Responses to Anonymous Referee #3

bg-2020-120

1 General Comments

While the authors provide sufficient literature context for their discussion, my biggest concern with the paper in its current form is that it does not clearly delineate novel insights or findings by the authors from pre-existing literature. In other words, I am not certain where the review of the historical literature ends, and the authors' analysis begins, which makes it difficult to evaluate exactly what the authors' primary contributions are here. For example, to what extent has the N2O site-preference example already been articulated in the literature? Site-preference measurements have been made for many years, and the authors cite other studies noting that precursor symmetry matters, so for readers less familiar with this application: is this simply an example compiled from existing studies to argue that more context is needed to interpret an Intra-ID, or is some component of the discussion new, like the subsequent discussion of reversibility? Some careful re-wording could clarify this and similar types of questions throughout. Similarly, it would be helpful if the authors could add at least one more sentence at the end of the oxygen-bearing minerals example synthesizing the broadly generalizable point that the authors are trying to convey.

Response: Thanks for the comments. You summarized our piece accurately. Per your criticism on a lack of clear distinction of prior work and our new insights in some of the writings, we have revised the text to add phrases and sentences like "It had been proposed" "In previous literature" "The measured $\delta^{13}C_{met}$ values from literature", "Nevertheless, the two previously proposed mechanisms can only explain the observed SP<0, but cannot provide information on reaction kinetics.", "Except for the above-mentioned features, the production of artificial and biological acetic acid has too many unconstrained parameters. Thus, our discussion will focus on the acetic acid derived from hydrous pyrolysis of oil-prone source rocks."

We have added a summary at the end of the oxygen-bearing minerals example. It reads:

"The use of a single-mineral geothermometer requires that oxygens at two different sites have attained equilibrium by exchanging with each other or with the same source oxygen (e.g. water). Unfortunately, it is difficult to meet this requirement. It is, therefore, not surprising that few successful single-mineral geothermometers exist if any at all."

2 Specific Comments

I do not really understand the meaning behind the "same carbon different elements" portion of the title. Unless further explanation is added to the text, I think it could be easily removed. "Same carbon different positions" or something similar seems better aligned with the focus of the paper, but does not necessarily improve the existing title.

Response: "Same carbon different elements" here means: "The position-specific

carbons at different positions are the same element but behave like different elements." In light of the fact that the other reviewer was also puzzled by the title, we have changed our title, reluctantly, to "Carbons at Different Positions Behave Like Different Elements- An Insight into Position-Specific Isotope Distributions".

It is not always clear throughout the text whether the term "Intra-ID" is being used to mean position-specific isotope analysis (i.e., a measurement) or a calculated distribution of isotopes (i.e., a prediction of an equilibrium state). Adding clarifying language throughout would be helpful.

Response: Thanks for the suggestion. The term Intra-ID refers to the isotope distribution that is measured, predicted, or purely conceptual. To make it more clear, we have specified the measured isotope distribution as "observed/measured/apparent Intra-ID", the calculated equilibrium state as "predicted/calculated equilibrium Intra-ID".

Line 34: the definition of position-specific isotope composition is circular since it uses the words 'specific' and 'position' again. Perhaps something along the lines of "at particular atomic sites within an individual compound" or "at structurally distinct atomic sites. . ." would be more clear.

Response: Thanks for the suggestion. We have revised as suggested. It now reads:

"Position-specific isotope composition refers to the isotope composition of an element at structurally-distinct atomic positions within an individual compound."

Line 38: "Intra-ID" is an abbreviation that has been introduced in prior papers, for example, He et al., 2020 GCA. It might be helpful to cite this or the earliest use of this phrase to show precedent.

Response: Thanks for the reminder. Initially, we have defined intramolecular isotope distribution as Intra-ID, and intermolecular isotope distribution as Inter-ID in He et al., 2018 RCM. We have added the two references: He et al., 2018 RCM and He et al., 2020 GCA here.

Line 39: can the authors clarify what they mean here? "Most common" in what sense? (i.e., in terms of calculations, measurements, publications, or something else)

Response: We were trying to say that technological development in the carbon position-specific isotope analysis (PSIA) has been very active in recent years. This is in addition to the ongoing debate among Galimov, Buchachenko, and Schmidt on position-specific carbon isotope distributions. However, we realized that PSIA on hydrogen and oxygen is also developing fast. Therefore, this sentence only added confusion. We have deleted it in the new version.

Line 47: "to compare to" does not add much, but could be replaced by "for interpreting position-specific isotope measurements" and I believe makes the authors' point clearer.

Response: Thanks for the suggestion. We have revised as suggested. It now reads: "At equilibrium, the difference in isotope composition between two positions depends on temperature only and therefore such difference has been considered as a reference for interpreting position-specific isotope compositions (Galimov, 1985; Hayes, 2001, 2004; He et al., 2018, 2020; Rustad, 2009; Piasecki et al., 2016)."

Line 54: I do not think "correlate loosely" is sufficiently clear here. Perhaps, "do not correlate well with" or "are poorly correlated with"

Response: Thanks for the suggestion. We have revised it to "are poorly correlated with".

Line 60: the point citing He et al., 2018 seems interesting and highly relevant to the discussion in this paper. Can the authors add one more sentence summarizing the finding of that study and why the correlation approach is invalid?

Response: We have added a few sentences here. It now reads:

"Such a $^{13}\beta$-$\delta^{13}C$ correlation is written as $\delta^{13}C - \delta^{13}C_{ave} = \chi(\beta - \beta_{ave}) \times 10^3$, where χ is the regression coefficient. … The $^{13}\beta$-$\delta^{13}C$ correlation implicitly normalized the $^{13}\beta$ and $\delta^{13}C$ values using the averages of a given system. It revealed that the unweighted arithmetic mean isotope composition of all the components was used as the reference of a system. Strictly, only the mass-weighted isotope composition of all components should represent that of a system (Hayes, 2001). In addition, assigning an arbitrary reference is not mathematically rigorous either (He et al., 2018). Therefore, a $^{13}\beta$-$\delta^{13}C$ correlation cannot be used as supporting evidence for Galimov's hypothesis that the theorem of minimum entropy production applies in biochemical systems."

Line 97: It would be helpful to add one summary sentence at the end here to clarify what general point the authors hope the readers will take away from this section.

Response: Thanks for the suggestion. We have added a summary sentence at the end. It reads:

"The use of a single-mineral geothermometer requires that oxygens at two different sites have attained equilibrium by exchanging with each other or with the same source oxygen (e.g. water). Unfortunately, this requirement is difficult to be met for most minerals. It is, therefore, not surprising that few successful single-mineral geothermometers exist if any at all."

Line 137: "mechanisms" might be more meaningful than "processes" here?

Response: We have revised as suggested.

In line 196, I do not understand why any references are needed. It is clear from the chemical formulae, for example, that H2O consists of H and O atoms and NO− 3 consists of N and O. Why are 7 references needed in this sentence?

Response: This paragraph serves to connect to the title "Carbons at Different Positions Behave Like Different Elements- An Insight into Position-Specific Isotope Distributions". All the references are the studies of bonded isotope effects – D and ^{18}O in H_2O, ^{15}N and ^{18}O in NO_3^-. We have revised this paragraph to cite the references in their respective places. It now reads:

"A simple comparison of position-specific isotope compositions in one sample, e.g. $ln^{13}\alpha_{carb\text{-}met}$ values of one acetic acid sample, offers little information on the reaction it involves. Although the position-specific atoms are the same elements, without an exchange mechanism, they behave independently as if they were different elements. The isotope fractionation relationship of different elements in the same compound, i.e. $(\alpha_A\text{-}1)/(\alpha_B\text{-}1)$, $ln\alpha_A/ln\alpha_B$, or $\Delta\delta_A/\Delta\delta_B$, (named bonded isotope effect, He and Bao, 2019), is useful in characterizing a reaction pathway. Some of the studied examples are δD and $\delta^{18}O$ in H_2O (Dansgaard, 1964;Craig, 1961), $\delta^{15}N$ and $\delta^{18}O$ in NO_3^- (Casciotti and McIlvin, 2007; Wankel et al., 2009), $\delta^{34}S$ and $\delta^{18}O$ in SO_4^{2-} (Antler et al., 2013), and $\delta^{13}C$ and δD in organic compounds (Elsner, 2010; Palau et al., 2017). The isotope composition difference of different elements in a molecule is useful only when the isotope fractionation relationships are considered and their isotope compositions are normalized, e.g. $\Delta(15,18) = (\delta^{15}N\text{-}\delta^{15}N_m)\text{-}(^{15}\alpha\text{-}1/^{18}\alpha\text{-}1)\times(\delta^{18}O\text{-}\delta^{18}O_m)$, in which $\delta^{15}N_m$ and $\delta^{18}O_m$ are the average isotope composition in a given ocean water column (Sigman et al., 2005). The normalization procedure was necessary because the source isotope compositions can affect the values of the product. Similarly, if the same element at different positions have different sources, their source isotope composition difference must also be considered."

I would expect to see Hayes, 2001 cited somewhere in the manuscript as another classic discussion of kinetic and metabolic controls on isotope signatures of biomolecules.

Response: We have cited Hayes (2001) in multiple places

Hayes (2001) did a comprehensive review of C and H isotope fractionation in biosystems. It focuses more on biochemical processes rather than isotope effects. All the isotope fractionations discussed in Hayes (2001) are apparent ones. It has not been tested that if those values are intrinsic KIEs or EIEs. Thus, at the end of the paper, Hayes also stated: "*It often seems that isotopic fractionations provide* ***too much*** *information about* ***too many*** *processes, combining it all in a package that is unmanageably intricate.*" Hayes (2004) gave a very good example of Intra-IDs of fatty acid controlled by KIE or EIE. However, since he did not clearly define the two "equilibrium" scenarios, that example and his interpretation can be interpreted in both ways.

Although Hayes has made important contributions in the area of PSIA, we could not find a publication in which Hayes took a stand in the Galimov-Buchachenko-Schmidt debate publicly. We do not want to second guess his words, therefore, we did not

comment on Hayes' idea in this debate.

3 Suggested minor technical corrections
Line 12 - "biosystem" should be plural
Response: Corrected.

Line 13 - "debates remain" should become "debate remains"
Response: Corrected.

Line 21 - "roots" → "is rooted"
Response: Corrected.

Line 24 - "to be isolated and to be controlled" → "to isolate and control"; "effect" should be plural
Response: Corrected.

Line 39 - "facing" → "faced"
Response: Corrected.

Line 42 - "termed in" → "described as being in"
Response: Corrected.

Line 53 - "contrary" → "contrast"
Response: Corrected.

Line 55 - "are also observed" is not needed
Response: Corrected.

Line 63 - "between" → "among"
Response: Corrected.

Line 71 - eliminate "in contrast to existing optimism"
Response: Corrected.

Line 95 - "can come from different sources"
Response: Corrected.

Line 179 - I believe "involving" should be "evolving" here
Response: Corrected.

Line 206 - "offer" → "offers"
Response: Corrected.

Line 218 - "at molecular level" → "at the molecular level"
Response: Corrected.