

Interactive comment on “Diel variations in the carbon isotope composition of respired CO₂ and associated carbon sources: a review of dynamics and mechanisms” by C. Werner and A. Gessler

Anonymous Referee #2

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The topic of the review “Diel variations in the carbon isotope composition of respired CO₂ and associated carbon sources: A review of dynamics and mechanisms” is very interesting and merits publication; the more so as a good review is more than the sum of citations. The article gives a good overview on recent developments and offers a “mechanistic explanation” for the observed variations and diurnal changes of d13C of respired CO₂. However, I suggest some revisions. On the one hand I would like to draw the authors’ attention to more formal objections, and on the other hand I offer some

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proposals with regards to contents which I think could further improve the manuscript.

From a formal point of view, I think more attention to exact wording is necessary and would facilitate the readers’ understanding in the following instances:

- 1) Page 2184 line 13: I know that the term “post-photosynthetic fractionation” is used quite often. Regardless the fact that a very wide range of anabolic and catabolic reactions of the secondary metabolism are “post-photosynthetic” or downstream to carbon assimilation, please replace the term by “post-photosynthetic isotope fractionation”. Please add “isotope” to “fractionation” also at other places in the manuscript.
- 2) Page 2184 line 25-27: Please use either “. . . alter the carbon isotope ratio . . .” or “. . . alter the 13C/12C ratio . . .” not both descriptions in one sentence. The d13C value is the relative deviation of the 13C/12C ratio of a sample from the 13C/12C ratio of an international standard.
- 3) Page 2187 line 20-21: Please replace “carbohydrates, starch” by something more appropriate as starch is also a member of the “carbohydrate family”. Perhaps “soluble and storage carbohydrates” or “mono-, oligo- and polysaccharide” etc.
- 4) Page 2200 line 14: Please rephrase “higher enrichment of d13Cres” with e.g. “more positive d13Cres values”. Pls check at other places in the manuscript. Page 2197 line 11: “leading to a depletion of d13Cres” → more negative d13C ?
- 5) Page 2192 title: Please replace “M1.3: Isotope effects during . . .” by something like “M1.3: Isotope fractionation during . . .”. Not every isotope effect will be expressed in vivo because of its dependence on turnover rates and metabolic branching points . . . (See also 14).
- 6) Page 2196 line 18: Please rephrase. The fragmentation fractionation per se cannot lead neither to an “isotope effect” (pls see definition of isotope effects: <http://goldbook.iupac.org/I03327.html>) nor to an “isotope fractionation”. Perhaps to a difference in d13C-values because of different d13C-values of the educts ?

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7) Page 2198 line 17/18: Please rephrase “Inversely, in the dark the reaction can be assumed to go to completion resulting in little net isotope effects”. Isotope effects do not depend on completion of reaction. They can depend on reaction conditions like temperature, pH etc. Isotope fractionation is depending on branching points and turnover rates.

The following points might improve the content of the review and therefore support the authors’ reasoning and argumentation:

8) Page 2188 lines 11-16: Perhaps the article by Villar & Merino on leaf construction costs (New Phytol 151, 213-226, 2001) or related work would be helpful for the discussion on why there are “largest diel variations in $\delta^{13}\text{C}$ ” in not fast-growing plant species. Additionally helpful could also be the article by Pinelli and Loreto (J exp Bot 54, 1761-1769, 2003) regarding the ratio of CO_2 emission in the light/ CO_2 emission in the dark being smaller for *Quercus* relative to two herbaceous species. The working group of F. Loreto has also published articles on the production of volatile organic emissions by plants.

9) Page 2192 lines 8-9: Gleixner et al. (Planta 207, 241-245, 1998) suggested that transitory starch is ^{13}C enriched relative to soluble sugars because of the isotope effects on the “aldolase-reaction” measured originally by Gleixner and Schmidt (1997). Interesting for the M1.2 discussion is certainly also the fact that transitory starch “does not provide substrates for respiratory and photorespiratory decarboxylations in irradiated photosynthesizing leaves.” (Ivanova et al., Photosynthetica 46, 84-90, 2008).

10) Page 2195 line 1ff: Please give citations for the light-inhibition of glycolysis (!) and Krebs-Cycle. Citations for the light-reduction of the TCA cycle activity could be e.g. Tcherkez et al. (Plant Physiol. 138, 1596-1606, 2005) and Nunes-Nesi et al. (Physiol. Plant. 129, 45-56, 2007) etc. According to Tcherkez et al. (Plant Physiol 151, 620-630, 2009) and Sweetlove et al. (Trends in Plant Science 15, 462-470, 2010) and cited literature therein, the Krebs-Cycle is not a real cycle during illumination.

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11) Page 2196 1st paragraph: Please be aware that pyruvate is also the starting point of corresponding biosyntheses e.g. biosynthesis of the amino acids alanine (and valine). The PDH reaction has been recognized to imply kinetic isotope effects on all 3 carbon atoms of pyruvate (Melzer and Schmidt 1987). In case of a non-quantitative conversion of pyruvate to acetyl-CoA and CO_2 + a branching point at pyruvate, the KIE on C1 of pyruvate will be expressed in vivo and the released CO_2 will be depleted in ^{13}C relative to C3/C4 of glucose. Please check also on Page 2198 line 9ff: The membrane of the mitochondria is permeable to pyruvate. This has as consequence the possibility for pyruvate to be distributed to other pathways besides the Krebs cycle. This metabolic branching (upstream to the PDH reaction) is certainly not only regulated by the substrate availability regulation of the PDH reaction.

12) Page 2196 2nd paragraph and corresponding comment of Referee 1 (“around 12%.”): Perhaps it would be good to include a chemical drawing of the monosaccharide and state there which C-atom has which relative ^{13}C -enrichment or ^{13}C -depletion according to Rossmann et al. (1991) and recent articles by the working group of R. Robins. This would be helpful also for the discussion in 1st paragraph on page 2197 and for the corresponding discussion of the PPP cycle on page 2199. It would also facilitate the fragmentation fraction issue (Page 2196 line 18). Please see attached draft (Fig. 1) as a non-binding suggestion, and in case pls check the correctness of the numbers.

13) Page 2197 line 10, literature citation Hayes 2001: Savidge and Blair (Oecologia 139, 178-189, 2004) discuss the possibility to use the ^{13}C -enrichment of the glutamate carboxyl groups and the C4 carboxyl group of aspartate as proxy to estimate the amount of anaplerotically fixed carbon in C3-plants. Melzer and O’Leary show similar results (Plant Physiol 84, 58-60, 1987; Planta 185, 368-371, 1991).

14) Page 2197 line 10-11: Regarding “. . .equilibrium and kinetic isotope effects. . .” in the Krebs cycle. Several isotope effects on reactions catalyzed by enzymes of the Krebs cycle have been measured (see Tcherkez & Farquhar (2005) and cited literature

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therein). But not in every case an isotope effect will lead to an observable isotope fractionation. E.g. the citrate synthase reaction in the mitochondrion will most probably not lead to an isotope fractionation in the acetyl part of the produced citrate molecule as the inner mitochondrial membrane is impermeable for acetyl-CoA (see e.g. cited Voet & Voet or other (plant) biochemistry text book). Acetyl-CoA can only be transported to the cytosol in form of citrate. This has as consequence that acetyl-CoA will react quantitatively with oxaloacetate to citrate! Quantitative (enzymically catalyzed) reactions can “have” isotope effects (as a sort of chemical property) but will not show a corresponding isotope fractionation. See e.g. JM Hayes' chapter 2.4 “isotopic fractionations” in <http://www.nosams.who.edu/docs/IsoNotesAug02.pdf>. A discussed carbon isotope fractionation by supposed isotope effects on other reactions catalyzed by enzymes from the Krebs cycle is questionable because of the (discussed) channeling principle of the Krebs cycle (e.g. Srere et al. 1996, Channeling in the Krebs tricarboxylic acid cycle. In: L Agius, HSA Sherratt (eds.): Channeling in intermediary metabolism. Portland Press, London, pp. 201-217). Intermediates of the Krebs cycle are transferred from one enzyme to the other in a manner that allows only restricted mixing of the enzyme-bound intermediates with free intermediates. Please check equation (1) and related text passages and also Figures 5 and 6 under this premise.

15) Page 2198 line 17/18: A more “philosophical” objection towards that sentence could be: At what time during a normal 24 h rhythm (illumination – darkening) the plants will grow? According to A. Walter's group (e.g. Walter et al. *Annu Rev Plant Biol* 60, 279ff, 2009) there are plants which mainly grow during dawn and others during dusk. According to Michael et al. (*PLOS* 6, 1887-1898, 2008) the gene expression for phytohormone growth pathway increases during the night with a maximum at dawn where the stem elongation is fast. There is obviously a not inconsiderable percentage of growth during night. This would most probably shift the Krebs cycle towards the anaplerotic side. But also alanine will be produced for proteins and other functions.

16) Page 2198 line 27: “fatty acids”. Please be aware that most fatty acids will be

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biosynthesized in the chloroplast and not in the mitochondria. According to Tovar-Méndez et al. (2003) “the de novo synthesis of fatty acids” in green plant parts “is light-driven and occurs exclusively” in plastids. Acetyl-CoA for this synthesis is supplied by plastidial PDH and the released CO₂ is most probably re-fixed by photosynthesis??

17) Page 2198 line 24ff and page 2200 line 1ff: Interesting approach. During isoprenoid biosynthesis through the DOXP/MEP pathway in chloroplast the ¹³C-enriched C1 of pyruvate will be released and under illumination possibly re-fixed by photosynthesis. During biosynthesis of isorenoids through the mevalonate pathway starting from acetyl-CoA in the cytosol as for the biosynthesis of flavonoids, polyketids, plant sterols etc. ¹³C-enriched CO₂ from pyruvate will be released. A corresponding release of the ¹²C-enriched acetyl-moiety of the pyruvate in form of e.g. terpenes would partly compensate the ¹³C-enrichment. Ghirardo et al. (*PLOS ONE* 6(2), e17393, 2011) give some numbers on BVOC production (under illumination) and show that the C loss in form of isoprene is mainly originating from recently assimilated CO₂.

18) Page 2199 line 19ff and literature citation Priault et al. (2009): Please be aware that in case of a non-cyclic Krebs cycle under illumination the C atom(s) released as CO₂ connected to the Krebs cycle will not originate from the carbon skeleton of acetyl-CoA (see the “way of carbon atoms” through the Krebs cycle on p. 539 in Voet & Voet 1995 cited by the authors). The (diurnal) CO₂ released under illumination by the isocitrate dehydrogenase (and the 2-oxoglutarate dehydrogenase, if applicable) will originate from C1 and C4 of oxaloacetate and not from acetyl-CoA. In any case there should be no possibility for an observable ¹³C-enrichment of this CO₂ during illumination in case of addition of pyruvate labelled with ¹³C in C2 or C3 position. Instead of this the “acetyl moiety” of pyruvate can be used for biosynthesis purposes (also for biosynthesis of BVOC).

19) Page 2232 Figure 5: Please be aware that PEPc will bind HCO₃⁻ to PEP. The resulting product is oxaloacetate and not malate as it is shown in Figure 5.

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20) Interesting for me personally would be: why is the $\delta^{13}\text{C}$ value of the CO_2 respired by plants during darkness getting more and more negative in the course of the night?

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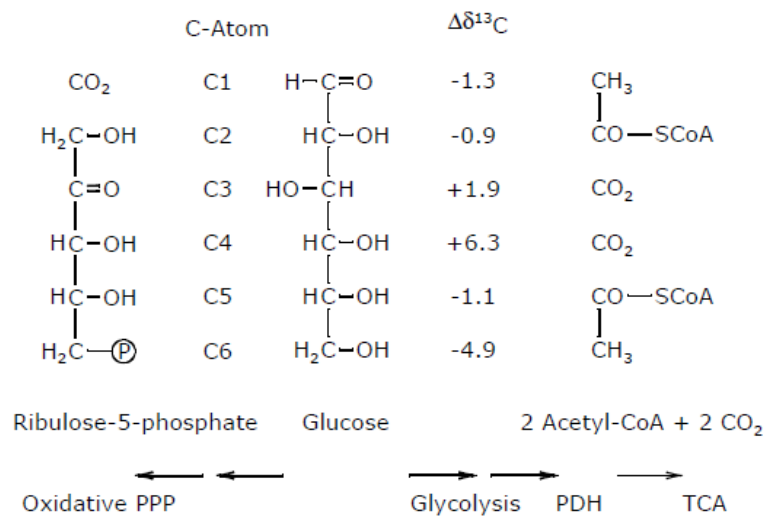


Figure 1

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