

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 N₂O isotope ratios on N₂O 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 N₂O 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 N₂O formation and dissipation, thereby contributing to proposals aimed at reducing N₂O 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

Comment #1 of Reviewer 1:

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. $25 \text{ m}^3 \text{ yr}^{-1}$ of dilution gas (< four 50 L high pressure cylinders at 150 bar) and $14.6 \text{ m}^3 \text{ yr}^{-1}$ 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; Ostrom 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_{N_2O} " and redN₂O with " f_{N_2} ".

Comment #5: L120. From this point onward, measurements have been conducted with the N₂O concentration set to 12 ppm. Please explain why this value was chosen—was it set to match the lowest expected N₂O 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 N₂O mole fraction (12 ppm) represents as a compromise for analyser sensitivity and non-linearity, which both degrade data quality at low N₂O concentrations and temporal coverage, i.e. sufficiently low N₂O 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. N₂, O₂, 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 ($d(\delta)/d(N_2O)$) 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 $d^{15}N_{bulk}$, $d^{18}O$, 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 δ_{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 ($\delta_{Cal1}^{int(+)}$ and $\delta_{Cal1}^{int(-)}$) during time $t_{Cal1}^{int(+)}$ and $t_{Cal1}^{int(-)}$ "* 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- $\delta^{18}\text{O}$ 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 $\delta^{18}\text{O}$ 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 $\delta^{18}\text{O}$ 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 N₂O 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:

$$\ln f = \frac{\delta_R - \delta_0}{\varepsilon}$$

Treating δ_0 and ε as constants allows us to propagate the error through the linear function:

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

e.g.:

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

Thus:

$$\sigma_{\ln f} = \frac{\sigma_{\delta_R}}{\varepsilon}$$

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, $\sigma_{f_{N2}}$ the relative uncertainty of f_{N2} , as:

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

Thus:

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

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 N₂O 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 $\delta^{18}\text{O}$ 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 $\text{d}^{15}\text{N}_{\text{bulk}}$ values for the standard operation of ca. -50‰ from Figure 5, $\text{d}^{15}\text{N}_{\text{bulk}}$ obtained for Cycle 1 here is higher by 40-60‰. Assuming the d^{15}N of NH_4 during the standard operation of 6-24‰ based on literature (Toyoda et al. 2011), the enhancement of d^{15}N of N_2O due to the labeled NH_4 is significantly smaller than the difference in d^{15}N of NH_4^+ ($100 - 6$ or $24 = 94$ to 76‰). If the measured d^{15}N of N_2O 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 $\delta^{15}\text{N}-\text{NH}_4^+$ and $\delta^{15}\text{N}-\text{N}_2\text{O}$ are based on a misunderstanding. In fact, none of the experiments is at ambient $\delta^{15}\text{N}-\text{NH}_4^+$ as assumed by the reviewer but both experiments shown on Figure 9 (cycle 1 and 2) are after $^{15}\text{N}-\text{NH}_4^+$ addition. While for cycle 1, $\delta^{15}\text{N}-\text{NH}_4^+$ is set by $^{15}\text{N}-\text{NH}_4^+$ addition to +100 ‰ for the consecutive cycle the actual $\delta^{15}\text{N}-\text{NH}_4^+$ is not provided but certainly above ambient as assumed by the reviewer. As the focus of this manuscript is on the optimisation / performance of the analytics, $\delta^{15}\text{N}-\text{NH}_4^+$ 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

Toyoda, S., Suzuki, Y., Hattori, S., Yamada, K., Fujii, A., Yoshida, N., et al. (2011). Iso-topomer analysis of production and consumption mechanisms of N₂O and CH₄ in an advanced wastewater treatment system. *Environmental Science and Technology*, 45, 917–922. <https://doi.org/10.1021/es102985u>

Reviewer 2

The manuscript by Keck and co-authors describes the deployment of an optical instrument for the analysis of N₂O isotopes at an experimental wastewater treatment plant. Despite such analysers being commercially available for over a decade, their operation is technically cumbersome and challenging, potentially limiting the use of these analysers. The fact that the authors have succeeded in operating such an analyser fully automated in the field with high performance is welcome and very newsworthy. The authors performed three experiments: 1) analysis of data from long-term deployment, 2) variation in dissolved oxygen, 3) addition of ¹⁵N-labelled compounds. The experiments provide conclusive results that demonstrate the ability of such measurements to deliver actionable data to wastewater treatment plant operators: isotope analysis in N₂O, along with knowledge on the isotopic composition of local tap water, indicate the realised/or remaining potential in N₂O emissions reduction. The study is comprehensive, very well thought out, and highly relevant. I therefore find it will be a very valuable contribution to AMT.

General comments:

In my view, this manuscript is likely to be of interest to spectroscopists, biogeochemists, but also to GHG emission-focused wastewater treatment plant operators, who may not be the typical AMT audience. With this in mind, I recommend making the rationale behind the presented isotope technique more accessible to a broader audience interested in WWTP operations, which may be minor or major revisions. The authors are affiliated with a world-leading isotope laboratory, and different approaches to interpret isotope signals is common practice for the authors. However, this may not be the case for the wider audience potentially interested in this technique. I'd therefore suggest going through the manuscript and improve the story telling, guide the reader through critical thinking processes you present, take more time to spell out what is happening in the WWTP and how that relates to what you observe in the isotopes. How can you then use isotope data to infer what is happening in the WWTP and how to mitigate emissions? What isotope scenarios may a WWTP operator observe and what does this say about the processes? How are these processes reflected in your isotope data? I'd expect this will maximise the impact and interdisciplinary uptake of this valuable publication.

Reviewer 2 - Reply to the general comments #1:

We would like to thank the reviewer for this positive assessment of the significance and potential of the measurement method presented.

Further interpretations of our data, focusing on process engineering aspects, have been published in Strubbe et al. (2026) in the journal Water Research, where we expect to reach readers with a corresponding thematic focus. Additional datasets interpreting variability in N₂O reduction and ¹⁵N-labelling are in preparation for publication. Although a detailed interpretation of data in conjunction with process parameters and supportive variables (e.g. N-substrate concentrations, carbon and oxygen content) is beyond the scope of this study we agree to add some context on the explanatory framework for the experimental results sections:

Page 21, line 424, section 3.2.1:

"Controlling the concentration of ammonium, nitrite, and dissolved oxygen nitrifier denitrification was successfully isolated from heterotrophic denitrification and became more active at lower dissolved oxygen concentrations. Lower dissolved oxygen, higher organic carbon availability, and lower pH increased N₂O production by heterotrophic denitrification during aeration. These new insights provide a systematic framework for understanding N₂O dynamics and support the development of mitigation strategies at full-scale."

Page 23, line 471, section 3.2.2:

"Isolating the N₂O-to-N₂ reduction step by on-line isotopic analysis for the first time provides insights into its key drivers, helping identify operational levers to reduce production and enhance consumption of N₂O, supporting the design of net-zero emission treatment systems. The detailed interpretation of positive or negative effects of operational factors on N₂O reduction beyond data shown here, i.e. pH, temperature, total air consumption, total suspended solids, will be published elsewhere."

Reviewer 2 - Replies to the specific comments:

Comment #2: Line 39: maybe "..., countries and WWTP facilities still rely on..."?

Reply: Thank you, we changed the text accordingly.

Comment #3: Line 41: However, different emission factors...

Reply: Thank you, we changed the text accordingly.

Comment #4: Line 79: maybe "mass-dependent isotope effect", instead of "normal isotope effect"?

Reply: Thank you, we changed the text accordingly.

Comment #5: Line 88: Here and everywhere else: use mole fraction or amount fraction instead of concentrations for gases. This differentiation is especially important as the manuscript includes concentrations of dissolved compounds. You could clarify that the term mole fraction is used for all gases within a gas matrix, concentrations for everything in a water matrix?

Reply: Thank you for pointing this out. We changed the wording accordingly.

Comment #6: Line 89: maybe "matrix effect" instead of "bulk gas composition"?

Reply: Thank you, we changed the text accordingly.

Comment #7: Line 97: "We demonstrate the first..."

Reply: Thank you, we changed the text accordingly.

Comment #8: Line 102: "...practical recommendation on how to..."

Reply: Thank you, we changed the text accordingly.

Comment #9: Line 116: "sections, optimal"

Reply: Thank you, we changed the text accordingly.

Comment #10: Line 117: "approaches, as"

Reply: Thank you, we changed the text accordingly.

Comment #11: Line 124: Can you comment on a potential N₂O blank?

Reply: The N₂O concentration in synthetic air was below 1 ppb; a respective statement was added to page 12, line 219, section 2.3.1 and Table 1

Comment #12: Line 133: "From every 10-minute measurement interval, the first..."?

Reply: Thank you, we changed the text accordingly.

Comment #13: Line 133: Why is the number of discarded minutes variable (3-5), and what does it depend on?

Reply: The analytical setup at the WWTP was periodically used for several projects in parallel, this required us to adjust our measurement interval lengths (8 or 10 minutes). For consistency, we decided to use the last 5 minutes of each measurement interval, which lead to discarding 3 or 5 minutes depending on the interval.

Comment #14: Line 138: Can the SA gas be added to Table 1?

Reply: We added the SA gas to Table 1.

Comment #15: Line 140: "isotopic composition analysed by EMPA", can you give a reference for the technique?

Reply: The analytical technique used for N₂O isotopic analysis at Empa is presented in Heil et al. (2014). The respective reference was added to the text (page 7, line 145, section 2.2.2).

Comment #16: Line 154: Here and everywhere else: it seems to me that the uncertainty might be within 1 significant digit? Suggest simplifying by rounding, i.e., 0.3, 0.9, 0.5, 0.2 and 0.9 permille?

Reply: Thank you, we adapted the text accordingly.

Comment #17: Line 157: Can you comment on potential offsets between EMPA and Science Tokyo from previous studies/publications?

Reply: Primary calibration gases applied at Empa were analyzed at Science Tokyo, and therefore several inter-laboratory comparisons indicated a good agreement in

N₂O isotope analyses between Empa and Science Tokyo. We added a respective statement and references to page 7, line 164, section 2.2.2:

"This discrepancy is higher than differences observed in past inter-laboratory comparisons between Empa and Science Tokyo (Mohn et al., 2014; Ostrom et al., 2018) but was not further addressed as it is within the uncertainty targets of our study."

Comment #18: Line 174: You show in Figure 2 that the N₂O mole fraction effect is variable with time. The magnitude of the time-dependent variation is massive in some examples. Can you provide a comment on your certainty that this effect is sufficiently captured across your experimental period of 1 year, even though it was only determined on 4 days? Also, I wonder if it's worth focusing on the time-dependent variation within the 11-13 ppm band, which is somewhat acceptable. The variation over the entire N₂O range is very interesting but could also be shown as an Appendix figure without the insert, which covers this for 3 out of 5 isotope ratios.

Reply: We agree that the observed temporal variability of the N₂O mole fraction effect on delta values is very high and four measurements within a one-year period are insufficient to account for this effect considering the full measurement range of the presented spectrometer. As correctly observed by reviewer #2 variations within the 11-13 ppm N₂O range applied are acceptable, which is demonstrated by the agreement of repeated target gas measurements.

We agree to show a better representation of the most relevant lower concentration range (0.5-20 ppm) and compress data in the higher concentration range, where fewer measurements were collected. This is in agreement with our response to comment #9 of reviewer #1.

Comment #19: Line 180: The authors describe data processing details before explaining the analytical setup, described in Figure 4. I wonder if describing the setup first would help the logical flow? Otherwise, when you talk about CO₂ removal here, can you give a reference to Figure 4?

Reply: Thank you for the suggestion, we moved the section about the analytical setup before the data processing section and now refer to the relevant section in text: "[...] since any produced CO₂ is removed prior to analysis (see section 2.3.1 for details)."

Comment #20: Line 182: If this is pure N₂, wouldn't this experiment vary O₂ as well as Ar at the same time, causing this to show the effect of simultaneously varying both gases? You speculate about the Ar effect in line 157, does it matter here?

Reply: We agree that in the described experiment both oxygen and argon concentrations are varied, which is not discussed. In fact, diluting Cal1 with N₂ and synthetic air to 12 ppm N₂O and a target O₂ concentration of 5% / 10%, e.g. a reduction in O₂ by 15.9% / 10.9%, reduces argon by 0.69% / 0.55%. According to our experiments published in Harris et al. (2020), gas matrix effects of argon are expected to increase oxygen effects by around 10%. We added this information to page 10, line 197, section 2.2.4:

"As N₂ did not contain Ar, in parallel with a decrease in O₂ by e.g. 16 % Ar dropped by 0.7 %. Earlier studies (Harris et al., 2020) indicate that the gas matrix effect attributed to O₂ for an OA-ICOS analyzer as tested here might therefore be overestimated by around 10 %, and our uncertainty estimates (section 2.5.2) are rather conservative."

In addition, we added a respective statement to the legend of figure 3 (page 11, Line 213, section 2.2.4): *"Presented O₂ gas matrix effects are conservative, i.e. overestimated, due to a parallel decrease in Ar mole fractions (0.5 % Ar per 10 % O₂)."*

Comment #21: Line 186: Maybe "...was linear over the entire..."?

Reply: Thank you, we adapted the text accordingly.

Comment #22: Line 189: Maybe "was measured using a PG..."?

Reply: Thank you, we adapted the text accordingly.

Comment #23: Line 206: The typesetting of the letters in the bracket seems a bit off.

Reply: Thank you for pointing this out. We fixed the typesetting in the new manuscript.

Comment #24: Line 220: It is not clear to me if all isotope measurements of the entire year are corrected for the same O₂ difference, or each 5 minute average isotope value for the O₂ difference corresponding to those 5 minutes?

Reply: Thank you, we reformulated the corresponding sentence to (page 16, line 311, section 2.5): *"With respect to the O₂ mole fraction correction of δ -values an offset correction to average O₂ mole fractions in the analyte gas (20.6 % O₂) was applied over the entire measurement period, instead of a correcting for actual O₂ mole fraction values"*

Comment #25: Line 229: I do not understand this section. Please clarify.

Reply: We reformulated the section as mentioned above in response to comment #13 of reviewer #1.

Comment #26: Line 235: What is the lower-left region? Does this correspond to a figure?

Reply: Yes, the statement refers to Fig. 5 (new manuscript). For clarity we highlighted the 5 lowest-ranked points and plotted the figure as Fig. 5 in the main text.

Comment #27: Line 236: What are the 5 lowest-ranked points? Are they shown in Figure A1, if so, can this be visualised/described with more clarity?

Reply: Please see reply above.

Comment #28: Line 239: I'd suggest moving Figure A1 into the main text.

Reply: Please see reply above.

Comment #29: Line 248: How is the repeatability of the instrument determined?

Reply: By the standard deviation of repeated drift-corrected measurements of the target gas. See our response to comment #15 of reviewer #1.

Comment #30: Line 269: How many measurements fell outside the 11-13 ppm band, what percentage was discarded?

Reply: During standard WWTP operations from September to December 2024 the proportion of data outside of the 11-13 ppm band was 53 %. We included this in the current manuscript as follows (page 12, line 219, section 2.3.1):

"The target mole fraction of the dilution system was set to 12 ppm N₂O and data with N₂O mole fractions outside the range of 11 to 13 ppm were discarded (53 % of data)."

Comment #31: Line 282: Could "retained" be a better word to describe what this filter does?

Reply: Thank you, yes indeed, we adapted the text accordingly.

Comment #32: Line 286: Does the CO₂ sensor not have a fixed flow rate?

Reply: The flow to the CO₂ sensor was throttled to approximately 80 ml / min, to ensure a constant overflow passing the sensor. We updated the Figure 5 (new manuscript) to include the overflow after the CO₂ sensor for clarification.

Comment #33: Line 294: "The reactors received subsamples of the local municipal wastewater"?

Reply: Thank you for indicating an unclear formulation. We changed the sentence to: *"The reactors were fed with local municipal wastewater taken directly from the sewer".*

Comment #34: Line 303: Maybe "The following representative datasets were obtained under three different experimental conditions and are used to demonstrate the usability and applicability of our analytical; setup."?

Reply: Thank you for proposing a better formulation that is now included in the text for better understanding.

Comment #35: Line 305: I suggest be more clear: "Experiment 1: To determine..." and then refer to this in the results/discussion section.

Reply: Thank you. We changed the text accordingly

Comment #36: Line 306: Here and elsewhere, the text talks about "standard operation", but it is not always clear if this refers to the measurement setup of the WWTP. Could this say "standard WWTP operation"?

Reply: Thank you. We changed the text accordingly

Comment #37: Line 319: "different operational conditions of the WWTP."?

Reply: Thank you. We changed the text accordingly

Comment #38: Line 335: "11 to 13 ppm N₂O target range."

Reply: Thank you. We changed the text accordingly

Comment #39: Line 348: "...the isotopic measurement presented here will be capable..."

Reply: Thank you. We changed the text accordingly

Comment #40: Line 349: It might be worth mentioning that these data are in fact "actionable", therefore high impact and potential to influence decisions by WWTP operators.

Reply: Thank you, we added the following text to 3.1 Performance of the measurement setup (page 19, line 390, section 3.1):

"Thus, the isotopic data are actionable, enabling the identification of the root source of emissions and their underlying drivers in near real time. This information can support evidence-based operational decisions and targeted mitigation strategies, thereby enhancing the ability of WWTP operators to reduce N₂O emissions effectively."

And to the conclusions (page 26, line 523, section 4):

"Thus, the isotopic data are actionable and directly relevant for improved process understanding and optimization to support evidence-based operational decisions targeting the reduction of N₂O emissions from WWTPs."

Comment #41: Line 351: I wonder why Figure 5 does not include SP?

Reply: We included SP in Figure 6 (new manuscript).

Comment #42: Line 357: "Experiment 1: Constraining..."? For a reader who is not familiar with the experiments, relating this section directly to the experiment description would make it a lot easier to follow. In my view, this is the text location from

where onwards it could be very beneficial to get more guidance on the assumptions and what has been done. Why do you do what you do, why are several data important.

Reply: Thank you for this indication leading to better readability of our manuscript. We suggest the addition of the following text:

"To determine the predominant microbial N₂O biological production pathway, we analysed the N₂O isotopic composition over several months (September to December 2024) under standard WWTP operation, i.e. at a dissolved O₂ (DO) concentration setpoint of 2 mg L⁻¹ (±0.5 mg L⁻¹) and without N-substrate additions."

To rationalize discrepancies between measurement results and indicated source signature areas the following text was added to page 21, line 415, section 3.2.1:

"This interpretation may strike the reader as odd, as the data points so obviously lay outside of the shaded source signature areas that are traditionally visualised as boxes in dual isotope plots. These source signature areas (see Fig. 7 and Fig. 8A) are based on a limited number of pure culture studies and laboratory incubations (Yu et al., 2020) and therefore cannot be perceived as hard limits but require refinement to fully capture the dynamics of complex mixed microbial communities as are found in wastewater treatment reactors. Thus, the isotopic source signatures and their overlaps will be subject to change with increased understanding of the complexities of managed and natural N₂O source systems. To determine system-specific source signature areas and to robustly differentiate between the contributions of nD and hD to N₂O formation, it requires further dedicated experiments that aim at stimulating both processes independent from each other [...]"

Comment #43: Line 361: I am not sure if "corrected" is the right concept here. The goal is to observe the difference between H₂O and N₂O. Why is that important, what does Dd18O tell us? Could this be described with readers in mind who are not isotope specialists?

Reply: We now included a section under "data processing" addressing the dependence of δ¹⁸O (N₂O) on δ¹⁸O of ambient water (page 14, line 256, section 2.4):

"As oxygen exchange between nitrogenous oxides and H₂O can occur in abiotic systems but also mediated by microbes (Kool et al 2007), the δ¹⁸O of N₂O partly reflects the

$\delta^{18}\text{O}$ of the dissolution water. This dependency was considered by subtracting the $\delta^{18}\text{O}$ signature of the local tap water ($\delta^{18}\text{O}(\text{H}_2\text{O}) = -11.2\text{‰}$) from the $\delta^{18}\text{O}$ of N_2O before further interpretation."

Comment #44: Line 363: It took me a while to understand that the slope of 0.37 is the slope you show in Fig 6 from your data of Experiment 1, and that it is not the slope reported by Yu 2020. Can this be clarified in the text, and maybe also included in the figure itself? At a later point, you mention the range in slopes reported by Yu 2020. Maybe the range reported by Yu 2020 can be presented here, when Yu 2020 is mentioned first to discuss your slope in the context of their findings?

Reply: We updated Fig. 7 (new manuscript) to include the regression line formula and changed the text to (page 21, line 408, section 3.2.1):

"Concurrent changes in SP and $\Delta\delta^{18}\text{O}(\text{N}_2\text{O}, \text{H}_2\text{O})$ along a straight line with a slope of 0.37 indicate successive reduction of N_2O to N_2 as the slope falls within the range defining the reduction line, spanning from 0.23 to 0.98 (Yu et al., 2020)."

Comment #45: "From Fig 6, we can infer nitrifier denitrification (nD) or heterotrophic denitrification (hD)..."

Reply: Thank you, we changed the text accordingly.

Comment #46: Line 369: Can you explain more broadly why you think your data show nD and hD as the most dominant sources, even though your data are outside the corresponding shaded areas in Fig 6?

Reply: Thank you, this question arises easily when looking at the dual plots. We changed the text accordingly to (page 21, line 411, section 3.2.1):

"From Fig. 7 we can infer nD or hD as the dominant N_2O production process, as measurements fall within the range of isotopic signatures expected for denitrification processes with a minimal effect of N_2O reduction (Yu et al., 2020) and are in accordance with other studies on wastewater treatment (Gruber et al., 2022; Wunderlin et al., 2013). Furthermore, the absence of elevated SP values ($> 30\text{‰}$) suggests that Hy was not a source term during our measurements (Frame and Casciotti, 2010; Sutka et al., 2006; Wunderlin et al., 2013; Yu et al., 2020). This interpretation may strike the reader as odd, as the data points so obviously lay outside of the shaded source signature areas

that we traditionally visualized as boxes in dual isotope plots. These source signature areas (see Fig. 7 and Fig. 8A) are based on a limited number of pure culture studies and laboratory incubations (Yu et al., 2020) and therefore cannot be perceived as decisive limits and require refinement to fully capture the dynamics of complex mixed microbial communities as are found in wastewater treatment reactors during regular operation. Thus, the isotopic source signatures and their overlaps are subject to change with increased understanding of the complexities of managed and natural N₂O source systems. To determine system-specific source signature areas and to robustly differentiate between the contributions of nD and hD to N₂O formation, it requires further dedicated experiments that aim at stimulating both processes independent from each other. A series of such experiments applying the analytical toolkit developed in this study is described in Strubbe et al. (2026)."

Comment #47: Line 377: Can you add the slope value or the equation for the linear fit to the figure?

Reply: We included the equation in Fig. 7 (new manuscript).

Comment #48: Line 381: "...with progressive heterotrophic N₂O reduction."?

Reply: We changed the text accordingly.

Comment #49: Line 383: "Experiment 2: Assessing..."?

Reply: Adapted accordingly.

Comment #50: Line 387: "The slope of our reduction line in Experiment 2 (0.8...)", or can it be clarified further that this slope is based on different data, excluding Experiment 1? Also, can you comment on why the slopes are different between Experiment 1 and 2? What does this tell someone who is not an expert in isotope techniques?

Reply: We reformulated the text to (page 22, line 441, section 3.2.2):

"The enrichment of SP, and $\delta^{18}\text{O}$ caused by N₂O reduction in experiment 2 is characterised by a slope of 0.80 (Fig. 8A), which falls within the data range defined by Yu et al. (2020) for such a process, supporting the validity of the here reported N₂O reduction fractions. As the two experiments (1 and 2) are separated in time, the difference in the

reduction line slopes is likely related to a shift in the active microbial community over time. This variability is within the range seen in scientific literature under similar settings (Gruber et al., 2022; Yu et al., 2020, Strubbe et al., 2026), but still represents an open research question worthwhile exploring in more detail."

Comment #51: Line 389: Here and everywhere else, consistent use of "DO concentrations" would help clarity.

Reply: Thank you, we changed the text accordingly.

Comment #52: Line 391: I am not sure I understand this section properly and suggest this to be clarified further. How do those redN₂O percentages relate to DO and isotopes? Can you guide the reader by simply explaining something general along the lines of "if this goes up, that goes down because of that and that." And then "what we see in our data is... suggesting that... Therefore, isotopes can highlight times when DO can be optimised to mitigate N₂O emissions. "

Reply: Thank you, we changed the wording to make it more accessible also for readers that are not familiar with isotopic analysis (page 23, line 447, section 3.2.2):

"In general, higher N₂O reduction was seen in the reactor that was set to a lower DO concentration compared to the reactor with a higher DO concentration setpoint. This can be seen in Fig. 8A, as the data from the reactor with a lower DO setpoint are higher up on the SP and $\delta^{18}\text{O}$ scales compared to those from the other reactor. Intuitively, this can be explained by the fact that enzymes responsible for N₂O reduction preferentially cleaving the bonds between lighter isotopes, i.e. between $^{14}\text{N}-^{16}\text{O}$ rather than between $^{15}\text{N}-^{16}\text{O}$ or $^{14}\text{N}-^{18}\text{O}$, which leads to an accumulation of molecules with ^{18}O or ^{15}N in the central position in the residual, unreduced N₂O fraction. When applying Eq. 8 to calculate the N_R for both scenarios, we can quantify the difference in f_{N_2} between the treatments. The low DO concentration setpoint of 0.5 mg L⁻¹ led to an increase in the fraction of reduced N₂O by 40 % (± 2 %) compared to the DO setpoint of 2 mg L⁻¹. Therefore, on-line isotopic analysis can highlight periods during which the DO setpoints can be optimised for N₂O emission reduction."

Comment #53: Line 397: Can "decreasing O₂ concentration" be changed to "decreasing DO concentration", or is this referring to O₂ mole fractions in the gas phase?

Reply: Thank you, we changed it accordingly.

Comment #54: Line 398: Maybe “In contrast, Rees....”?

Reply: Thank you, we changed it accordingly.

Comment #55: Line 401: What species are you thinking of, “related to microbial species”?

Reply: Thank you, indeed we refer to microbial species here.

Comment #56: Line 405: I can’t see the colour/time gradient in the plotted symbols in A and B. Can the slope be written in the figure? The caption talks about % uncertainty, the axis is in fractions of 1, which seems inconsistent. Can all be in %?

Reply: We changed the colour gradient and converted the y-axis to percent.

Comment #57: Line 413: “Experiment 3: 15N...”?

Reply: Adopted.

Comment #58: Line 421: Can you expand the explanation of your thinking about the stagnation, i.e., 15N bulk initially increases because of that, then stagnates when NH₄ is depleted, while Dd18O still increases. What is happening when Dd18O still changes, but not 15N bulk? Why is NO₂ still changing for a bit, and why is NO₃ changing throughout the entire experiment? I can’t see much difference in the colours of the symbols in panel A. Is there a better way to show the length of time that has passed for each data point?

Reply: We thank the reviewer for this insightful comment. We agree that the observed behavior of the isotopic signatures during the labelled-N experiment raises several interesting process-related questions. However, we would like to emphasize that the primary objective of the present manuscript is the development and validation of the analytical approach and the demonstration that the system is capable of resolving low-level ¹⁵N labelling of the nitrogen pool. A detailed mechanistic interpretation of the isotope dynamics observed during the incubation experiments is beyond the scope of this methodology-focused paper. We are currently preparing a follow-

up manuscript that will specifically focus on the interpretation of the labelled-N experiments, including the temporal evolution of the ^{15}N and oxygen isotope signatures and the associated nitrogen transformation processes. Rather than providing a necessarily speculative and possibly incomplete discussion here, we believe that a dedicated treatment of these results is more appropriate in that context. To avoid overstating our interpretation, we have therefore kept the discussion in the present manuscript focused on the analytical performance and proof-of-capability aspects of the experiment.

Regarding the Figure 9 (new manuscript), we appreciate the reviewer's observation. We have revised the color gradient in Panel A to improve the distinction between symbols and to provide a clearer visualization of the temporal progression of the experiment.

Comment #59: Line 423: Isotope conventions are -27 should be shown as -27 , delta symbols are italicised.

Reply: Thank you, we adjusted "-" signs and δ accordingly.

Comment #60: Line 430: "standard WWTP operation"?

Reply: Thank you, we adapted the text accordingly.

Comment #61: Line 436: This is an unconventional way to refer to site preference. Why not use SP?

Reply: Thank you, we changed the text accordingly.

Comment #62: Line 452: "...greenhouse gas emission reduction."?

Reply: Thank you, we adapted the text accordingly.

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