Answer reviewer comments for manuscript [egusphere-2025-5010]

Comments from the reviewers are marked as "**rev {reviewer no./comment no.}**" in italicized font. The answers of the authors are indented and follow immediately after the reviewer comments. General statements made by the reviewers are not reproduced here. highlighted and ~~strikethrough~~ text shows changes in the manuscript. Line numbers (L) mentioned in our replies correspond to the original single-column (unrevised) version of the manuscript.

Reviewer 3

High level comments

rev 3/1 *Regarding protocol complexity: on L8 the authors write that their simplified approach eliminates the need for "complex purification protocols, specialized equipment, and experimental designs that yield little CO2 fixation and high uncertainties." To us it seems that the cavity ringdown spectrometer is a specialized piece of equipment and its use introduces the need for additional preparatory steps (e.g., filtration, dilution) that produce some measurement artifacts (L320). It would help to simply describe what the key equipment is and why it is cheaper, simpler, or more accessible than the standard approach. This would improve the abstract, introduction (L80) and discussion.*

We revised the abstract and the end of the Introduction to more clearly explain why the approach is cheaper, simpler, and more accessible than standard methods. These revisions make the advantages of the workflow clearer to the reader (see also **rev 2/1**).

rev 3/2 *Moreover, as is made clear near L195, calibration of the Apollo-Picarro system was done by comparison with IRMS. If an IRMS is required for calibration, then the equipment demands of this protocol are really no simpler than the standard approach.*

We thank the reviewer for raising this point. An IRMS is not required for determining $\epsilon_{\mathrm{Rubisco}}$ using the Apollo–Picarro workflow. IRMS measurements were used only to independently validate the Apollo–Picarro isotope data and were not used for routine calibration or for calculating fractionation factors. We clarified this distinction by revising Abstract and end of the Introduction (see **rev 2/1**) as well as the Methods section accordingly (see below).

L190-192: "To independently validate the Apollo-Picarro results, the carbon isotope composition of DIC was also measured using a GasBench II system (Thermo Fisher Scientific, Germany) equipped with an autosampler (CTC Analytics AG, Switzerland), coupled to a ConFlo IV interface and a Delta V Plus isotope ratio mass spectrometer (Thermo Fisher Scientific). The same in-house $NaHCO_3$ isotope standards used in the Apollo–Picarro setup were also employed in a standard bracketing procedure..."

rev 3/3 *Regarding novelty: the issue of rubisco purity has also been previously addressed by Estep et al. 1978 Plant Physiol but see further discussion below on why the community tends to not cite this paper. The authors should cite this work and generally avoid excessive claims of novelty. The paper is an excellent resource without. Moreover, it represents the first measurement of the G. oceanica rubisco KIE, which bolsters the low KIE value from E. huxleyi and S. costatum by Boller et al. 2011 and 2015 respectively.*

We thank the reviewer for this important point. We agree that the issue of Rubisco purity has been previously addressed by Estep et al.[6], and we have now cited this work and clarified its relevance in the Introduction (see below).

L78: " ~~The need~~ The requirement for highly purified enzymes adds complexity and time to an already demanding protocol, ~~and its necessity has not been experimentally validated yet~~ yet this requirement was experimentally validated primarily in early studies and has not been systematically re-evaluated using modern analytical approaches (Estep et al.,1978)."

rev 3/4 *Regarding correction for rubisco side reactions (L74): As far as we understand, the side reactions are not expected to affect the KIE even though they would affect the net rate of carboxylation. Moreover, it is common and appears to be defensible to monitor rubisco reactions to ≈50% completion to fit the Rayleigh curve and derive the KIE (see Guy et al. 1993).*

We appreciate the suggestion to clarify the relevance of the correction for side reactions. We propose to clarify in lines 68-74:

L72-74: "...In cases where RuBP was limiting, the assumption of full substrate consumption becomes questionable~~, particularly given the lack of correction for~~ . This can be particularity problematic when DIC concentration is not measured directly and changes in reaction rate caused by inhibitor formation ~~by~~ from Rubisco side reactions ~~over time~~ are not accounted for in the estimation of DIC depletion (Wang et al., 2023a, b; Pearce, 2006)..."

rev 3/5 *On clarity: because the methods section precedes the results, it was unclear to us what the model of rubisco inactivation is for. On first reading we thought that the model of activation state was directly related to the KIE measurement. We only realized later upon re-reading that its purpose is to estimate the right amount of rubisco to assay. This could be made clearer by simply stating the purpose of this model up front, e.g. in the methods section and appropriate text. For example, the text near L290 could be moved to the beginning of S3.2*

We thank the reviewer for this suggestion. We have clarified the overall rationale for the kinetic model in the final paragraph of the Introduction (see **rev 2/1**), noting that it accounts for DIC consumption dynamics during incubations and provides a rational basis for selecting appropriate enzyme concentrations and sampling intervals.

Additionally, at the beginning of Section 3.2 (Lines 265–268), we state: "The concentration of Rubisco used in the assay was a critical parameter, as it directly

influenced the rate of CO_2 consumption, the extent of dissolved inorganic carbon (DIC) depletion, and the optimal timing of sample collection. To optimize both the enzyme concentration and the sampling schedule, we developed a kinetic model to simulate DIC dynamics throughout the course of the reaction..."

And in the Methods (Section 2.6, Lines 117–118), we introduce the model as follows: "To improve calculation of the needed Rubisco concentration and optimal sampling times, we modeled the concentration dynamics of DIC during the *in vitro* Rubisco catalyzed CO_2 fixation assay using Michaelis-Menten kinetics... "

rev 3/6 *A technical comment on the kinetic model of rubisco inhibition: it seems that the authors fit a 2-parameter model (unknown k_{acc} and v_{max}) from a single time-course. It seems that this would produce ambiguous fits with high uncertainty because the same trace might be compatible with either lower v_{max} or higher k_{acc}. Some uncertainty quantification, e.g., estimating posterior parameter ranges, would be helpful here as the authors present this fitting procedure as an integral part of their method.*

We appreciate the reviewer suggestion to clarify this. We have now detailed in line 181 that our datasets contain sufficient time points to distinguish both the curvature defining k_{acc} and the peak defining v_{max}, therefore allowing us to constrain both paramters.

L179-L281: "...The inhibitor accumulation rate (k_{acc}) was treated as a free parameter. Similarly, while v_{max} could in principle be constrained from known k_{cat} values and Rubisco concentrations, the resulting fits were unsatisfactory, so v_{max} was also treated as a free fitting parameter. Our dataset contained sufficient time points to distinguish both the curvature defining k_{acc} and the peak defining v_{max}, therefore allowing us to constrain both parameters..."

rev 3/7 *On transparency of analysis: we did not see any links to source code for the data analysis performed. Please publish all relevant code – this is an essential component of scientific reproducibility and especially important for a methods paper.*

We agree with the reviewer that transparency and reproducibility are essential, particularly for a methods-focused manuscript. The kinetic modeling and parameter estimation were performed using Microsoft Excel, specifically employing the built-in Solver function to minimize the root mean square (RMS) error. As such, no standalone source code exists.

However, all equations, model formulations, and fitting procedures are fully described in Section 2.5 of the Methods, which allows the analysis to be readily reproduced in other computational environments (e.g., R, Python, or MATLAB). To further improve transparency, we have revised the Methods section to explicitly state that Excel Solver was used and to describe the optimization procedure in more detail (see below).

L236-238: "...The parameters v_{max} and k_{acc} were estimated by fitting the model to experimental data. Parameter optimization was achieved by minimizing the root mean square deviation between the modeled and measured DIC concentrations. For this purpose, we used the built-in Solver function in Microsoft Excel."

rev 3/8 *On citation of unpublished work: the authors cite an unpublished study of their own. This citation is not essential to any of the arguments presented and could be omitted.*

We thank the reviewer for raising this point. As described by the reviewer, the cited unpublished study is not essential for any of the arguments or conclusions presented in this manuscript. We retain the citation because this study, developed in parallel and submitted simultaneously, provides further example of the use of this method for $\epsilon_{\text{Rubisco}}$ determinations across a broader range of taxa than described here. We will update this citation when this paper progresses towards publication.

rev 3/9 *On evolutionary constraints in section 3.6: We found this discussion of evolutionary constraints on rubisco to be out of place in an otherwise excellent methodological paper. The review of prior literature is somewhat out of date, omitting key references that posit alternative mechanisms that can affect rubisco carbon KIEs (Tcherkez et al. 2013 Biochemistry; Tcherkez et al. 2013 Plant Cell Environ; Bathellier et al. 2020 PNAS; Tcherkez and Farquhar 2021 J Plant Phys). In addition, if the authors do want to rely on the Tcherkez et al. 2006, they must also measure rubisco oxygen KIEs as a key aspect of that argument is that the oxygen KIE does not vary with specificity while the carbon KIE does. We strongly recommend that the authors omit or heavily trim this section.*

We appreciate the reviewers thoughtful comments regarding the scope and framing of Section 3.6. Although this manuscript has a strong methodological focus, it also presents new $\epsilon_{\text{Rubisco}}$ data for an additional Rubisco lineage. As is standard practice in studies reporting new $\epsilon_{\text{Rubisco}}$ values (e.g., Boller et al.[2,3], Thomas et al.[13]), we believe it is important to place these data within the broader biological and evolutionary context of previously reported values.

That said, we agree with the reviewer that the discussion can be strengthened. In particular, we recognize that there is an active debate regarding whether biochemical trade-offs or phylogenetic constraints play the dominant role in shaping Rubisco kinetics and associated $\epsilon_{\text{Rubisco}}$. Both perspectives are supported by existing evidence, and we have revised Section 3.6 to reflect this debate more explicitly and fairly.

Specifically, we now acknowledge alternative biochemical and kinetic mechanisms proposed to influence $\epsilon_{\text{Rubisco}}$ values and have expanded Section 3.6 to include discussion of Bathellier et al.[1], Tcherkez[9], Tcherkez and Farquhar[10], Tcherkez et al.[11], which extend beyond the framework of Tcherkez et al.[12]. At the same time, we now also cite studies emphasizing the role of phylogenetic constraints (Bouvier et al.[4], Bouvier and Kelly[5]), providing a more balanced treatment of the literature.

We further acknowledge the reviewer's point that a rigorous test of the hypothesis proposed by Tcherkez et al.[12] would require measurements of the oxygen kinetic isotope effect. We now clarify that such measurements are beyond the scope of the present study and identify them as an important direction for future work.

To address these points, we have revised Section 3.6 as follows:

L445-L448: "... As more measurements accumulate across phylogenetically diverse Rubisco families, it is becoming increasingly clear that no universal correlation exists between isotopic fractionation and specificity. Instead, different Rubisco lineages may follow distinct evolutionary trajectories, with isotope effects shaped by lineage-specific structural and mechanistic constraints. Recent studies that explicitly compare Rubisco kinetic properties with evolutionary origin support this interpretation, whereas other work suggests that phylogenetic effects may play a secondary role relative to biochemical constraints (Bouvier (2021); Tcherkez (2021); Bouvier (2023)). We note that additional biochemical and kinetic mechanisms affecting $\epsilon_{\text{Rubisco}}$ have been proposed (e.g., Tcherkez (2013); Tcherkez (2013); Bathellier (2020)), and a full evaluation of these hypotheses — including measurements of oxygen kinetic isotope effects — should be considered in future studies."

rev 3/10 *Figure 1: since these data are presented quantitatively in the text, please give the quantification in a second panel, e.g. as a bar plot.*

The purpose of Figure 1 is to provide a qualitative visual assessment of Rubisco purity in the semi-purified and fully purified extracts using SDS-PAGE. Quantitative estimates of purification are already presented and discussed in detail in the first Results section. Adding a separate quantitative panel was therefore not considered necessary, as it could overemphasize an aspect that is not central to the main objectives of the study.

Moreover, including quantitative information directly alongside the SDS-PAGE image could be misleading, as the gel itself is intended to be interpreted qualitatively, while quantification was performed using complementary approaches described in the text. We believe that the current presentation provides a clear and balanced view of both the visual and quantitative aspects of Rubisco purification.

rev 3/11 *Figure 2: the number of colors used in the figure is excessive. A legend would help a lot. Also, the dark green marks in panel (a) are not described in the figure or caption, but only in the text. In principle, Figure 3 could be part of this figure.*

We thank the reviewer for the careful reading of the figure. We have added a sentence to the caption to clarify the meaning of the dark green marks in panel (a) (see **rev 2/6**), and we have also added a legend to Figure 2.

A consistent color code is used throughout the manuscript to represent the three different organisms and their respective controls, which helps the reader follow the experimental results. All colors have been tested for color-blind accessibility, and there are no restrictions on the number of colors used in figures. Given these considerations, we chose to retain the current color scheme, which effectively conveys the experimental distinctions.

rev 3/12 *Figure 4: a legend would help here to define what the diamonds/triangles are.*

We agree. A legend has been added to Figure 4 to clearly define the symbols (diamonds and triangles).

rev 3/13 *Figure 6: again, why proliferate colors?*

We refer to our response to **rev 3/11**. A consistent color scheme is used throughout the manuscript to distinguish organisms and experimental conditions, which we believe improves readability and continuity across figures. The colors were chosen to clearly separate datasets and have been checked for color-blind accessibility. Given these considerations, and because the current color scheme effectively conveys the relevant distinctions, we have left the figure unchanged.

rev 3/14 *Figure 7: it is irresponsible to report an R2 value to a manually selected subset of the data. We strongly encourage the authors to (1) omit this fit line from the figure and (2) tone down their discussion of its evolutionary implications. There is simply too little data to draw solid conclusions from. Even in the case of rubisco reaction kinetics (e.g., Flamholz Biochem 2019), where there is far more data, such conclusions are not easy to come by.*

We do not consider including the subset and its associated R^2 value as irresponsible. The subset selection is not arbitrary, but based on Rubisco forms, which reflect fundamental structural and evolutionary differences among enzymes from different lineages. While some forms appear to follow a global correlation, others do not. The fit is intended solely to illustrate that no universal correlation between $\epsilon_{\mathrm{Rubisco}}$ and $S_{\mathrm{c/o}}$ is observed across the currently available data.

We have also tempered the discussion in the text to avoid overinterpreting this limited dataset and to focus on highlighting variation among lineages rather than drawing firm evolutionary conclusions (see also **rev 3/9**). The figure therefore serves as a visual aid to contextualize our new measurement, not to imply definitive evolutionary trends.

rev 3/15 *Table A1: This table should be expanded and provided in excel or CSV. It would be helpful to specify which reference provides the KIE and which the specificity. It is worth citing multiple measurements when available and useful to report additional kinetic parameters, e.g., $k_c$at and K_m values, by examination of recent meta-analyses, e.g. Flamholz et al. Biochem 2019 and Iñiguez et al.[8] Iniguez et al. 2020. Please also comment in the text and caption as to whether this collection of rubisco carbon KIEs is complete.*

We appreciate the reviewer's detailed suggestions regarding Table A1

In response to the reviewer's request for improved clarity, we have revised Table A1 to separate references for $\epsilon_{\mathrm{Rubisco}}$ and $S_{\mathrm{c/o}}$ into distinct columns, making it explicit which sources provide which parameter. We have also added a statement to the table caption noting that, to the best of our knowledge, this compilation represents a complete collection of published $\epsilon_{\mathrm{Rubisco}}$ for which corresponding Sc/o values are available at the time of writing.

However, we believe that the scope of this table should remain focused. The primary purpose of Table A1 is to document the data underlying Figure 7, which relates published $\epsilon_{\mathrm{Rubisco}}$ to $S_{\mathrm{c/o}}$. Accordingly, the table is intentionally limited to the parameters required for this comparison. Additional kinetic parameters such as k_{cat} and K_m are not discussed elsewhere in the manuscript, and including them would go beyond the scope of the present study.

Similarly, while multiple determinations may exist for some Rubiscos, Figure 7 uses averaged values where appropriate, and Table A1 reflects the data required to support this visualization rather than all individual measurements. A comprehensive meta-analysis of Rubisco kinetics, as presented in studies such as Flamholz et al.[7], Iñiguez et al.[8] is outside the intent of the present work.

Regarding data format, we prefer to provide all supplementary information within a single SI file. We do not see a clear advantage in additionally providing the table in Excel or CSV format, as all data are already fully accessible. That said, should the editor specifically request submission in an alternative format, we would of course comply.

Specific Comments

rev 3/16 *L39: refer to table A1 here.*

We agree and have added a reference to Table A1 at this point in the manuscript.

rev 3/17 *L41: this sentence is cuttable, especially as it cites an unpublished work.*

Please see **rev 3/8**

rev 3/18 *L45: why is KIE variation important?*

KIE variation is not "important" in itself; however, characterizing this variation is crucial because it determines how $\epsilon_{\text{Rubisco}}$ values can be applied when interpreting carbon isotope records. We clarified this point in the Introduction by adding the following sentence:

L44-45: "...This wide variation has been documented in only a limited number of species, suggesting that additional values are yet to be discovered. Characterizing this variation is essential, as $\epsilon_{\text{Rubisco}}$ directly influences interpretations of carbon isotope records used to reconstruct past biological activity and environmental conditions. However,.."

rev 3/19 *L71: explain why this method no longer requires accounting for oxygenation-derived 3PGA.*

Because we do not measure the reaction product but instead follow isotopic changes in the substrate pool, the revised text clarifies that the substrate depletion method no longer requires accounting for oxygenation-derived 3-PGA (see below).

L71-L72 "Although the substrate depletion method no longer requires accounting for oxygenation-derived 3-PGA because the measurement focuses on isotopic changes in the substrate rather than the reaction products, some applications have reported high variability in $\epsilon_{\text{Rubisco}}$ estimates within the same species."

rev 3/20 *L75: give reference to studies that report experiments with < 30% DIC consumption*

We added the references. We also rewrote the sentence to clarify that these studies, despite exhibiting low DIC conversion, still obtained reproducible results, and to more clearly articulate the limitations associated with low DIC consumption (see text below):

L74-L77: "...Moreover, several studies report experiments in which DIC consumption remained below ~~with less than~~ 30 % DIC ~~consumption~~ — and in some cases even ~~less than~~ below 6 % — in at least some replicates, yet still yielded reproducible $\epsilon_{\text{Rubisco}}$ values. While these results suggest the assay can yield consistent outcomes under low substrate turnover, such low conversion rates inherently reduce the reliability of the linearization required for Rayleigh fractionation, increasing uncertainty and potentially compromising the accuracy of the derived $\epsilon_{\text{Rubisco}}$ values (Wang et al.,2023a, b; Boller et al., 2011, 2025; Thomas et al., 2019). ~~casting doubt on the reliability of the linearization required for Rayleigh fractionation, increasing uncertainty and potentially compromising the accuracy of the derived $\epsilon_{\text{Rubisco}}$ values.~~"

rev 3/21 *L78: cite Estep et al. 1978 Plant Physiol for prior work on testing whether rubisco purity matters. See their Table 2 for carbon KIEs from spinach prepared to different purities; they conclude, like the authors here, that "It can be seen that fractionation is independent of enzyme purity." This is a landmark study in our field, but it is unfortunately infrequently cited because the absolute KIE values are off for reasons unrelated to the important conclusions about purity and metalation state.*

We have now cited Estep et al.[6] and revised the surrounding text to clarify its relevance (see also **rev 3/3**).

rev 3/22 *L83: "a single instrument" – specify which instrument.*

We have specified the instrument as suggested; see **rev 2/1** for details.

rev 3/23 *L275: worth noting other other reasons why rubisco deviates from Michaelis-Menten kinetics beyond inhibitor formation. For example, the activation state can be changed, and many organisms express catalytic chaperones (rubisco activases) that catalyze the disinhibition of the enzyme complex, etc.*

We agree that other factors can cause deviations from simple Michaelis–Menten kinetics, such as changes in Rubisco activation state. In the revised manuscript, we now acknowledge these additional mechanisms (see below). Rubisco activases, however, function primarily *in vivo* and are unlikely to affect in vitro assays, so we have not included them in this context.

L279-L181: "...Similarly, while v_{max} could in principle be constrained from known k_{cat} values and Rubisco concentrations, the resulting fits were unsatisfactory, so v_{max} was also treated as a free fitting parameter. We note that other factors, such as changes in Rubisco activation state, can also contribute to deviations from simple Michaelis–Menten kinetics, but these were not considered in this model."

rev 3/24 *L324: why is the dilution required?*

The dilution is required to allow injection of a larger sample volume into the Apollo–Picarro system, which improves the accuracy and precision of the isotope measurements.

L122-224: "...This discrepancy likely stems from differences in sample processing: samples analyzed using the GasBench were taken directly from the reaction assay, whereas those measured on the Apollo-Picarro system were first diluted with 2 mL of reaction buffer (110 mM EPPS) prior to injection to permit a larger injection volume, thereby improving measurement accuracy and precision...."

rev 3/25 *Table 1: what does it mean when you write "2-3"? That you pooled samples? Please clarify in place.*

Yes. The entry "2–3" indicates that the reported $\epsilon_{\text{Rubisco}}$ value is a pooled estimate derived from replicates 2 and 3. We added a footnote to clarify this further:

Footnote: Indicates which replicates were used to calculate $\epsilon_{\text{Rubisco}}$ using the Pitman estimator.

rev 3/26 *L425: This filtration step seems like it exposes the rubisco reaction to air, which deserves more prominent mention and discussion than it is given. Please find a place to explain why this does not affect the KIE measurement much.*

We agree that exposure to air during this step warrants careful consideration. We minimized contact with air during filtration as much as possible, and to explicitly test whether this step affected the kinetic isotope effect, we performed control experiments with *S. oleracea* Rubisco both with and without the filtration step. The resulting $\epsilon_{\text{Rubisco}}$ values were statistically indistinguishable. We have now added a two-sided t-test to quantitatively demonstrate that inclusion of the filtration step does not result in a significant change in $\epsilon_{\text{Rubisco}}$. We refer the reader to **rev 2/8** for additional details. In addition, we added the following clarification to the main text:

L424-425: "...To mitigate this issue, we introduced an additional filtration step for *G. oceanica* and *Synechococcus* sp. Rubisco assays , taking care to minimize the samples exposure to air..."

References

[1] Bathellier, C., Yu, L.-J., Farquhar, G. D., Coote, M. L., Lorimer, G. H., and Tcherkez, G. (2020). Ribulose 1,5-bisphosphate carboxylase/oxygenase activates o¡sub¿2¡/sub¿ by electron transfer. *Proceedings of the National Academy of Sciences*, 117(39):24234–24242.

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[3] Boller, A. J., Thomas, P. J., Cavanaugh, C. M., and Scott, K. M. (2015). Isotopic discrimination and kinetic parameters of rubisco from the marine bloom-forming diatom, *Skeletonema costatum*. *Geobiology*, 13(1):33–43.

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[5] Bouvier, J. W. and Kelly, S. (2023). Response to tcherkez and farquhar: Rubisco adaptation is more limited by phylogenetic constraint than by catalytic trade-off. *Journal of Plant Physiology*, 287:154021.

[6] Estep, M. F., Tabita, F. R., Parker, P. L., and Van Baalen, C. (1978). Carbon isotope fractionation by ribulose-1,5-bisophosphate carboxylase from various organisms 1. *Plant Physiology*, 61(4):680–687.

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[8] Iñiguez, C., Capó-Bauçà, S., Niinemets, Ü., Stoll, H., Aguiló-Nicolau, P., and Galmés, J. (2020). Evolutionary trends in rubisco kinetics and their co-evolution with co_2 concentrating mechanisms. *The Plant Journal*, 101(4):897–918.

[9] Tcherkez, G. (2013). Modelling the reaction mechanism of ribulose-1,5-bisphosphate carboxylase/oxygenase and consequences for kinetic parameters. *Plant, Cell & Environment*, 36(9):1586–1596.

[10] Tcherkez, G. and Farquhar, G. D. (2021). Rubisco catalytic adaptation is mostly driven by photosynthetic conditions – not by phylogenetic constraints. *Journal of Plant Physiology*, 267:153554.

[11] Tcherkez, G. G. B., Bathellier, C., Stuart-Williams, H., Whitney, S., Gout, E., Bligny, R., Badger, M., and Farquhar, G. D. (2013). D2o solvent isotope effects suggest uniform energy barriers in ribulose-1,5-bisphosphate carboxylase/oxygenase catalysis. *Biochemistry*, 52(5):869–877.

[12] Tcherkez, G. G. B., Farquhar, G. D., and Andrews, T. J. (2006). Despite slow catalysis and confused substrate specificity, all ribulose bisphosphate carboxylases may be nearly perfectly optimized. *Proceedings of the National Academy of Sciences*, 103(19):7246–7251.

[13] Thomas, P. J., Boller, A. J., Satagopan, S., Tabita, F. R., Cavanaugh, C. M., and Scott, K. M. (2019). Isotope discrimination by form ic rubisco from *Ralstonia eutropha* and *Rhodobacter sphaeroides*, metabolically versatile members of 'proteobacteria' from aquatic and soil habitats. *Environmental Microbiology*, 21(1):72–80.