Leaf-scale quantification of the effect of photosynthetic gas exchange on $\Delta^{17}$O of atmospheric CO$_2$

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Abstract. Understanding the processes that affect the triple oxygen isotope composition of atmospheric CO$_2$ during gas exchange can help constrain the interaction and fluxes between the atmosphere and the biosphere. We conducted leaf cuvette experiments under controlled conditions using three plant species. The experiments were conducted at two different light intensities and using CO$_2$ with different $\Delta^{17}$O. We directly quantify the effect of photosynthesis on $\Delta^{17}$O of atmospheric CO$_2$ for the first time. Our results demonstrate the established theory for $\delta^{18}$O is applicable to $\Delta^{17}$O(CO$_2$) at leaf level, and we confirm that the following two key factors determine the effect of photosynthetic gas exchange on the $\Delta^{17}$O of atmospheric CO$_2$. The relative difference between $\Delta^{17}$O of the CO$_2$ entering the leaf and the CO$_2$ in equilibrium with leaf water and the back-diffusion flux of CO$_2$ from the leaf to the atmosphere, which can be quantified by the $c_m/c_a$ ratio, where $c_a$ is the CO$_2$ mole fraction in the surrounding air and $c_m$ is the one at the site of oxygen isotope exchange between CO$_2$ and H$_2$O. At low $c_m/c_a$ ratios the discrimination is governed mainly by diffusion into the leaf, and at high $c_m/c_a$ ratios it is governed by back-diffusion of CO$_2$ that has equilibrated with the leaf water. Plants with a higher $c_m/c_a$ ratio modify the $\Delta^{17}$O of atmospheric CO$_2$ more strongly than plants with a lower $c_m/c_a$ ratio. Based on the leaf cuvette experiments, the global value for discrimination against $\Delta^{17}$O of atmospheric CO$_2$ during photosynthetic gas exchange is estimated to be $-0.57 \pm 0.14$ ‰ using $c_m/c_a$ values of 0.3 and 0.7 for C$_4$ and C$_3$ plants, respectively. The main uncertainties in this global estimate arise from variation in $c_m/c_a$ ratios among plants and growth conditions.

1 Introduction

Stable isotope measurements of CO$_2$ provide important information about the magnitude of the CO$_2$ fluxes between atmosphere and biosphere, which are the largest components of the global carbon cycle (Farquhar et al., 1989, 1993; Ciais et al., 1997a, b; Flanagan and Ehleringer, 1998; Yakir and Sternberg, 2000; Gillon and Yakir, 2001; Cuntz et al., 2003a, b). A better understanding of the terrestrial carbon cycle is essential for predicting future climate and atmospheric CO$_2$ mole fractions (Booth et al., 2012). Gross primary productivity (GPP), the total carbon dioxide uptake by vegetation during photosynthesis, can only be determined indirectly and remains poorly constrained (Cuntz, 2011; Welp et al., 2011). For example, Beer et al. (2010) estimated global GPP to be 102–135 PgC yr$^{-1}$ (85% confidence interval, CI) using machine learning techniques by extrapolating from a database of eddy covariance measurements of CO$_2$. This estimate has since then been widely used as target for terrestrial vegetation models (Sitch et al., 2003a, b) and replicated based on cross-consistency checks with atmospheric inversions, sun-induced fluorescence (SIF), and global vegetation models (Jung et al., 2020). As an alternative, Welp et al. (2011) estimated global GPP to be 150–175 PgC yr$^{-1}$ using variations in $\delta^{18}$O of atmospheric CO$_2$ after the 1997/98 El Niño event; see Eq. (1) for definition of the $\delta$ value.

The concept behind the latter study was that atmospheric CO$_2$ exchanges oxygen isotopes with leaf and soil water, and this isotope exchange mostly determines the observed variations in $\delta^{18}$O of CO$_2$ (Francey and Tans, 1987; Yakir, 1998).
Following the 97/98 El Niño–Southern Oscillation (ENSO) event, the anomalous $\delta^{18}O$ signature imposed on tropical leaf and soil waters was transferred to atmospheric CO$_2$, before slowly disappearing as a function of the lifetime of atmospheric CO$_2$. This in turn is governed by the land vegetation uptake of CO$_2$ during photosynthesis, as well as soil invasion of CO$_2$ (Miller et al., 1999; Wingate et al., 2009). For the photosynthesis term, the equilibration of CO$_2$ with water is an uncertain parameter in this calculation, partly because the $\delta^{18}O$ of water at the site of isotope exchange in the leaf is not well defined. Importantly, a significant $\delta^{18}O$ variation can occur in leaves due to the preferential evaporation of H$_2^{18}$O relative to H$_2^{16}$O (Gan et al., 2002, 2003; Farquhar and Gan, 2003; Cernusak et al., 2016), which induces a considerable uncertainty in estimating $\delta^{18}O$ of CO$_2$. Similar considerations for the transfer of the $\delta^{18}O$ signature of precipitation into the soils, and then up through the roots, stems, and leaves makes $^{18}O$ of CO$_2$ a challenging measurement to interpret (Peylin et al., 1999; Cuntz et al., 2003a, b).

Classical isotope theory posits that oxygen isotope distributions are modified in a mass-dependent way. This means that the $^{17}O/^{16}O$ ratio changes by approximately half of the corresponding change in $^{18}O/^{16}O$ (Eq. 2), and it applies to the processes involved in gas exchange between atmosphere and plants. However, in 1983 Thiemens and co-workers (Heidenreich and Thiemens, 1983, 1986; Thiemens and Heidenreich, 1983) reported a deviation from mass-dependent isotope fractionation in ozone (O$_3$) formation called mass-independent isotope fractionation ($\Delta^{17}$O, Eq. 3). In the stratosphere, the $\Delta^{17}$O of O$_3$ is transferred to CO$_2$ via isotope exchange of CO$_2$ with O($^{17}$D) produced from O$_3$ photolysis (Yung et al., 1991, 1997; Shaheen et al., 2007), which results in a large amount of $\Delta^{17}$O in stratospheric CO$_2$ (Thiemens et al., 1991, 1995; Lyons, 2001; Lämerzahl et al., 2002; Thiemens, 2006; Kawagucci et al., 2008; Wiegel et al., 2013).

Once $\Delta^{17}$O has been created in stratospheric CO$_2$, the only process that modifies its signal is isotope exchange with leaf water, soil water and ocean water at the Earth’s surface, after CO$_2$ has reentered the troposphere (Boering, 2004; Thiemens et al., 2014; Liang and Mahata, 2015; Hofmann et al., 2017). Isotope exchange with leaf water is more efficient relative to ocean water due to the presence of the enzyme carbonic anhydrase (CA), which effectively catalyzes the conversion of CO$_2$ and H$_2$O to HCO$_3^-$ and H$^+$ and vice versa (Francey and Tans, 1987; Friedli et al., 1987; Badger and Price, 1994; Gillon and Yakir, 2001). The isotope exchange in the atmosphere is negligible due to lower liquid water content, lower residence time, and the absence of carbonic anhydrase (Mills and Urey, 1940; Miller et al., 1971; Johnson, 1982; Silverman, 1982; Francey and Tans, 1987).

$\Delta^{17}$O of CO$_2$ has been suggested as an additional independent tracer for constraining global GPP (Hoag et al., 2005; Thiemens et al., 2013; Hofmann et al., 2017; Liang et al., 2017b; Koren et al., 2019) because the processes involved in plant–atmosphere gas exchange are all mass dependent. Therefore, $\Delta^{17}$O at the CO$_2$–H$_2$O exchange site in the leaf will vary much less than $\delta^{18}O$. Nevertheless, mass-dependent isotope fractionation processes with slightly different three-isotope fractionation slopes are involved, which have been precisely established in the past years. Figure 1 shows how the different processes affect $\Delta^{17}$O of the H$_2$O and CO$_2$ reservoirs involved. The triple isotope slope of oxygen in meteoric waters is taken as reference slope, $\lambda_{\text{Ref}} = 0.528$ (Meijer and Li, 1998; Barkan and Luz, 2007; Landais et al., 2008; Luz and Barkan, 2010; Uemura et al., 2010), and we assume that soil water is similar to meteoric water. Due to transpiration and diffusion in the leaf, $\Delta^{17}$O of leaf water gets modified following a humidity-dependent three-isotope slope $\theta_{\text{trans}} = 0.522 – 0.008 \times h$ (Landais et al., 2006). Exchange of oxygen isotopes between leaf water and CO$_2$ follows $\delta_{\text{CO}_2-\text{H}_2\text{O}} = 0.5229$ (Barkan and Luz, 2012), which determines the $\Delta^{17}$O of CO$_2$ inside the leaf at the CO$_2$–H$_2$O exchange site. Finally, the $\Delta^{17}$O of the CO$_2$ is modified when CO$_2$ diffuses into and out of the leaf with $\lambda_{\text{diff}} = 0.509$ (Young et al., 2002).

In the first box model study of Hoag et al. (2005), the small deviations in $\Delta^{17}$O of CO$_2$ due to differences in three-isotope slopes were neglected and exchange with water was assumed to reset $\Delta^{17}$O to 0. Hofmann et al. (2017) included the different isotope effects shown in Fig. 1 in their box model. Koren et al. (2019) incorporated all the physicochemical processes affecting $\Delta^{17}$O of CO$_2$ in a 3D atmospheric model and investigated the spatiotemporal variability of $\Delta^{17}$O and its use as tracer for GPP. Using these and other similar models, numerous measurements of $\Delta^{17}$O in atmospheric CO$_2$ from different locations have been performed and used to estimate GPP (Liang et al., 2006; Barkan and Luz, 2012; Thiemens et al., 2014; Liang and Mahata, 2015; Laskar et al., 2016; Hofmann et al., 2017). The three-isotope slopes of the processes involved in the gas exchange (Fig. 1) have been precisely determined in idealized experiments. In the advanced models mentioned above it is assumed that when all the pieces are put together they result in a realistic overall modification of $\Delta^{17}$O of CO$_2$ in the atmosphere surrounding the leaf. However, this has not been confirmed by measurements previously. In this study we report the effect of photosynthesis on $\Delta^{17}$O of CO$_2$ in the surrounding air at the leaf scale. We measured $\Delta^{17}$O of CO$_2$ entering and leaving a leaf cuvette to calculate the isotopic fractionation associated with photosynthesis for three species that are representative for three different biomes. The fast-growing annual herbaceous C$_3$ species Helianthus annuus (sunflower) has a high photosynthetic capacity ($A_{\text{s}}$) and high stomatal conductance ($g_{\text{s}}$) and is representative for temperate and tropical crops (Freden et al., 1991). The slower-growing perennial evergreen C$_3$ species Hedera hibernica (ivy) is representative for forests and other woody vegetation and stress-subjected habitats (Pons et al., 2009). The fast-growing, agronomically im-
In the case of oxygen isotopes, the isotope ratios are expressed as the deviation of the heavy-to-light isotope ratio in a sample relative to a reference ratio and is denoted as \( \delta \). Isotopic composition is expressed as the deviation of the reference ratio and is denoted as \( \delta \). Notation and definition of \( \delta \) values

Isotopic composition is expressed as the deviation of the heavy-to-light isotope ratio in a sample relative to a reference ratio and is denoted as \( \delta \), expressed in per mill (‰). In the case of oxygen isotopes, the isotope ratios are \( ^{18}\text{R}=[^{18}\text{O}]/[^{16}\text{O}] \) and \( ^{17}\text{R}=[^{17}\text{O}]/[^{16}\text{O}] \) and the reference material is Vienna Standard Mean Ocean Water (VSMOW):

\[
\delta^n \text{O} = \frac{n^{R\text{sample}}}{n^{R\text{VSMOW}}} - 1, \quad n \text{ refers to 17 or 18.} \tag{1}
\]

For most processes, isotope fractionation depends on mass, and therefore the fractionation against \( ^{17}\text{O} \) is approximately half of the fractionation against \( ^{18}\text{O} \) (Eq. 3).

\[
\ln \left( \delta^{17}\text{O} + 1 \right) = \lambda \times \ln \left( \delta^{18}\text{O} + 1 \right) \tag{2}
\]

The mass-dependent isotope fractionation factor \( \lambda \) ranges from 0.5 to 0.5305 for different molecules and processes (Matsuhisa et al., 1978; Thiemens, 1999; Young et al., 2002; Cao and Liu, 2011). \( ^{17}\text{O} \) is used to quantify the degree of deviation from Eq. (2) (see Eq. 3). Note that \( ^{17}\text{O} \) changes not only by mass-independent isotope fractionation processes but also by mass-dependent isotope fractionation processes with a different \( \lambda \) value from the one used in the definition of \( ^{17}\text{O} \) (Barkan and Luz, 2005, 2011; Landais et al., 2006, 2008; Luz and Barkan, 2010; Pack and Herwartz, 2014).

\[
^{17}\text{O} = \ln \left( \delta^{17}\text{O} + 1 \right) - \lambda \times \ln \left( \delta^{18}\text{O} + 1 \right) \tag{3}
\]

The choice of \( \lambda \) is in principle arbitrary, and in this study we use \( \lambda = 0.528 \), which was established for meteoric waters (Meijer and Li, 1998; Landais et al., 2008; Brand et al., 2010; Luz and Barkan, 2010; Barkan and Luz, 2012; Sharp et al., 2018). Equation (3) can be linearized to \( ^{17}\text{O} = \delta^{17}\text{O} - \lambda \times \delta^{18}\text{O} \) (Miller, 2002), but this approximation causes an error that increases with \( \delta^{18}\text{O} \) (Miller, 2002; Bao et al., 2016).
crimination, as described in Farquhar and Richards (1984), Farquhar et al. (1989), and Farquhar and Lloyd (1993). We use the symbol $\Delta$ for discrimination due to assimilation in this paper since the commonly used $\Delta$ is already used for the definition of $\Delta^{17}O$ (see Eq. 3). $\Delta$ quantifies the enrichment or depletion of carbon and oxygen isotopes of CO$_2$ in the surrounding atmosphere relative to the CO$_2$ that is assimilated (Farquhar and Richards, 1984). It can be calculated from the isotopic composition of the CO$_2$ entering and leaving the leaf cuvette (Evans et al., 1986; Gillon and Yakir, 2000a; Barbour et al., 2016) as follows:

$$\Delta^{18}O_{\text{obs}} = \frac{nR_a - 1 - nO_2 - n\delta^18O}{1 + \delta^18O} = \frac{\zeta \times (\delta^18O_a - \delta^18O)}{1 + \delta^18O_a - \zeta \times (\delta^18O_a - \delta^18O)},$$

where the indices $e$, $a$, and $R$ refer to CO$_2$ entering and leaving the cuvette and being assimilated, respectively. $\zeta = \frac{c_x}{c_x - c_y}$, where $c_x$ and $c_y$ are the mole fractions of CO$_2$ entering and leaving the cuvette. For quantifying the effect of photosynthesis on $\Delta^{17}O$ in our experiments, the $\Delta A^{17}O$ is calculated from $\Delta A^{18}O$ and $\Delta A^{17}O$ using the three-isotope slope $\lambda_{RI} = 0.528$, similar to Eq. (3). In previous studies slightly different formulations have been used to define the effect of photosynthesis on $\Delta^{17}O$, and a comparison of the different definitions is provided in the Supplement (Eqs. S37–S40).

It is important to note that when the logarithmic definition of $\Delta^{17}O$ or $\Delta A^{17}O$ is used, values are not additive (Kaiser et al., 2004). In linear calculations, the error gets larger when the relative difference in $\delta^{18}O$ between the two CO$_2$ gases increases regardless of the $\Delta^{17}O$ of the individual CO$_2$ gases (Fig. S1 in the Supplement). Therefore, $\Delta A^{17}O$ values have to be calculated from the individual $\Delta A^{18}O$ and $\Delta A^{18}O$ values and not by linear combinations of the $\Delta^{17}O$ of air entering and leaving a plant chamber.

3 Materials and methods

3.1 Plant material and growth conditions

Sunflower (Helianthus annuus L. cv “sunny”) was grown from seeds in 0.6 L pots with potting soil (Primasta, the Netherlands) for about 4 weeks. All leaves appearing above the first leaf pair were removed to avoid shading. Established juvenileivy (Hedera hibernica L.) plants were pruned and planted in 6 L pots for 6 weeks. Ivy leaves that had developed and matured were used for the experiments. Maize (Z. mays L. cv “saccharate”) was grown from seed in 1.6 L pots for at least 7 weeks. For maize, the fourth or higher leaf number was used for the experiments when it was mature. A section of the leaf at about one-third from the tip was inserted into the leaf cuvette. They were placed on a subirrigation system that provided water during the growth period in a controlled-environment growth chamber, with an air temperature of 20 °C, relative humidity of 70 %, and CO$_2$ mole fraction of about 400 ppm. The photosynthetic photon flux density (PPFD) was about 300 μmol m$^{-2}$s$^{-1}$ during a daily photoperiod of 16h measured with a PPFD meter (Li-Cor LI-250A, Li-Cor Inc, NE, USA).

3.2 Gas exchange experiments

Gas exchange experiments were performed in an open system where a controlled flow of air enters and leaves the leaf cuvette, similar to the setup used by Pons and Welschen (2002). A schematic for the gas exchange experimental setup is shown in Fig. 2. The leaf cuvette had dimensions of 7 × 7 × 7 cm$^3$ (l × w × h) and the top part of the cuvette was transparent. The temperature of the leaf was measured with a K type thermocouple. The leaf chamber temperature was controlled by a temperature-controlled water bath kept at 20 °C (Tamson TLC 3, The Netherlands). A halogen lamp (Pradovit 253, Ernst Leitz Wetzlar GmbH, Germany) in a slide projector was used as a light source. Infrared was excluded by reflection from a cold mirror. The light intensity was varied with spectrally neutral filters (Pradovit 253, Ernst Leitz Wetzlar GmbH, Germany).

The CO$_2$ mole fraction of the incoming and outgoing air was measured with an infrared gas analyzer (IRGA, model LI-6262, Li-Cor Inc., NE, USA). The isotopic composition and mole fraction of the incoming and outgoing water vapor were measured with a triple water vapor isotope analyzer (WVIA, model 911-0034, Los Gatos Research, USA). Compressed air (ambient outside air without drying) was passed through soda lime to scrub the CO$_2$. The CO$_2$-free air could be humidified depending on the experiment conditions (see Fig. 2). The humidity of the inlet air was monitored continuously with a dew point meter (HYGRO-M1, General Eastern, Watertown, MA, USA). Pure CO$_2$ (either normal CO$_2$ or isotopically enriched CO$_2$) was mixed with the incoming air to produce a CO$_2$ mole fraction of 500 ppm. The isotopically enriched CO$_2$ was prepared by photochemical isotope exchange between CO$_2$ and O$_2$ under UV irradiation (Adnew et al., 2019).

An attached leaf or part of it was inserted into the cuvette, the composition of the inlet air was measured, and both IRGA and WVIA were switched to measure the outlet air. Based on the CO$_2$ mole fraction of the outgoing air the flow rate of the incoming air to the cuvette was adjusted to establish a drawdown of 100 ppm CO$_2$ due to photosynthesis in the plant chamber. The water vapor content entering the cuvette was adjusted depending on the transpiration rate relative to CO$_2$ uptake to avoid condensation (Fig. 2). The outgoing air was measured continuously until a steady state was reached for CO$_2$ and H$_2$O mole fractions and $\delta$D and $\delta^{18}O$ of the water vapor. After a steady state was established, the air was directed to the sampling flask while the IRGA and WVIA were switched back to measure the inlet air. The air
passed through a Mg(ClO₄)₂ dryer before entering the sampling flask.

After sampling, the leaf area inside the cuvette was measured with a LI-3100C area meter (Li-Cor Inc., USA). Immediately afterward, the leaf was placed in a leak-tight 9 mL glass vial and kept in a freezer at −20 °C until leaf water extraction.

3.3 Calibration of the water vapor isotope analyzer (WVIA) and leaf water analysis

The WVIA was calibrated using five water standards provided by IAEA (Wassenaar et al., 2018) for both δ¹⁸O and δD (Fig. S2). We did not calibrate the WVIA for δ¹⁷O, so the δ¹⁷O data are not used in the quantitative evaluation. The isotopic composition of the water standards ranged from −50.93‰ to 3.64‰ and −396.98‰ to 25.44‰ for δ¹⁸O and δD, respectively. The detailed characterization and calibration of the WVIA is provided in the Supplement (Figs. S2 to S4).

Leaf water was extracted by cryogenic vacuum distillation for 4 h at 60 °C following a well-established procedure as shown in Fig. S5 (Wang and Yakir, 2000; Landais et al., 2006; West et al., 2006). Details are provided in the Supplement. The δ¹⁷O and δ¹⁸O of leaf water were determined at the Laboratoire des Sciences du Climat et de l’Environnement laboratory using a fluorination technique as described in Barkan and Luz (2005) and Landais et al. (2006, 2008).

3.4 Carbon dioxide extraction and isotope analysis

CO₂ was extracted from the air samples in a system made from electropolished stainless steel (Fig. S6). Our system used four commercial traps (MassTech, Bremen, Germany). The first two traps were operated at dry ice temperature (−78 °C) to remove moisture and some organics. The other two traps were operated at liquid nitrogen temperature (−196 °C) to trap CO₂. The flow rate during extraction was 55 mL min⁻¹ controlled by a mass flow controller (Brooks Instruments, the Netherlands). The reproducibility of the extraction system was 0.030‰ for δ¹⁸O and 0.007‰ for δ¹³C determined on 14 extractions (1σ standard deviation, Table S1 in the Supplement).

The Δ¹⁷O of CO₂ was determined using the CO₂–O₂ exchange method (Mahata et al., 2013; Barkan et al., 2015; Adnew et al., 2019). The CO₂–O₂ exchange system used at Utrecht University is described in Adnew et al. (2019).
short, equal amounts of CO$_2$ and O$_2$ were mixed in a quartz reactor containing a platinum sponge catalyst and heated at 750°C for 2 h. After isotope equilibration, the CO$_2$ was trapped at liquid nitrogen temperature, while the O$_2$ was collected with 1 pellet of a 5Å molecular sieve (1.6 mm, Sigma Aldrich, USA) at liquid nitrogen temperature. The isotopic composition of the isotopically equilibrated O$_2$ was measured with a Delta Plus XL isotope ratio mass spectrometer in dual-inlet mode with reference to a pure O$_2$ calibration gas that has been assigned values of δ$^{17}$O = 9.254‰ and δ$^{18}$O = 18.542‰ by Eugeni Barkan at the Hebrew University of Jerusalem. The reproducibility of the δ$^{17}$O measurement was better than 0.01‰ (Table S1).

3.5 Leaf cuvette model

We used a simple leaf cuvette model to evaluate the dependence of $\Delta_A$δ$^{17}$O on key parameters. In this model, the leaf is partitioned into three different compartments: the intercellular air space, the mesophyll cell, and the chloroplast. In the leaf cuvette model, we used a 100 ppm down-draw of CO$_2$, similar to the leaf exchange experiment, i.e., the CO$_2$ mole fraction decreases from 500 ppm in the entering air ($c_e$) to 400 ppm in the outgoing air ($c_o$), which is identical to the air surrounding the leaf ($c_a$) as a result of thorough mixing in the cuvette. The assimilation rate is set to 20.0 µmol m$^{-2}$ s$^{-1}$. The leaf area and flow rate of air are set to 30 cm$^2$ and 0.7 L min$^{-1}$, respectively. The isotopic composition of leaf water at the site where the H$_2$O–CO$_2$ exchange occurs is δ$^{17}$O = 5.39‰ and δ$^{18}$O = 10.648‰, which is the mean of the measured δ$^{17}$O and δ$^{18}$O values of bulk leaf water in our experiments. The leaf water temperature is set to 22°C (similar to the experiment). In the model, the δ$^{18}$O of the CO$_2$ entering the cuvette is set to 30.47‰ for all the simulations, as in the normal CO$_2$ experiments, but the assigned Δ$^1^8$O values range from −0.5‰ to 0.5‰, which encompasses both the stratospheric intrusion and combustion components. The corresponding δ$^{17}$O of the CO$_2$ entering the cuvette is calculated from the assigned δ$^{18}$O value (30.47‰) and Δ$^1^7$O values (−0.5‰ to 0.5‰). For the calculations with this model, we assumed an infinite boundary layer conductance. The leaf cuvette model is illustrated in the Supplement (Fig. S7), and the detailed code and description is available at https://git.wur.nl/leaf_model (last access: 23 March 2020, Koren et al., 2020).

4 Results

4.1 Gas exchange parameters

Table 1 summarizes the isotopic composition and mole fraction of the CO$_2$ used in this study for sunflower, ivy, and maize. The Δ$^1^7$O of CO$_2$ used in this study varies from −0.215‰ to 0.44‰, while the δ$^{18}$O value is close to 30‰ for all the experiments. For all the experiments, the mole fraction of CO$_2$ entering the leaf ($c_a$) is 400 ppm, whereas the mole fraction of the CO$_2$ in the intercellular air space ($c_i$), at the CO$_2$–H$_2$O exchange site ($c_m$), and in the chloroplast ($c_c$) varies depending on the assimilation rate and metabolism type of the plants. Estimating the mesophyll conductance is described in the companion paper. A detailed description for estimating $c_m$ and $c_c$ is provided in the Supplement. A list of variables and parameters used in this study are summarized in Table 2.

4.2 Discrimination against $^{18}$O of CO$_2$

Figure 3a shows discrimination against $^{18}$O associated with photosynthesis (Δ$^1^8$O) for sunflower, ivy, and maize as a function of the $c_m$/$c_a$ ratio. Δ$^1^8$O varies with $c_m$/$c_a$, as found in previous studies (Gillon and Yakir, 2000a; Barbour et al., 2016). For sunflower, we observe Δ$^1^8$O values between 29‰ and 64‰ for $c_m$/$c_a$ between 0.54 and 0.86. Ivy shows relatively little variation in Δ$^1^8$O around a mean of 22‰ for $c_m$/$c_a$ between 0.48 and 0.58. For maize, Δ$^1^8$O is lower than for the C$_3$ plants measured in this study, with values between 10‰ and 20‰ for $c_m$/$c_a$ between 0.15 and 0.37.

For sunflower, changing the irradiance from 300 µmol m$^{-2}$ s$^{-1}$ (low light, hereafter LL) to 1200 µmol m$^{-2}$ s$^{-1}$ (high light, hereafter HL) leads to a clear decrease in Δ$^1^8$O (average 22‰). For maize, the Δ$^1^8$O change is only 4.4‰ on average. For ivy, changing the light intensity does not significantly change the observed Δ$^1^8$O. The solid lines in Fig. 3a show the results of leaf cuvette model calculations, where the dependence of Δ$^1^8$O on $c_m$/$c_a$ is explored for a set of calculations with otherwise fixed parameters. The model agrees well with the experimental results, except for ivy, where the model overestimates the discrimination.

4.3 Discrimination against $^{17}$O of CO$_2$

The discrimination of photosynthesis against Δ$^1^7$O of CO$_2$ (Δ$_A$Δ$^1^7$O) is shown in Fig. 3b. Δ$_A$Δ$^1^7$O is negative for all experiments, it depends strongly on the $c_m$/$c_a$ ratio, and $|$Δ$_A$Δ$^1^7$O$|$ increases with $c_m$/$c_a$ ratio. For instance, for Δ$^1^7$O of CO$_2$ entering the cuvette of −0.215‰, Δ$_A$Δ$^1^7$O is −0.25‰ for maize with $c_m$/$c_a$ ratio of 0.3, −0.3‰ for ivy with $c_m$/$c_a$ ratio of 0.5‰, and −0.5‰ for sunflower with $c_m$/$c_a$ ratio of 0.7 (Fig. 3b). For sunflower and ivy, Δ$_A$Δ$^1^7$O is also strongly dependent on the Δ$^1^7$O of CO$_2$ supplied to the cuvette, whereas no significant dependence is found for maize. For an increase in Δ$^1^7$O of CO$_2$ entering the cuvette from −0.215‰ to 0.435‰, Δ$_A$Δ$^1^7$O increases from −0.3‰ to −0.9‰ at $c_m$/$c_a$ ratio of 0.5 for ivy. For sunflower, an increase Δ$^1^7$O of CO$_2$ entering the cuvette from −0.215‰ to 0.31‰ increases Δ$_A$Δ$^1^7$O from −0.8‰ to −1.7‰ at $c_m$/$c_a$ ratio of 0.8. The leaf cuvette model results illustrate the shape of the dependence on the $c_m$/$c_a$ ratio and agree well with the experiments. For the leaf cuvette model,
the $\Delta^{17}O$ value of the water is assigned a constant value of $-0.122 \%e$ (average $\Delta^{17}O$ value for the bulk leaf water).

Figure 4b shows the same values of $\Delta A \Delta^{17}O$ as a function of the difference between $\Delta^{17}O$ of CO$_2$ entering the leaf and the calculated $\Delta^{17}O$ of leaf water at the evaporation site where CO$_2$–H$_2$O exchange takes place ($\Delta^{17}O_{wes}$). The leaf cuvette model results (solid lines in Fig. 4b) suggest a linear dependence between $\Delta A \Delta^{17}O$ and ($\Delta^{17}O_{a}$–$\Delta^{17}O_{wes}$). The experimental results agree with the hypothesis that $\Delta A \Delta^{17}O$ is linearly dependent on $\Delta^{17}O_{a}$–$\Delta^{17}O_{wes}$ at a certain $c_m/c_a$ ratio. Figure 4a shows the corresponding relation where $\Delta A \Delta^{17}O$ is divided by $\Delta^{17}O_{a}$–$\Delta^{17}O_{wes}$. All the values follow the same relationship as a function of the $c_m/c_a$ ratio, which can be approximated quite well by an exponential function (Eq. 5). This function quantifies the dependence of $\Delta A \Delta^{17}O$ on $c_m/c_a$ and thus the effect of the diffusion of isotopically exchanged CO$_2$ back to the atmosphere, which increases with increasing $c_m/c_a$ ratio.

\[
\frac{\Delta A \Delta^{17}O}{\Delta^{17}O_{a} - \Delta^{17}O_{wes}} = -0.150 \times \exp(3.707 \times c_m/c_a) + 0.028 \quad (5)
\]

Figure 5a and c show results from the leaf cuvette model that illustrates in more detail how $\delta^{18}O_e$ and $\Delta^{17}O_{wes}$ affect $\Delta^{17}O_{a}$ and $\Delta A \Delta^{17}O$ and their dependence on $c_m/c_a$.

At lower $c_m/c_a$, only a very small fraction of CO$_2$ that has undergone isotopic equilibration in the mesophyll diffuses back to the atmosphere, and therefore $\Delta^{17}O_{a}$ stays close to

Table 1. Summary of gas exchange parameters and isotopic compositions of maize, sunflower, and ivy. Mole fraction at the site of exchange ($c_m$) is calculated assuming complete isotopic equilibrium with the water at the CO$_2$–H$_2$O exchange site. The water at the CO$_2$–H$_2$O exchange site is assumed to be the same as the isotopic composition at the site of evaporation. Numbers in parentheses are the standard deviations of the mean ($\sigma$).

| Parameter       | Unit          | Sunflower | Ivy | Maize | Irradiance (µmol m$^{-2}$ s$^{-1}$) |
|-----------------|---------------|-----------|-----|-------|-----------------------------------|
| $A_m$           | µmol mol$^{-1}$ m$^{-2}$ s$^{-1}$ | 18 (0.7)  | 12 (0.7) | 17 (2) | 300                               |
| $g_s$           | mol m$^{-2}$ s$^{-1}$ | 0.45 (0.14) | 0.11 (0.02) | 0.08 (0.01) | 300                               |
| $\delta^{18}O_e$ | %e           | 27.26 to 31.80 | 28.28 to 30.48 | 27.26 to 30.48 |                                  |
| $\Delta^{17}O_e$ | %e           | $-0.227$ to $0.409$ | $-0.215$ to $0.435$ | $-0.215$ to $0.310$ |                                  |
| $\delta^{18}O_a$ | %e           | 33.25 to 43.87 | 32.64 to 35.86 | 34.04 to 29.764 |                                  |
| $\Delta^{17}O_a$ | %e           | $-0.333$ to 0.163 | $-0.276$ to 0.327 | $-0.270$ to 0.296 |                                  |
| $\Delta^{18}O_{obs}$ | %e         | 57.12 (4.70) | 22.20 (1.32) | 17.23 (1.32) | 300                               |
| $\delta^{18}O_{obs}$ | %e         | 34.48 (3.25) | 24.35 (3.09) | 12.78 (0.83) | 1200                              |
| $\Delta A \Delta^{17}O_{obs}$ | %e       | $-2.61$ to $-0.43$ | $-1.03$ to $-0.19$ | $-0.36$ to $-0.09$ |                                  |
| $\delta^{18}O_m$ | %e           | 52.02 (1.24) | 47.17 (1.17) | 52.62 (0.52) | 300                               |
| $\Delta^{17}O_m$ | %e           | $-0.41(0.001)$ | $-0.35(0.001)$ | $-0.40(0.01)$ | 1200                              |
| $c_a$           | ppm          | 402 (3)  | 403 (3)  | 403 (3)  |                                  |
| $c_i$           | ppm          | 357 (10) | 284 (0.1) | 194 (20) | 300                               |
| $c_c$           | ppm          | 277 (15) | 188 (30) | 194 (15) | 1200                              |
| $c_m$           | ppm          | 320 (10) | 220 (10) | 134 (15) | 300                               |

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Table 2. List of symbols and variables.

| Symbol | Description | Unit/calculation/value |
|--------|-------------|------------------------|
| $A_n$  | Rate of CO$_2$ assimilation | $\frac{\mu A}{2}(c_e - c_a\left(\frac{1 - w_a}{1 - w_e}\right))$, mol m$^{-2}$ s$^{-2}$ |
| $E$    | Transpiration rate | $\frac{\mu A}{2}(\frac{w_e - w_a}{1 - w_e})$, mol m$^{-2}$ s$^{-2}$ |
| $w_i$  | Mole fraction of water vapor inside leaf | $613.65 e^{\left(\frac{17.92 + P}{17.92 + P_{atm}}\right)} \times 10^{-5}$, mol mol$^{-1}$ |
| $w_a$  | Mole fraction of water vapor leaving the cuvette or leaf surrounding | mol mol$^{-1}$ |
| $w_e$  | Mole fraction of water vapor entering the cuvette | mol mol$^{-1}$ |
| $c_e$  | Mole fraction of CO$_2$ entering the cuvette | mol mol$^{-1}$ |
| $c_a$  | Mole fraction of CO$_2$ in the leaf surrounding or leaving the cuvette | mol mol$^{-1}$ |
| $u_e$  | Flow rate of air entering the cuvette | mol s$^{-1}$ |
| $s$    | Surface area of the leaf inside the cuvette | m$^2$ |
| $P$    | Atmospheric pressure | bar |
| $T_{leaf}$ | Leaf temperature | °C |
| $g_s$(H$_2$O) | Stomatal conductance for water vapor | $\delta_{H_2O} \times \delta_{H_2O} / \delta_{H_2O} - \delta_{H_2O}$ |
| $g_b$(H$_2$O) | Boundary layer conductance for water vapor | Calibrated for the cuvette we used |
| $g_s^t$ | Conductance for water vapor through the boundary layer and stomata | $E \left(1 - \left(\frac{\delta_{H_2O} - \delta_{H_2O}}{\delta_{H_2O} - \delta_{H_2O}}\right)\right)$, mol m$^{-2}$ s$^{-1}$ |
| $g_s$  | Stomatal conductance for CO$_2$ | $\delta_{CO_2} / 1.0$ |
| $g_b$  | Boundary conductance for CO$_2$ | $\delta_{CO_2} / 1.37$ |
| $g_s^{CO_2}$ | Conductance for CO$_2$ through the boundary layer and stomata | $\delta_{CO_2} / 1.0$ |
| $\Gamma^*$ | CO$_2$ compensation point | 45 µmol m$^{-2}$ s$^{-1}$ |
| $\delta_{m13}$ | CO$_2$ conductance from intercellular air space to the site of carboxylation calculated using $\Delta^{13}$C (for C$_3$ plants only) | mol m$^{-2}$ s$^{-1}$ bar$^{-1}$ |
| $\delta_{m18}$ | CO$_2$ conductance from intercellular air space to CO$_2$--H$_2$O exchange sites calculated using $\Delta^{18}$O | mol m$^{-2}$ s$^{-1}$ bar$^{-1}$ |
| $\delta_{m17}$ | CO$_2$ conductance from intercellular air space to CO$_2$--H$_2$O exchange sites calculated using $\Delta^{17}$O | mol m$^{-2}$ s$^{-1}$ bar$^{-1}$ |
| $\delta_{m18\Delta17}$ | CO$_2$ conductance from intercellular air space to CO$_2$--H$_2$O exchange sites calculated using $\Delta_{A}^{18}$O | mol m$^{-2}$ s$^{-1}$ bar$^{-1}$ |
| $c_i$  | Mole fraction of CO$_2$ in the intercellular air space | $\left(\frac{\delta_{CO_2} - \delta_{CO_2}}{\delta_{CO_2} + \delta_{CO_2}}\right)c_a - A_n$, mol mol$^{-1}$ |
| $c_s$  | Mole fraction of CO$_2$ at the leaf surface | $c_s = \frac{A_n}{E}$ |
| $c_m$  | Mole fraction of CO$_2$ at the site of CO$_2$--H$_2$O exchange | mol mol$^{-1}$ |
| $c_c$  | Mesophyll conductance to the chloroplast (for C$_3$ plants) | $c_i - \frac{A_n}{\delta_{m13}}$, mol mol$^{-1}$ |
| $t^{13}$ | Ternary correction for $^{13}$CO$_2$ | $\frac{(1 + n_{13})E}{2\delta_{CO_2}}$ |
| $t^{18}$ | Ternary correction for $^{18}$O | $\frac{(1 + n_{18})E}{2\delta_{CO_2}}$ |
| $t^{17}$ | Ternary correction for $^{17}$O | $\frac{(1 + n_{17})E}{2\delta_{CO_2}}$ |
| $R_D$  | Dark respiration rate | 0.8 µmol m$^{-2}$ s$^{-1}$ |
| $R_L$  | Day respiration rate | $0.5 \times R_D$, µmol m$^{-2}$ s$^{-1}$ |
Table 2. Continued.

| Symbol | Description                                                                 | Unit/calculation/value                                                                 |
|--------|-----------------------------------------------------------------------------|----------------------------------------------------------------------------------------|
| $\delta_k^{18}$ | Kinetic fractionation of water vapor in air                                    | $\frac{28\delta_8+19\delta_9}{8\delta_8+8\delta_9}, \%e$                               |
| $\delta_{\text{eq}}^{18}$ | Equilibrium fractionation between liquid and gas phase of water vapor        | $2.644 - 3.206 \left( \frac{10^5}{T_{\text{ref}}} \right) + 1.534 \left( \frac{10^6}{T_{\text{ref}}} \right), \%e$ |
| $\alpha_{13bs}$ | Weighted fractionation for $^{13}$COO as CO$_2$ diffuses through the boundary layer and stomata | $\frac{(C_1-C_2)\delta_{13}^{13} + (C_2-C_3)\delta_{13}^{18} + (C_3-C_4)\delta_{13}^{17}}{c_{a}-v_{t}}, \%e$ |
| $\alpha_{17bs}$ | Weighted fractionation for C$^{17}$OO as CO$_2$ diffuses through the boundary layer and stomata | $\frac{(C_1-C_2)\delta_{17}^{13} + (C_2-C_3)\delta_{17}^{18} + (C_3-C_4)\delta_{17}^{17}}{c_{a}-v_{t}}, \%e$ |
| $\alpha_{18bs}$ | Weighted fractionation for C$^{18}$OO as CO$_2$ diffuses through the boundary layer and stomata | $\frac{(C_1-C_2)\delta_{18}^{13} + (C_2-C_3)\delta_{18}^{18} + (C_3-C_4)\delta_{18}^{17}}{c_{a}-v_{t}}, \%e$ |
| $\alpha_{13bs}$ | Weighted fractionation for $^{13}$COO as CO$_2$ diffuses through the boundary layer and stomata | $\frac{(C_1-C_2)\delta_{13}^{13} + (C_2-C_3)\delta_{13}^{18} + (C_3-C_4)\delta_{13}^{17}}{c_{a}-v_{t}}, \%e$ |
| $\alpha_{17bs}$ | Weighted fractionation for C$^{17}$OO as CO$_2$ diffuses through the boundary layer and stomata | $\frac{(C_1-C_2)\delta_{17}^{13} + (C_2-C_3)\delta_{17}^{18} + (C_3-C_4)\delta_{17}^{17}}{c_{a}-v_{t}}, \%e$ |
| $\alpha_{18bs}$ | Weighted fractionation for C$^{18}$OO as CO$_2$ diffuses through the boundary layer and stomata | $\frac{(C_1-C_2)\delta_{18}^{13} + (C_2-C_3)\delta_{18}^{18} + (C_3-C_4)\delta_{18}^{17}}{c_{a}-v_{t}}, \%e$ |
| $\alpha_{17bs}$ | Weighted fractionation for C$^{17}$OO as CO$_2$ diffuses through the boundary layer and stomata | $\frac{(C_1-C_2)\delta_{17}^{13} + (C_2-C_3)\delta_{17}^{18} + (C_3-C_4)\delta_{17}^{17}}{c_{a}-v_{t}}, \%e$ |
| $\Pi_{17}$ | Weighted fractionation of C$^{17}$OO as it diffuses through the boundary layer, stomata, and liquid phase in series | $\frac{(C_1-C_2)\delta_{17}^{13} + (C_2-C_3)\delta_{17}^{18} + (C_3-C_4)\delta_{17}^{17}}{c_{a}-v_{t}}, \%e$ |
| $\alpha_{18}$ | Weighted fractionation of C$^{18}$OO as it diffuses through the boundary layer, stomata, and liquid phase in series | $\frac{(C_1-C_2)\delta_{18}^{13} + (C_2-C_3)\delta_{18}^{18} + (C_3-C_4)\delta_{18}^{17}}{c_{a}-v_{t}}, \%e$ |
| $\alpha_{13b}$ | Fractionation in $^{13}$CO$_2$ as CO$_2$ diffuses through the boundary layer | 2.9 ‰ |
| $\alpha_{13s}$ | Fractionation in $^{13}$CO$_2$ as CO$_2$ diffuses through the stomata          | 4.4 ‰ |
| $\sigma_m$ | Fractionation factor for dissolution and diffusion through water             | 1.8 ‰ |
| $\delta$ | Fractionation factor for photosynthesis (decarboxylation of glycine)         | 16 ‰ |
| $\epsilon$ | Fractionation factor for day respiration                                     | $R_D + \epsilon, \%e$ |
| $\epsilon^*$ | Apparent fractionation for day respiration                                    | $\delta^{11}C_{H} - \delta^{13}C_{\text{A}} - \delta^{13}C_{\text{substrate}}, \%e$ |
| $b$ | Fractionation factor for uptake by RuBisCO                                | 29 ‰ |
| $\alpha_f$ | Fractionation due to photosynthesis (decarboxylation of glycine)            | 1 + $\delta$ |
| $\alpha_e$ | Fractionation due to day respiration                                         | 1 + $\epsilon$ |
| $\alpha_b$ | Fractionation due to uptake by RuBisCO                                     | 1 + $b$ |
| $\alpha_{17b}$ | Fractionation of C$^{17}$OO as CO$_2$ diffuses through the boundary layer | 2.9 ‰ |
| $\alpha_{17s}$ | Fractionation in C$^{17}$OO as CO$_2$ diffuses through stomata              | 4.4 ‰ |
| $\alpha_{18b}$ | Fractionation of C$^{18}$OO as CO$_2$ diffuses through the boundary layer | 5.8 ‰ |
| $\alpha_{18s}$ | Fractionation in C$^{18}$OO as CO$_2$ diffuses through stomata              | 8.8 ‰ |
| $\alpha_{17w}$ | Fractionation in C$^{17}$OO due to diffusion and dissolution in water       | 0.382 ‰ |
| $\alpha_{18w}$ | Fractionation in C$^{18}$OO due to diffusion and dissolution in water       | 0.8 ‰ |
| $\delta_{18}^{18}$ | Equilibrium fractionation of CO$_2$ and water for C$^{18}$OO               | $\frac{17604}{T_{\text{ref}}} - 17.93, \%e$ |
| $\delta_{18}^{18}$ | kinetic fractionation of water vapor in air                                  | $\frac{28\times\delta_8+19\times\delta_9}{8\times\delta_8+8\times\delta_9}, \%e$ |
| $\delta_{18}^{18}$ | equilibrium fractionation between liquid- and gas-phase water              | $2.644 - 3.206 \times \left( \frac{10^3}{T} \right) + 1.534 \times \left( \frac{10^6}{T} \right)$ |
Table 2. Continued.

| Symbol   | Description                                                                 | Unit/calculation/value                              |
|----------|-----------------------------------------------------------------------------|-----------------------------------------------------|
| $\delta^{17}O_A$ | $\delta^{17}O$ of the assimilated CO$_2$ | $\frac{\delta^{17}O_a - \delta^{17}O}{\delta^{17}O_a} = \delta^{17}O_a - \frac{\epsilon_{18}}{\epsilon_{17}} (\delta^{17}O_a - \delta^{17}O)$ |
| $\delta^{18}O_A$ | $\delta^{18}O$ of the assimilated CO$_2$ | $\frac{\delta^{18}O_a - \delta^{18}O}{\delta^{18}O_a} = \delta^{18}O_a - \frac{\epsilon_{18}}{\epsilon_{17}} (\delta^{18}O_a - \delta^{18}O)$ |
| $\delta^{17}O_{iso}$ | $\delta^{17}O$ of CO$_2$ in the intercellular air space ignoring ternary correction | $\delta^{17}O_{iso} (1 - \frac{\epsilon_{17}}{\epsilon_{18}}) (1 + a_{17bs}) + \frac{\epsilon_{18}}{\epsilon_{17}} (\delta^{17}O_a - a_{17bs}) + a_{17bs}, \%e$ |
| $\delta^{18}O_{iso}$ | $\delta^{18}O$ of CO$_2$ in the intercellular air space ignoring ternary correction | $\delta^{18}O_{iso} (1 - \frac{\epsilon_{18}}{\epsilon_{17}}) (1 + a_{18bs}) + \frac{\epsilon_{18}}{\epsilon_{17}} (\delta^{18}O_a - a_{18bs}) + a_{18bs}, \%e$ |
| $\delta^{17}O_i$ | $\delta^{17}O$ of CO$_2$ in the intercellular air space | $\delta^{17}O_{iso} + \frac{\epsilon_{17}}{\epsilon_{18}} (\delta^{17}O_a (\frac{\epsilon_{18}}{\epsilon_{17}}) - 1) - \frac{\epsilon_{17}}{\epsilon_{18}} \frac{a_{17bs}}{a_{18bs}}, \%e$ |
| $\delta^{18}O_i$ | $\delta^{18}O$ of CO$_2$ in the intercellular air space | $\delta^{18}O_{iso} + \frac{\epsilon_{18}}{\epsilon_{17}} (\delta^{18}O_a (\frac{\epsilon_{18}}{\epsilon_{17}}) - 1) - \frac{\epsilon_{18}}{\epsilon_{17}} \frac{a_{18bs}}{a_{17bs}}, \%e$ |
| $\delta^{18}O_{trans}$ | $\delta^{18}O$ of transpired water vapor | $\left( \frac{\Delta_{\text{w-air}}}{\Delta_{\text{trans}}} \right) (\delta^{18}O_{\text{trans}} - \delta^{18}O_{\text{w-air}}) + \delta^{18}O_{\text{w-air}}, \%e$ |
| $\delta^{18}O_{wes}$ | $\delta^{18}O$ of water at the evaporation site | $\delta^{18}O_{wes} = \delta^{18}O_{\text{trans}} + \epsilon_{18}^{18} + \epsilon_{18}^{18} + \frac{\epsilon_{18}^{18}}{\epsilon_{17}^{18}} (\delta^{18}O_{\text{w-air}} - \epsilon_{18}^{18} + \epsilon_{18}^{18})$ |
| $\delta^{17}O_m$ | $\delta^{17}O$ of CO$_2$ at the site of CO$_2$-H$_2$O exchange | $(\delta^{17}O_{wes} + 1) \times (1 + \epsilon_{17}^{18} - 1), \%e$ |
| $\delta^{18}O_m$ | $\delta^{18}O$ of CO$_2$ at the site of CO$_2$-H$_2$O exchange | $(\delta^{18}O_{wes} + 1) \times (1 + \epsilon_{18}^{18} - 1), \%e$ |
| $\delta^{13}C_{\text{substrate}}$ | Isotope ($^{13}$C) ratio of substrate used for dark respiration | $\frac{\delta^{13}C_{\text{substrate}} - \delta^{13}C_{\text{atm}}}{\delta^{13}C_{\text{atm}}} - \%e$ |
| $\Delta^{12}C$ | $^{13}$C-photosynthetic discrimination | $\frac{\epsilon^{13}C_{\text{atm}} - \epsilon^{13}C_{\text{substrate}}}{1 + \epsilon^{13}C_{\text{atm}} (1 - \epsilon^{13}C_{\text{substrate}})}, \%e$ |
| $\Delta^{13}C_{\text{obs}}$ | $^{13}$C-photosynthetic discrimination (Farquhar model) | $(1 - \frac{\epsilon^{13}C_{\text{atm}} - \epsilon^{13}C_{\text{substrate}}}{\epsilon^{13}C_{\text{atm}}}) \left( \frac{\epsilon^{13}C_{\text{atm}} - \epsilon^{13}C_{\text{substrate}}}{\epsilon^{13}C_{\text{atm}}} \right) + \frac{\epsilon^{13}C_{\text{atm}} - \epsilon^{13}C_{\text{substrate}}}{\epsilon^{13}C_{\text{atm}}} \left( \frac{\epsilon^{13}C_{\text{atm}} - \epsilon^{13}C_{\text{substrate}}}{\epsilon^{13}C_{\text{atm}}} \right)$ |
| $\Delta^{13}C_{\text{c}}$ | $^{13}$C-photosynthetic discrimination (assuming no mesophyll conductance, i.e., $c_1 = c_1$) | $(1 - \frac{\epsilon^{13}C_{\text{atm}} - \epsilon^{13}C_{\text{substrate}}}{\epsilon^{13}C_{\text{atm}}}) \left( \frac{\epsilon^{13}C_{\text{atm}} - \epsilon^{13}C_{\text{substrate}}}{\epsilon^{13}C_{\text{atm}}} \right) + \frac{\epsilon^{13}C_{\text{atm}} - \epsilon^{13}C_{\text{substrate}}}{\epsilon^{13}C_{\text{atm}}} \left( \frac{\epsilon^{13}C_{\text{atm}} - \epsilon^{13}C_{\text{substrate}}}{\epsilon^{13}C_{\text{atm}}} \right)$ |
| $\Delta^{18}O$ | $^{18}$O-photosynthetic discrimination | $\frac{\epsilon^{18}O_{\text{atm}} - \epsilon^{18}O_{\text{substrate}}}{1 + \epsilon^{18}O_{\text{atm}} (1 - \epsilon^{18}O_{\text{substrate}})}, \%e$ |
| $\Delta^{17}O$ | $^{17}$O-photosynthetic discrimination | $\frac{\epsilon^{17}O_{\text{atm}} - \epsilon^{17}O_{\text{substrate}}}{1 + \epsilon^{17}O_{\text{atm}} (1 - \epsilon^{17}O_{\text{substrate}})}, \%e$ |
| $\Delta^{17}O_{\text{FPM}}$ | Farquhar model for $^{17}$O-photosynthetic discrimination | $\frac{\epsilon^{17}O_{\text{FPM}}}{1 + \epsilon^{17}O_{\text{FPM}} (1 - \epsilon^{17}O_{\text{FPM}})}, \%e$ |
| $\Delta^{18}O_{\text{FPM}}$ | Farquhar model for $^{18}$O-photosynthetic discrimination | $\frac{\epsilon^{18}O_{\text{FPM}}}{1 + \epsilon^{18}O_{\text{FPM}} (1 - \epsilon^{18}O_{\text{FPM}})}, \%e$ |
| $\delta^{17}O_e$ | $\delta^{17}O$ of CO$_2$ entering the cuvette | \%e |
| $\delta^{18}O_e$ | $\delta^{18}O$ of CO$_2$ entering the cuvette | \%e |
| $\delta^{18}O_{w}$ | $\delta^{18}O$ of CO$_2$ leaving the cuvette | \%e |
| $\delta^{17}O_{ma}$ | $\delta^{17}O$ of CO$_2$ leaving with the leaf water at the evaporating site relative to the CO$_2$ leaving the cuvette | $\frac{\delta^{17}O_{ma} - \delta^{17}O_{ca}}{1 - \delta^{18}O_{ca}}, \%e$ |
| $\delta^{18}O_{ma}$ | $\delta^{18}O$ of CO$_2$ leaving with the leaf water at the evaporating site relative to the CO$_2$ leaving the cuvette | $\frac{\delta^{18}O_{ma} - \delta^{18}O_{ca}}{1 - \delta^{18}O_{ca}}, \%e$ |
| $\delta^{18}O_{w}$ | $\delta^{18}O$ of water vapor entering the cuvette | \%e |
| $\delta^{18}O_{w}$ | $\delta^{18}O$ of water vapor leaving the cuvette or leaf surrounding | \%e |
the incoming $\Delta^{17}O$, modified by the fractionation during CO$_2$ diffusion through the stomata (Fig. 5a). Figure 5c confirms that at low $c_m/c_a$, $\Delta_A^{17}O$ approaches the fractionation constant expected for diffusion, $-0.170\%e$. This diffusional fractionation is independent of the isotopic composition of the CO$_2$ entering the leaf, and therefore at low $c_m/c_a$, the $\Delta_A^{17}O$ curves for the different values of the anomaly of the CO$_2$ entering the leaf converge. For a high $c_m/c_a$ ratio, the back-diffusion flux of CO$_2$ that has equilibrated with water becomes the dominant factor, and, in this case, the isotopic composition of the outgoing CO$_2$ converges towards this isotope value, independent of the isotopic composition of the incoming CO$_2$ (Fig. 5a). This can lead to a very wide range of values for the discrimination against $\Delta^{17}O$ because now the effect on $\Delta^{17}O$ of the ambient CO$_2$ depends strongly on the difference in isotopic composition between incoming CO$_2$ and CO$_2$ in isotopic equilibrium with the leaf water.

In the model calculations shown in Fig. 5b and d, the isotopic composition of the water was changed from $\Delta^{17}O_{wes} = -0.122\%e$ to $0.300\%e$, whereas all other parameters were
kept the same. The value of $\Delta^{17}O_e$ for which $\Delta^{17}O_a$ does not depend on $c_m/c_a$ is shifted accordingly, again being similar to $\Delta^{17}O_{eq}$. At low $c_m/c_a$, $\Delta A^{17}O$ converges to the same value as in Fig. 5c, confirming the role of diffusion into the stomata as discussed above.

Figure 6 shows how $\delta^{18}O$ and $\Delta^{17}O$ vary in key compartments of the leaf cuvette system that determine the oxygen isotope effects associated with photosynthesis, based on the previously established three-isotope slopes of the various processes (Fig. 1). The irrigation water has a $\Delta^{17}O$ value of 0.017 ‰. The measured bulk leaf water is 6 % to 16 % enriched in $^{18}O$ and its $\Delta^{17}O$ value is lower by $-0.075%$ to $-0.200%$ (mean value $-0.121%$) than the irrigation water, calculated using a three-isotope slope of $\theta_{\text{trans}} = 0.516\%$ at 80 % humidity (Landais et al., 2006). $\Delta^{17}O$ of leaf water at the evaporation site, calculated from the transpired water, has slightly lower $\Delta^{17}O$, with values between $-0.119%$ and $-0.237$ (average $-0.184%$). Note that the bulk leaf water was not measured for all the experiments. For the experiments where the bulk leaf water is measured, $\Delta^{17}O$ of leaf water at the evaporation site ranges from $-0.160%$ to $-0.231%$ with an average value of $-0.190 \pm 0.020%$. The calculated isotopic composition of water at the exchange site was thus similar but slightly lower in $\Delta^{17}O$ than the values measured for bulk leaf water. CO₂ exchanges with the water in the leaf with a well-established fractionation constant (see Eq. S17) and a three-isotope slope of $\theta_{\text{CO}_2-\text{H}_2\text{O}} = 0.5229$ (Barkan and Luz, 2012), leading to the lower $\Delta^{17}O$ values of the equilibrated CO₂. In our experiments, the $\Delta^{17}O$ value of CO₂ in equilibrium with leaf water is lower than the $\Delta^{17}O$ value of CO₂ entering the leaf. The $\Delta^{17}O$ of the CO₂ in the intercellular air space is a mixture between two end-members, the $\Delta^{17}O$ of the CO₂ entering the leaf and $\Delta^{17}O$ of the CO₂ in equilibrium with leaf water. This explains why the observed values of $\Delta A^{17}O$ are negative for the experiments performed in this study.

5 Discussion

5.1 Discrimination against $\delta^{18}O$ of CO₂

The higher $\Delta^{18}O_{\text{obs}}$ values for sunflower compared to maize and ivy (Fig. 3a) are mainly due to a higher back-diffusion flux $(c_m/(c_a - c_m))$. The back-diffusion flux is higher for the C₃ plants sunflower and ivy than for the C₄ plant maize, a consequence of the lower stomatal conductance and higher assimilation rate of C₃ plants (Gillon and Yakir, 2000a; Barbour et al., 2016). In C₃ plants most of the CO₂ entering
the stomata is carboxylated by phosphoenolpyruvate carboxylase (PEPC), resulting in a lower CO$_2$ mixing ratio in the mesophyll, which results in a lower back-diffusion flux. The increase in assimilation rate with higher light intensity decreases the $c_m/c_a$ ratio and thus leads to a lower back-diffusion flux, which explains the decreases in $\Delta^{18}O_{\text{obs}}$ for maize and most clearly for sunflower. A similar trend of increase in $\Delta^{18}O_{\text{obs}}$ with an increase in $c_m/c_a$ ratio has been reported in previous studies (Gillon and Yakir, 2000b, a; Osborne et al., 2017). For ivy, $\Delta^{18}O_{\text{obs}}$ and $\Delta^{17}O_{\text{obs}}$ do not decrease with an increase in irradiance because the change in assimilation rate with irradiance is small. Thus, $c_m$ will not decrease strongly and the effect on the back diffusion is smaller than the variability in $\Delta^{18}O_{\text{obs}}$ of different leaves of the same plant.

In our experiments, photosynthesis causes an enrichment in the $\delta^{18}O$ of atmospheric CO$_2$ for both C$_3$ and C$_4$ plants, i.e., positive value of $\Delta^{18}O$. In principle, $\Delta^{18}O$ can also be negative if the $\delta^{18}O_m$ is depleted relative to the ambient CO$_2$. This is in contrast to $\Delta^{13}C$, which will always be positive since it is determined by the fractionation due to the PEPC and RuBisCO enzyme activity (Figs. S8 and S9). In general, in our experiments the $\Delta^{18}O_{\text{obs}}$ values are about 5 times larger than $\delta^{18}O_m$–$\delta^{18}O_e$, the $\delta^{18}O$ difference between CO$_2$ entering and leaving the cuvette (Figs. S10 to S12). This is easy to understand from the definition of $\Delta_A$. Taking $\Delta^{18}O$ as an example, $\Delta^{18}O_{\text{obs}} = \frac{\delta^{18}O_e - \delta^{18}O_m}{1 + \delta^{18}O_m/\zeta (\delta^{18}O_e - \delta^{18}O_m)} \approx \delta^{18}O_e - \delta^{18}O_m$, and in our experiments $\zeta = c_e/(c_e - c_a) \approx 500/(500 - 400) = 5$.

5.2 Discrimination against the $\Delta^{17}O$ of CO$_2$

The leaf cuvette model includes the isotope fractionations of all the individual processes that have been quantified in dedicated experiments previously (Fig. 1). The good agreement of the model results with the measurements (Fig. 3a) demonstrates that when all these processes are combined in the quantitative description of a gas exchange experiment, they actually result in a correct quantification of the isotope effects associated with photosynthesis. This has already been demonstrated before for $\Delta^{18}O_{\text{obs}}$ but has now been confirmed for $\Delta_A^{17}O$.

Unlike ivy and sunflower, maize does not show a significant change in $\Delta_A^{17}O$ when CO$_2$ gases with different $\Delta^{17}O$ are supplied to the plant. The C$_4$ plant maize has a small back-diffusion flux due to its high assimilation rate and low stomatal conductance, leading to a low $c_m/c_a$ ratio. At low $c_m/c_a$ ratios, $\Delta_A^{17}O$ is expected to be close to the weighted fractionation due to diffusion through boundary layer and stomata. In general, the effect of diffusion on $\Delta^{17}O$ of atmospheric CO$_2$ can be expressed as follows:

$$\Delta^{17}O_{\text{Modified}} = \Delta^{17}O_2 + (\lambda_{RL} - \theta_{\text{CO}_2-\text{diff}}) \times \ln \alpha_{\text{diffusion}}, \quad (6)$$

where $\Delta^{17}O_2$ is the $\Delta^{17}O$ of the CO$_2$ surrounding the leaf; $\Delta^{17}O_{\text{modified}}$ is the $\Delta^{17}O$ of the CO$_2$ modified due to diffusional fractionation; and $\theta_{\text{CO}_2-\text{diff}}, \lambda_{RL}$, and $\alpha_{\text{diffusion}}$ are the oxygen three-isotope relationships during diffusion from the CO$_2$–H$_2$O exchange site to the atmosphere, the reference slope used, and the fractionation against $^{18}O$ for CO$_2$ during diffusion through the stomata. Using the values $\lambda_{RL} = 0.528, \theta_{\text{CO}_2-\text{diff}} = 0.509$ (Young et al., 2002), and $\alpha_{\text{diffusion}} = 0.9912$ (Farquhar and Lloyd, 1993), the effect of diffusional fractionation on the $\Delta^{17}O$ of atmospheric CO$_2$ is

Figure 6. Isotopic composition of various relevant oxygen reservoirs that affect the $\Delta^{17}O$ of atmospheric CO$_2$ during photosynthesis: irrigation water (gray triangle), calculated leaf water at the evaporation site (brown circles), measured bulk leaf water (brown star), CO$_2$ entering the cuvette (black circles), CO$_2$ leaving the leaf cuvette (green circles), CO$_2$ equilibrated with leaf water at the evaporation site (blue circles), and CO$_2$ equilibrated with bulk leaf water (blue stars). $\Delta^{17}O$ is calculated with $\lambda = 0.528$. 

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−0.168 ‰ regardless of the anomaly of the CO2 entering the leaf, and the model results confirm this at low \(c_m/c_a\) ratios (Fig. 5c and d, inset).

At a high \(c_m/c_a\) ratio, \(\Delta^{17}O\) is dominated by the back-diffusion flux of CO2 that has equilibrated with water. As a consequence, \(\Delta^{17}O\) converges to a common value that is independent of the anomaly of the CO2 entering the cuvette and is determined by the isotopic composition of leaf water. Figure 5 confirms that the end-member is equal to the \(\Delta^{17}O\) of CO2 in equilibrium with leaf water, \(\Delta^{17}O_m\). In fact, when \(\Delta^{17}O_a = \Delta^{17}O_m\), \(\Delta^{17}O\) does not change with \(c_m/c_a\), indicating that in this case the \(\Delta^{17}O\) of the CO2 diffusing back from the leaf is the same as the \(\Delta^{17}O\) of leaf water entering the cuvette.

\(\pi_{18}\) is the overall discrimination occurring during the diffusion of \(^{12}C^{18}O^{16}O\) from the ambient air surrounding the leaf to the CO2−H2O exchange site (see Table 2 for the list of variables). In our study \(\pi_{18}\) ranges from 5 ‰ to 7.2 ‰, lower than the literature estimate of 7.4 ‰ (Farquhar et al., 1993). \(\pi_{18}\) depends on the ratio of stomatal conductance, which is associated with a strong fractionation of 8.8 ‰, to mesophyll conductance with an associated fractionation of only 0.8 ‰. Therefore, the higher the ratio \(g_s/gm\) the lower the \(\pi_{18}\) (Table S2). The difference in \(\pi_{18}\) of 2.4 ‰ between the literature value of 7.4 ‰ and the lowest \(\pi_{18}\) estimate in this study will introduce an error of only 0.046 ‰ in the \(\Delta^{17}O\) value (see Eq. 6). The uncertainty \(\pi_{18}\) has lower influence on the \(\Delta_A\) of C3 plants compared to C4 plants since the diffusional fractionation is less important at the higher \(c_m/c_a\) ratio where C3 plants operate.

### 5.3 Global average value of \(\Delta_A\) for tropospheric CO2

We can use the established relationship between \(\Delta_A\) and \(\Delta^{17}O\) (Eq. 8) to determine \(\Delta_A\) of CO2 based on the \(\Delta^{17}O\) of atmospheric CO2 for tropospheric CO2 based on the \(\Delta^{17}O\) of leaf and \(c_m/c_a\) ratio. In Fig. 4b, the vertical dashed black line indicates \(\Delta^{17}O_{a}−\Delta^{17}O_{wes}\) obtained from the 3D global model (Koren et al., 2019). The results of the global estimate and parameters used for the extrapolation of a leaf-scale study to the global scale are summarized in Table 3.

The \(\delta^{17}O\) value of atmospheric CO2 (21.53 ‰) is calculated from the global \(\delta^{18}O\) and \(\Delta^{17}O\) values (41.5 ‰ and −0.168 ‰, respectively) (Koren et al., 2019). The \(\delta^{17}O\) and \(\delta^{18}O\) values of global mean leaf water are calculated from the soil water. A global mean \(\delta^{18}O\) value of soil water is −8.4 ‰ assuming soil water to be similar to precipitation (Bowen and Revenaugh, 2003; Koren et al., 2019). The \(\delta^{17}O\) value of soil water is −4.4 ‰, calculated using Eq. (7) (Luz and Barkan, 2010).

\[
\ln \left( \delta^{17}O_{\text{soil}} + 1 \right) = 0.528 \times \ln \left( \delta^{18}O_{\text{soil}} + 1 \right) + 0.033 \tag{7}
\]

\(\delta^{17}O\) and \(\delta^{18}O\) of leaf water are calculated from \(\delta^{17}O\) and \(\delta^{18}O\) of soil water with fractionation factors of 1.0043 and 1.0084, respectively (Hoffmann et al., 2017; Koren et al., 2019). The fractionation factor for \(\delta^{18}O\) is calculated using \(\alpha = (\alpha)^{\text{trans}}\) with \(\lambda^{\text{trans}} = 0.516\), assuming relative humidity to be 75 % (Landais et al., 2006). The \(\delta^{17}O\) and \(\delta^{18}O\) values of global mean leaf water are then −0.136 ‰ and −0.131 ‰, respectively. Thus, the difference between global atmospheric CO2 and leaf water is \(\delta^{17}O_{\text{CO2−water}} = 21.666 \pm 0.001\) and \(\delta^{18}O_{\text{CO2−water}} = 41.631 \pm 0.001\). This yields \(\Delta^{17}O_{\text{CO2−water}} = −0.101 ‰\), and this value is indicated as a dashed line in Fig. 4. The gray shaded area indicates the propagated error using the standard deviation of the relevant parameters in 180 × 360 grid boxes for 12 months of leaf water and 45 × 60 grid boxes for 24 months for CO2 (Koren et al., 2019). In Fig. 4b, the intersection between the vertical dashed black line and the discrimination lines for the representative \(c_m/c_a\) ratios of C3 and C4 plants corresponds to the \(\Delta_A\) of leaf water value of C3 and C4 plants. For C4 plants \((c_m/c_a = 0.3)\) this yields \(\Delta_A\) of −0.3 ‰ (dashed gray line in Fig. 4b), and for C3 plants it yields \((c_m/c_a = 0.7)\) \(\Delta_A\) of −0.65 ‰ (dashed black line in Fig. 4b).

Three main factors contribute to the uncertainty of the extrapolated \(\Delta_A\) value. The first is the measurement error, which contributes 0.25 ‰ (standard error for individual experiments). The second factor is the uncertainty in the difference between \(\Delta^{17}O\) of atmospheric CO2 and leaf water, and we use results from the global model to estimate an error. For \(\Delta^{17}O\) of atmospheric CO2, statistics for all 45 × 60 grid boxes for 24 months (2012–2013) show a range of −0.218 ‰ to −0.151 ‰, with a mean of −0.168 ‰ and a standard deviation of 0.013 ‰ (Fig. S13). For \(\Delta^{17}O\) of the leaf water statistics for all 180 × 360 grid boxes for 12 months show a range of −0.236 ‰ and −0.027 ‰ (Fig. S14). The mean is −0.067 ‰ with a standard deviation of 0.041 ‰. From the combined errors we estimate the error in \((\Delta^{17}O_{a}−\Delta^{17}O_{wes})\) to be 0.043 ‰. The third uncertainty in the extrapolation of...
Table 3. Summary of the parameters used for the extrapolation of leaf-scale experiments to the global scale and the results obtained, as well as an overview of available $\Delta^{17}$O measurements.

| Parameter                                      | Value                          | ref                        |
|------------------------------------------------|-------------------------------|----------------------------|
| $f_{C4}$                                       | 120 PgC yr$^{-1}$              | Beer et al. (2010)         |
| $f_{C3}$                                       | 23%                           | Still et al. (2003)        |
| $c_{m}/c_{a}$ (C$_3$)                         | 77%                           | Still et al. (2003)        |
| $c_{m}/c_{a}$ (C$_4$)                         | 0.7                            | Hoag et al. (2005)         |
| $\Delta^{17}$O leaf water (global mean, modeled) | $-0.067 \pm 0.04\%_e$         | Koren et al. (2019)       |
| $\Delta^{17}$O CO$_2$ (global mean, modeled)   | $-0.168 \pm 0.013\%_e$        | Koren et al. (2019)       |
| $\Delta_A\Delta^{17}$O (global mean for C$_4$) | $-0.3 \pm 0.18\%_e$          | Fig. 5b, for $c_{m}/c_{a}$ ratio of 0.3 |
| $\Delta_A\Delta^{17}$O (global mean for C$_3$) | $-0.65 \pm 0.18\%_e$         | Fig. 5b, for $c_{m}/c_{a}$ ratio of 0.7 |
| $\Delta_A\Delta^{17}$O (global mean for whole vegetation) | $-0.57 \pm 0.14\%_e$         | Eq. (13)                  |
| $\Delta_A\Delta^{17}$O-isoflux (global mean for C$_4$) | $-7.3 \pm 4\%$ PgC yr$^{-1}$ | Eq. (14), only for C$_4$ |
| $\Delta_A\Delta^{17}$O-isoflux (global mean for C$_3$) | $-53 \pm 15\%$ PgC yr$^{-1}$ | Eq. (14), only for C$_3$ |
| $\Delta_A\Delta^{17}$O-isoflux (global mean for whole vegetation) | $-77\%$ PgC yr$^{-1}$        | Hoag et al. (2005)        |
| $\Delta_A\Delta^{17}$O-isoflux (global mean for whole vegetation) | $-47\%$ PgC yr$^{-1}$        | Hoag et al. (2005)        |
| $\Delta_A\Delta^{17}$O value of tropospheric CO$_2$ | $-42\%$ PgC yr$^{-1}$ to $-92\%$ PgC yr$^{-1}$ | Hofmann et al. (2017) |

$\Delta^{17}$O comes from the uncertainty in the $c_{m}/c_{a}$ ratio. For C$_3$ and C$_4$ plants, these errors are indicated by the light orange and light blue shadings in Fig. 4b.

Taking these uncertainties into account leads to a mean value of $\Delta_A\Delta^{17}$O $= -0.3 \pm 0.18\%_e$ for C$_4$ plants and $\Delta_A\Delta^{17}$O $= -0.65 \pm 0.18\%_e$ for C$_3$ plants. The leaf-scale discrimination against $\Delta^{17}$O is then extrapolated to global vegetation using these representative values of $\Delta_A\Delta^{17}$O and the relative fractions of photosynthesis by C$_4$ and C$_3$ plants, respectively, as follows:

$$\Delta_A^{17}O_{\text{global}} = f_{C4} \times \Delta_A^{17}O_{C4} + f_{C3} \times \Delta_A^{17}O_{C3},$$

(8)

where $f_{C4}$ and $f_{C3}$ are the photosynthesis-weighted global coverage of C$_4$ and C$_3$ vegetation. $\Delta_A\Delta^{17}$O$_{C4}$ and $\Delta_A\Delta^{17}$O$_{C3}$ quantify the discrimination against $\Delta^{17}$O by C$_4$ and C$_3$ plants, which are calculated using estimated values of $c_{m}/c_{a}$ from a model. Using assimilation-weighted fractions of 23% for C$_4$ and 77% for C$_3$ vegetation (Still et al., 2003), the global mean value of $\Delta_A\Delta^{17}$O obtained from Eq. (8) is $-0.57 \pm 0.14\%_e$.

Isosflux is the product of isotope composition and gross mass flux of the molecule. In the case of assimilation, the net flux $F_A = F_{AL} - F_{LA}$ is multiplied with the discrimination associated with assimilation (Ciais et al., 1997a). $F_{AL}$ and $F_{LA}$ are total CO$_2$ fluxes from leaf to the atmosphere and from atmosphere to leaf, respectively. The global-scale $\Delta^{17}$O$_A$ isosflux is calculated by multiplying the discrimination with the assimilation flux as follows:

$$F_A \times \Delta_A^{17}O = A \times \left( f_{C4} \times \Delta_A^{17}O_{C4} + f_{C3} \times \Delta_A^{17}O_{C3} \right).$$

(9)
where \( A = 0.88 \times \text{GPP} \) is the terrestrial assimilation rate. The factor 0.88 accounts for the fraction of CO\(_2\) released due to autotrophic respiration (Ciais et al., 1997a). The \( \Delta_A \Delta^{17}O \) isoflux due to photosynthesis is calculated using a GPP value of 120 PgC yr\(^{-1}\) (Beer et al., 2010) and \( A = 0.88 \times \text{GPP} \), resulting in an isoflux of \(-60 \pm 15\%\) PgC yr\(^{-1}\) globally. This is the first global estimate of \( \Delta_A \Delta^{17}O \) based on direct measurements of the discrimination during assimilation. Our value is in good agreement with previous model estimates. Hofmann et al. (2017) estimated an isoflux ranging from \(-42\%\) PgC yr\(^{-1}\) to \(-92\%\) PgC yr\(^{-1}\) (converted to a reference line with \( \lambda = 0.528 \)) using an average \( c_m/c_a \) ratio of 0.7 for both C\(_4\) and C\(_3\) plants and \( \Delta^{17}O \) of \(-0.147\%\) for atmospheric CO\(_2\). A model-estimated value from Hoag et al. (2005) is \(-47\%\) PgC yr\(^{-1}\) (converted to our reference slope of \( \lambda = 0.528 \)), derived with a more simple model and using \( \Delta^{17}O \) of \(-0.146\%\) with \( c_m/c_a \) ratio of 0.33 and 0.66 for C\(_4\) and C\(_3\) plants, respectively.

The main uncertainty in the extrapolation of \( \Delta_A \Delta^{17}O \) from the leaf experiments to the global scale is the uncertainty in the \( c_m/c_a \) ratio. The error from the uncertainty in \( c_m/c_a \) ratio increases when the relative difference in \( \Delta^{17}O \) between CO\(_2\) and leaf water increases (Fig. 5b). It is difficult to determine a single representative \( c_m \) value for different plants because this value would need to be properly weighted with temperature, irradiance, CO\(_2\) mole fraction, and other environmental factors (Flexas et al., 2008, 2012; Shrestha et al., 2019). Recent developments in laser spectroscopy techniques (McManus et al., 2005; Nelson et al., 2008; Tuzson et al., 2008; Kammer et al., 2011) might enable more and easier measurements of \( c_m/c_a \) both in the laboratory and under field conditions. This could lead to a better understanding of variations in the \( c_m/c_a \) ratio among plant species temporarily, spatially, and environmentally.

6 Conclusions

In order to directly quantify the effect of photosynthetic gas exchange on the \( \Delta^{17}O \) of atmospheric CO\(_2\), gas exchange experiments were carried out in leaf cuvettes using two C\(_3\) plants (sunflower and ivy) and one C\(_4\) plant (maize) with isotopically normal and slightly anomalous (\( ^{17}O\)-enriched) CO\(_2\). Results for \( ^{18}O \) agree with results reported in the literature previously. Our results for \( \Delta^{17}O \) confirm that the formalism developed by Farquhar and others for \( \delta^{18}O \) is also applicable to the evaluation of \( \Delta^{17}O \). In particular, our experiments confirm that two parameters determine the effect of photosynthesis on CO\(_2\): (1) the \( \Delta^{17}O \) difference between the incoming CO\(_2\) and CO\(_2\) in equilibrium with leaf water and (2) the \( c_m/c_a \) ratio, which determines the degree of backflux of isotopically exchanged CO\(_2\) from the mesophyll to the atmosphere. At low \( c_m/c_a \) ratios, \( \Delta_A \Delta^{17}O \) is mainly influenced by the diffusion fractionation. Under our experimental conditions, the isotopic effect increased with \( c_m/c_a \), e.g., \( \Delta_A \Delta^{17}O \) was \(-0.3\%\) and \(-0.65\%\) for maize and sunflower with \( c_m/c_a \) ratios of 0.3 and 0.7, respectively. However, experiments with mass independently fractionated CO\(_2\) demonstrate that the results depend strongly on the \( \Delta^{17}O \) difference between the incoming CO\(_2\) and CO\(_2\) in equilibrium with leaf water. This is supported by calculations with a leaf cuvette model.

\( \delta^{18}O \) is largely affected by kinetic and equilibrium processes between CO\(_2\) and leaf water, and also leaf water isotopic inhomogeneity and dynamics. The \( \Delta^{17}O \) variation is much smaller compared to \( \delta^{18}O \) and is better defined since conventional biogeochemical processes that modify \( \delta^{17}O \) and \( \delta^{18}O \) follow a well-defined three-isotope fractionation slope. Results from the leaf exchange experiments were upscaled to the global atmosphere using modeled values for \( \Delta^{17}O \) of leaf water and CO\(_2\), which results in \( \Delta_A \Delta^{17}O = -0.57 \pm 0.14\% \) and a value for the \( \Delta^{17}O \) isoflux of \(-60 \pm 15\%\) PgC yr\(^{-1}\). This is the first study that provides such an estimate based on direct leaf chamber measurements, and the results agree with previous \( \Delta^{17}O \) calculations. The largest contribution to the uncertainty originates from uncertainty in the \( c_m/c_a \) ratio and the largest contributions to the isoflux come from C\(_3\) plants, which have both a higher share of the total assimilation and higher discrimination. \( \Delta_A \Delta^{17}O \) is less sensitive to \( c_m/c_a \) ratios at lower values of \( c_m/c_a \), for instance for C\(_4\) plants such as maize.

\( \Delta^{17}O \) of tropospheric CO\(_2\) is controlled by photosynthetic gas exchange, respiration, soil invasion, and stratospheric influx. The stratospheric influx is well established and the effect of photosynthetic gas exchange can now be quantified more precisely. To untangle the contribution of each component to the \( \Delta^{17}O \) atmospheric CO\(_2\), we recommend measuring the effects of foliage respiration and soil invasion both in the laboratory and at the ecosystem scale.

Code and data availability. The data used in this study are included in the paper either with figures or tables. The python code for the cuvette model is available at https://git.wur.nl/leaf_model (last access: 23 March 2020, Koren et al., 2020).

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Author contributions. GAA and TR designed the main idea of the study. GAA and TP designed the leaf cuvette setup. TP monitors plant growth. GAA and TR designed the CO\(_2\) extraction and CO\(_2\)–O\(_2\) exchange system. GAA conducted all the measurements. GK provided the leaf cuvette model. WP enabled the work within the ASICA project. All authors discussed the results at different steps of the project. GAA and TR prepared the manuscript with contributions from all the co-authors.
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Competing interests. The authors declare that they have no conflict of interest.

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