated on in Tab. 1. Therefore, we are confident that the calibration can be extrapolated linearly throughout our measurement ranges.

We added the following changes to the text at page 7, line 156, section 2.2.2:

"*We acknowledge that δ-values of Cal1 and Cal2 do not cover most of the measurement range relevant for WWTP. The accuracy of measurement results, however, was assessed using target gas measurements (Tab. 1), at an isotopic compositions typical for the WWTP application. Measurements were performed …*"

Comment #7: L150. Do "blocks" mean the four dates described in the next line?

Reply: Thank you, yes, indeed it referred to the measurement dates. For clarity we use "experiments" instead:

Page 7, line 156, section 2.2.2: "*The accuracy of measurement results, however, was assessed using target gas measurements (Tab. 1), at an isotopic composition typical for the WWTP application performed in four experiments distributed over the complete*

measurement period (24th September 2024, 30th and 31st January 2025, 11th September 2025)."

Comment #8: L159. It would be helpful for readers unfamiliar with this method if the authors explain following points in the introduction or discussion section: the reasons and principles behind why isotope ratio measurements using infrared absorption spectroscopy depend so heavily on concentration of target gas and coexisting gases.

Reply: Details on the dependency of apparent delta values on target gas concentration, gas matrix and spectral interferants is provided in the cited literature (Harris et al., 2020). We added the following text to the Introduction section to forewarn the reader on challenges connected with process gas measurements and changing gas composition.

Page 3, line 88, section 1:

"*The fundamental reasoning behind these phenomena is the difference in pressure broadening caused by different bulk gases (e.g. N_2, O_2, Ar) and spectral interferences by non-target gaseous species with absorptions in the analysed spectral range. Furthermore, changes in analyte gas concentration can lead to inaccurate apparent delta values due to detector non-linearities, residual baseline effects, or other phenomena. It is therefore good practice to apply isotopic reference gases, which mimic the composition of the sample in target gas concentration, matrix composition, and relevant trace gases. In this respect, process studies with strong changes in gas composition pose an inevitable challenge, as the reference gas can only be adapted to the most relevant or average sample gas composition, while residual variability has to be monitored and delta values post-corrected, if changes exceed a critical threshold.*"

Comment #9: L168–169. It is unclear how the authors are using the term “strong”. Does it mean that the slope of delta value versus N2O (d(delta)/d(N2O)) is large? If so, it would be better to show the concentration ranges lower than 6 ppm more closely, as in the case of 8-16 ppm range. The horizontal scale of Figure 2 is too coarse, making it difficult to read the data. Simply adding a secondary scale would help. Also, because the inset figure of 8-16 ppm range hides the plots of d15Nbulk, d18O, and SP at >50 ppm, one cannot examine the relationship for high concentration ranges.

Reply: As suggested by the reviewer we changed figure 2; i.e. included two different scales for the x-axis and removed the small zoom-in plot, as details are easier to spot in the new figure, including concentrations (< 6 ppm).

In addition, changed the term "was strongest" to "showed largest nominal values" (page 8, line 177, section 2.2.3).

Comment #10: L178. I think air or oxygen is injected not only to oxidize ammonium to nitrite, but also oxidize organics using microorganisms.

Reply: Very true. We changed the wording in the text as follows (page 10, line 191, section 2.2.4): "*In nitrifying zones of WWTPs, air is injected to oxidize NH_4^+ to NO_2^- and NO_3^- as well as organic matter to CO_2.*"

Comment #11: L213–214. Notation of variables are not fully explained and are partly inconsistent or confusing. For example, is the integral sign attached to delta_cal1 in line 213 the same as "Int" attached to the parameters in equation 3? Moreover, eq. 3 itself is not correct if I follow the explanation described in L211–213. It seems that the coefficients of the time-difference terms in the denominator are reversed.

Reply: Thank you, we exchanged the integral signs with "int", abbreviating "interval" and included "t" in our explanation. Furthermore, we reformulated the sentence (page 15, line 303, section 2.5) to "*For each sample interval at time t_S, the drift-related offset was determined by subtracting the overall mean based on all Cal1 intervals recorded during an experiment ($\delta_{Cal1}^{Mean,Raw}$) from the linear interpolation of δ-values of the two nearest bracketing Cal1 intervals ($\delta_{Cal1}^{\mathrm{int}(+)}$ and $\delta_{Cal1}^{\mathrm{int}(-)}$) during time $t_{Cal_1}^{\mathrm{int}(+)}$ and $t_{Cal_1}^{\mathrm{int}(-)}$*" In that logic we need to subtract the time of int(-) from the time of int(+) in the denominator of equation 3, thus the equation remains correct.

Comment #12: L215. I think subscript "sa" in equation 4 denotes sample, but the authors have already used "SA" to mean synthetic air. To avoid confusion, replace with other expression.

Reply: Thank you, we now refer to "sample" with "S" only and continue using "SA" for synthetic air.

Comment #13: L237–238. I think estimating the isotope ratios at the time of formation based on the distribution of observed values is a second-best approach, but it is not clearly explained how the reduction line was estimated. To calculate the "perpendicular distance" of the data point and the reduction line, the latter must be estimated independently with the observation. While the slope can be determined using literature values, how was it decided which points on the graph the line passes through (or what the y-intercept should be)?

Reply: We agree that the procedure to evaluate δ_0 should be described better in the text. In addition, we justify our choice of using system specific δ_0 values, as the range of literature data is too broad and would introduce large uncertainties. The updated procedure is provided on page 16, line 327, section 2.5.1:

"*As relevant δ_0 values for SP reported in literature span from −7.5 to 1.9 ‰ (hD: −7.5 to 3.7 ‰; nD: −13.6 to 1.9 ‰; Yu et al. 2020), we estimated our system specific δ_0 value based on our dataset. First, it was ensured that the SP-$\delta^{18}O$ regression slope fell within the expected range reported in literature (0.23 to 0.98; Yu et al. 2020), then the value of δ_0 was estimated for SP by ranking observations according to the sum of their $\delta^{18}O$ and SP values of which we retained the five lowest-ranked points. Further, the perpendicular distance of each of the lowest-ranked observations in the SP and $\delta^{18}O$ space to the regression line was calculated and the observations with the smallest distance selected. The corresponding SP value was defined as δ_0 (Fig. 5).*"

Comment #14: L242. I agree that d18O of N2O is related to d18O of water, but do the authors argue that N2O and water is under full isotopic equilibrium? I wonder the changes in relative contributions from Hy and nD/hD or the changes in dissolved oxygen isotope ratios due to partial consumption might affect d18O of N2O or precursors like NO2-.

Reply: We agree with the arguments of the reviewer and adapted the approach to calculate the fraction of residual N_2O as described in our reply to comment #13 of reviewer #1.

Comment #15: L248. Does "repeatability of the instrument" include uncertainty of drift correction?

Reply: Yes, as stated in Table 2, repeatability refers to the standard deviation for repeated drift-corrected measurements. No changes were made to the text.

Comment #16: L260. It would be helpful if the authors explain derivation of equation 9. What does "n" mean?

Reply:

Reply: Thank you, indeed, "n" needs to be explained in the text page 18, line 356, section 2.5.2. We derived equation 10 (numbering according to new manuscript version) from Eq. 1 as follows:

Reformulation of Eq. 1 to linearize it:

$$lnf = \frac{\delta_R - \delta_0}{\varepsilon}$$

Treating δ0 and ε as constants allows us to propagate the error through the linear function:

$$lnf = \frac{1}{\varepsilon} * \sigma_{\delta_R} - \frac{\delta_0}{\varepsilon}$$

e.g.:

$$\sigma_y = |m| \sigma_x$$

Thus:

$$\sigma_{lnf} = \frac{\sigma_{\delta_R}}{\varepsilon}$$

Including the uncertainty reduction due to averaging of repeated measurements of δ_R:

$$\sigma_{\delta_R} = \frac{\sigma_i}{\sqrt{n}}$$

Leads to:

$$\sigma_f = \frac{\sigma_i}{|\varepsilon| \sqrt{n}}$$

Where n is the number of measurements, and notably, σf_{N2} the relative uncertainty of f_{N2}, as:

$$\frac{d(lnf)}{df} = \frac{1}{f}$$

Thus:

$$\sigma_{lnf} = \frac{\sigma_f}{f}$$

Comment #17: Figure 4. I would like to confirm the accuracy of the description in this figure regarding the gas sampling method from the reactors. A portion of the water's surface is covered, and something resembling a chimney is depicted. Is the gas being actively drawn from the water surface, or is it passively carried by the air-flow generated by aeration? This question concerns whether the representativeness of the collected gas is ensured.

Reply: This is a misunderstanding; in fact, the complete reactor is covered, so the analyte gas is representative of the sampled reactor. In addition, the statement that a subsample is drawn from the reactor head space is misleading as the analyte gas was extracted from an emission duct purged with aeration air (incl. the N_2O analyte). Comparing aeration gas flows and extracted analyte gas flows, only 0.3% to 2% of the gas is diverted to the analytic equipment. With a gas flow rate in the chimney of 0.2 to 2 m/s, diffusive gas transport backwards through the chimney to the reactor headspace can be excluded (see Strubbe et al. (2026) for more information).

We edited Figure 4 to represent the actual setup more closely and added a statement to page 14, line 253, section 2.4:

"During aeration periods the air volume in the reactors head space was constantly replaced by the aeration gas bubbling through the reactors liquid phase (Fig. 4). A subsample of the off-gas was constantly drawn from the emission duct to the analytic equipment and analyzed for isotopic signatures. Only data from the aeration phases were used for further analysis."

Comment #18: L380–382. It seems the authors assume that isotopic signature of produced N2O is constant over time and that the variation in dO and SP is simply caused by difference in the progress of N2O reduction. This assumption should be clearly stated along with its basis, together with the reason why the slope of the line in Figure 6 is different from that in Figure 7 or Figure A1. To my eyes, variation around the regression line seems to indicate variation in the produced N2O.

Reply: This is a misunderstanding, we do not assume that the isotopic signatures of $\delta^{18}O$ and SP of the initially formed N_2O, not affected by N_2O reduction, are constant over time. We provide an improved description of our procedure in response to reviewer#1 comment #13/14, as shown above.

In addition, we added an explanatory sentence to the legend of Figure 7 (new manuscript; page 22, line 435, section 3.2.1): "*Differences in reduction slopes observed for different experiments are likely due to variations in the microbial community.*"

Comment #19: L391–393. This statement is difficult to confirm looking at Figure 7B, mainly because the color gradients corresponding to the time are difficult to distinguish.

Reply: We updated the figure 8 (new manuscript) using a more visible color gradient.

Comment #20: L417–421. Although the caption of Figure 8 mentions grey dots representing the results from standard operation, I cannot see the data points. If I estimate the d15Nbulk values for the standard operation of ca. -50‰ from Figure 5, d15Nbulk obtained for Cycle 1 here is higher by 40-60‰. Assuming the d15N of NH4 during the standard operation of 6-24‰ based on literature (Toyoda et al. 2011), the enhancement of d15N of N2O due to the labeled NH4 is significantly smaller than the difference in d15N of NH4+ (100 – 6 or 24 = 94 to 76‰). If the measured d15N of N2O here is correct, what would be the reason for the discrepancy?

Reply: We agree and improved the contrast between the grey dots and the background in Figure 9 (new manuscript) for better visibility.

Interpretations of reviewer #1 on differences in $\delta^{15}N\text{-}NH_4^+$ and $\delta^{15}N\text{-}N_2O$ are based on a misunderstanding. In fact, none of the experiments is at ambient $\delta^{15}N\text{-}NH_4^+$ as assumed by the reviewer but both experiments shown on Figure 9 (cycle 1 and 2) are after $^{15}N\text{-}NH_4^+$ addition. While for cycle 1, $\delta^{15}N\text{-}NH_4^+$ is set by $^{15}N\text{-}NH_4^+$ addition to +100 ‰ for the consecutive cycle the actual $\delta^{15}N\text{-}NH_4^+$ is not provided but certainly above ambient as assume by the reviewer. As the focus of this manuscript is on the optimisation / performance of the analytics, $\delta^{15}N\text{-}NH_4^+$ analysis and detailed interpretation of experiments is beyond the scope of this study but will be published elsewhere.

Comment #21: P28. Fix the citation of Morley et al..

Reply: This has been fixed. Thank you.

Comment #22: Figure A1. I can see only a single “blue area”. If the authors really plotted both light blue and dark blue areas, please differentiate them by improving the color contrast.

Reply: We increased the contrast between the light blue and the dark blue area in Fig. 5 (new manuscript).

References

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