

Part A: Letter to the Editor

Dear Mister Wang

We discussed the remaining issue raised, the oxygen from atmospheric CO₂ as major source of photosynthates, already in our response to Referee#1. Here we describe in more detail the oxygen exchange and fractionation processes that occur during the dissolution of atmospheric CO₂ in the leaf water prior to assimilation and the incorporation into the organic matter as documented by a vast body of literature. We included a statement about the oxygen of CO₂ as source of photosynthtates in the revised manuscript.

We hope that our response clarifies this issue and that now you can consider the revised version for publication in Biogeosciences.

Yours sincerely,

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Part B: Editors comments to the authors

"As suggested by one reviewer, CO₂ uptaken during photosynthesis is an important source that must be considered for oxygen. While most of the CO₂ goes into the photosynthetates, most of the water vapor goes into the plant water with subsequent or simultaneous isotope exchange without uptake. Therefore, the concept of the three labels is questionable as these sources are not linked to each other. The authors need clarify this aspect."

Part C: Authors response

We agree, that the oxygen of the carbohydrates is introduced into the cell and into the Calvin-Cycle via CO₂. However, BEFORE CO₂ is incorporated it dissolves in the water coating on the intercellular cell membranes. In this dissolved form, the oxygen atoms exchange between the CO₂ and the water molecules, such that the CO₂ now carries the oxygen atom of the leaf water and vice versa. However, since the ratio of the CO₂:H₂O molecules is between 1 : 500–1000 the oxygen isotopic signal derived from the atmospheric CO₂ cannot be detected in the leaf water. Therefore the CO₂ molecule carries the oxygen isotope signal of the water by 99.8% to 99.9% BEFORE it is introduced into the Calvin-Cycle and transformed into carbohydrates (organic matter). Therefore it is correct to state that the carbohydrates carry predominantly the oxygen of the leaf water. This has been demonstrated in numerous experiments, which is documented in a large number of publications. The dissolution of the CO₂ in the leaf water and its oxygen equilibration prior to photosynthesis is the basis for whole research areas (see e.g. Werner et al., 2012), as for example the reconstruction of paleoclimate based on tree ring analysis.

In the following we take the reader through the most essential steps and hope that we could clarify this essential part of the paper. We describe the principal oxygen fractionations and exchanges occurring along the pathway of oxygen (bonded to CO₂ and H₂O) from the atmosphere into the organic matter (Figure 1) and give examples from the literature to support our statements.

Further, we would like to refer here explicitly to two publications. The isotopic oxygen composition of atmospheric CO₂, of the CO₂ dissolved in the leaf water and diffusing out of the leaf and its relation to the H₂O isotopic signatures are discussed in Yakir & Sternberg (2000; e.g. see Figure 1). The imprint of the leaf water isotopic oxygen signature into the signature of different organic compounds is highlighted by Schmidt, Werner, & Rossmann (2001; e.g. see Figure 8).

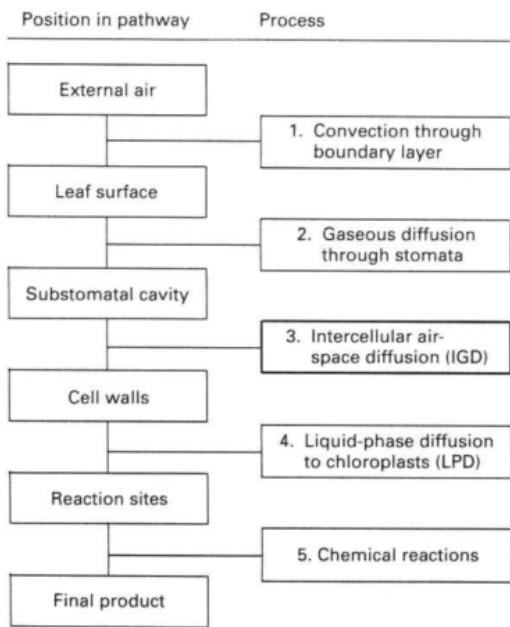


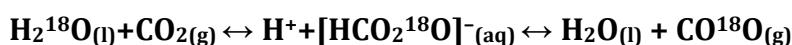
Figure 1: Generalized pathway (left) and processes (right) involved in the uptake of a gas (e.g. CO₂ and H₂O) by a leaf (Parkhurst, 1994).

Step 1: Diffusion of CO₂ (external air -> leaf surface -> substomatal cavity)

- CO₂ (and H₂O) molecules diffuse through the boundary layer and the stomata into the leaf air spaces (described in detail by Parkhurst, 1994).
- Diffusion is driven by the concentration gradients between the leaf substomatal cavity and the atmospheric air, but it is always bi-directional (e.g. see Figure 7 in Gillon & Yakir 2000).
- The flux of CO₂ back to the atmosphere leads to an apparent fractionation of the oxygen in the emitted CO₂ at the leaf surface compared to the external air (outside the boundary layer) due to equilibration with the leaf water isotopic signature (Gillon & Yakir, 2000).

Step 2: Dissolution of CO₂ in the leaf water (substomatal cavity -> cell walls -> reaction sites)

- **Before** the CO₂ enters the cells and the chloroplast (reaction sites) it dissolves in water.
- The dissolution is catalysed by carbonic anhydrase (as recently demonstrated by Uchikawa & Zeebe, 2012) and accompanied with a temperature dependent isotopic fractionation of 41 ‰ δ¹⁸O at 25°C compared to the leaf water (Yakir & Sternberg, 2000):



- The equilibration (oxygen exchange between CO₂ and water until chemical equilibration) can be regarded as complete under the given conditions (C3 plant, low assimilation due to limited light availability; Gillon & Yakir, 2000).
- Thus the **dissolved CO₂ bears the isotopic signature of the leaf water plus the isotopic fractionation of ca. 41 ‰ δ¹⁸O**.
- The isotopic composition of the leaf water remains nearly unchanged, since the amount of water involved in the above Equation is several magnitudes larger than the amounts of CO₂ (ca. CO₂: H₂O = 1 : 500-1000) and thus the signal change in the leaf water due to the oxygen derived from the atmospheric CO₂ is not detectable (dilution effect),

Step 3: Incorporation of dissolved CO₂ into fresh assimilates (reaction sites -> final product)

- Within the chloroplast **the dissolved CO₂ is incorporated into organic matter** (AFTER the equilibrated O-exchange) during the Calvin-Cycle (of C3 plants). Thus the isotopic composition of dissolved CO₂ (reflecting the leaf water isotopic signature plus ca. 41 ‰ δ¹⁸O) is the primary source for photosynthates.
- However, as discussed in the manuscript, the isotopic signature of the photosynthates is partially lost later due to the O-exchange of carbonyl groups with water and fractionation processes (Schmidt et al., 2001).

References

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Parkhurst, D. F. (1994). Tansley review no. 65. Diffusion of CO₂ and other gases inside leaves. *New Phytologist*, 126, 449–479.

Schmidt, H.-L., Werner, R. A., & Rossmann, A. (2001). O-18 pattern and biosynthesis of natural plant products. *Phytochemistry*, 58, 9–32.

Uchikawa, J., & Zeebe, R. E. (2012). The effect of carbonic anhydrase on the kinetics and equilibrium of the oxygen isotope exchange in the CO₂-H₂O system: Implications for δ¹⁸O vital effects in biogenic carbonates. *Geochimica et Cosmochimica Acta*, 95, 15–34.

Werner, C., Schnyder, H., Cuntz, M., Keitel, C., Zeeman, M. J., Dawson, T. E., ... Gessler, A. (2012). Progress and challenges in using stable isotopes to trace plant carbon and water relations across scales. *Biogeosciences*, 9, 3083–3111.

Yakir, D., & Sternberg, L. da S. L. (2000). The use of stable isotopes to study ecosystem gas exchange. *Oecologia*, 123, 297–311.

Part D: Changes in the revised manuscript

In our calculations the enrichment of the oxygen in the dissolved CO₂ relative to the leaf water is not relevant since we calculate the maximum label strength as excess to the unlabelled control. Thus the equilibrium fractionation (which can be assumed to be constant in this experiment with controlled temperature conditions) is automatically omitted by the calculation of the label strength. To clarify this we included another sentence in the method part of the manuscript (section 2.5 last paragraph).

"In a second step we corrected the isotopic ratios (¹⁸O/¹³C and ²H/¹³C) with the maximum label strength of the precursor of the plant organic matter, i.e. the maximum label strength of fresh assimilates (Eq. 7). The maximum ¹³C label strength was approximated by the ¹³C excess atom fraction relative to the unlabelled control (x^E) measured in the leaf water-soluble organic matter (wsOM) and the maximum ¹⁸O and ²H label strength by the excess atom fraction measured in the leaf water. **The leaf water is the direct source of hydrogen in assimilates and the indirect source of oxygen via the atmospheric CO₂ dissolved in water (Schmidt, Werner, & Eisenreich, 2003; Schmidt et al., 2001). The oxygen isotope composition of dissolved CO₂ equilibrates immediately with the leaf water signature, whereby carbonic anhydrase catalyzes this process and induces a temperature dependent kinetic ¹⁸O fractionation (Gillon & Yakir, 2000; Uchikawa & Zeebe, 2012). The fractionation was assumed to be constant in this experiment with controlled temperature and was thus omitted by the calculation of the excess atom fraction."**

In the discussion section of the manuscript we discuss the different oxygen and hydrogen sources for organic matter synthesized AFTER assimilation (e.g. out of the photosynthates). For these products oxygen from the atmospheric CO₂ is not an additional source (e.g. see Schmidt et al., 2001).