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

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1. Discrimination against \(^{13}\text{C} \) of CO\(_2\)

The isotope discrimination against \(^{13}\text{C} \) (\( \Delta^{\text{A}^{13}\text{C}} \)) associated with photosynthesis for the three-plant species is shown in Figure S8 a) and b) as a function of \( c/c_a \) and \( c_i/c_a \) for C\(_3\) and C\(_4\) plants, respectively. For the C\(_3\) plants, \( c_c \) is calculated following (Farquhar and Cernusak, 2012) as:

\[
 c_c = c_i - \left( \frac{1 - \ell^{13}}{1 + \ell^{13}} \right) \left( \frac{\Delta^{\text{A}^{13}\text{C}} c_i - \Delta^{\text{A}^{13}\text{C}}_{\text{obs}} Pc_a}{b - a_m - \frac{\alpha_b}{\alpha_e} \frac{R_d}{R_d + A}} \right)
\]

where \( \Delta^{\text{A}^{13}\text{C}}_{\text{obs}} \) is the observed discrimination against \(^{13}\text{C} \) and \( \Delta^{\text{A}^{13}\text{C}}_{\text{i}} \) is the discrimination that can be calculated for infinite mesophyll conductance (no mesophyll resistance). \( \ell^{13} \) is a ternary correction factor (considers the collisions between air and CO\(_2\), air and H\(_2\)O, CO\(_2\) and H\(_2\)O), \( b \) the fractionation due to uptake by Rubisco, and \( a_m \) the sum of the fractionations associated with \(^{13}\text{CO}_2\) dissolution in and diffusion through water, respectively. \( e \), \( R_d \), \( \alpha_e \), \( \alpha_b \) and \( P \) are the fractionations during day respiration (decarboxylation), the day respiration rate, the fractionation factor for day respiration with respect to net assimilation, the fractionation factor for C\(_3\) carboxylation, and the pressure of the air surrounding the leaf, respectively. A detailed description of the equations, best fit parameters and definitions of discrimination factors are given in Table S3.

Experiments at different light intensities are shown in different colors, blue for LL and yellow for HL. For ivy and sunflower, \( \Delta^{\text{A}^{13}\text{C}} \) and \( c/c_a \) are linearly correlated. As irradiance increases, \( c/c_a \) and \( \Delta^{\text{A}^{13}\text{C}} \) decrease. \( \Delta^{\text{A}^{13}\text{C}} \) for ivy increases from 13\(\%\) to 16\(\%\) when \( c/c_a \) increases from 0.34 to 0.55 while for sunflower \( \Delta^{\text{A}^{13}\text{C}} \) ranges from 13\(\%\) to 25 \(\%\) for \( c/c_a \) ratios of 0.37 to 0.8. This is due to the lower assimilation rate and generally higher back-diffusion flux at a higher \( c/c_a \) ratio. When irradiance increases, the assimilation rate increases, \( c/c_a \) decreases, and less of the CO\(_2\) that has entered the stomata diffuses back to the atmosphere. As a result, \( \Delta^{\text{A}^{13}\text{C}} \) decreases with an increase in light intensity. For maize,
Δ_\text{13}C is much smaller than for the C_3 plants, ranging from 2.4‰ to 3.5‰ for c_i/c_a ratios of 0.42 to 0.55, and we did not observe a strong correlation between Δ_\text{13}C and c_i/c_a. Δ_\text{13}C vs c_i/c_a is shown in Figure S9 of the for both C_3 plants (sunflower and ivy) and a C_4 plant (maize).

2. Leaf cuvette model

In the simple leaf cuvette model, we partitioned the leaf into three different compartments: the intercellular air space, the mesophyll cell, and the chloroplast, as shown in Figure S6. For this model, we assumed an infinite boundary conductance. The conductance from the intercellular air space to the chloroplast, where assimilation takes place (mainly for the C_3 plants), is represented by g_m13. The conductance from the intercellular air space to the mesophyll, where the CO_2-H_2O exchange occurs is expressed as g_m18.

CO_2 balance

First, we solve for the CO_2 mole fractions in the atmosphere (c_a), the intercellular air space (c_i), the mesophyll cell (c_m) and the chloroplast (c_c). The main assumptions in the leaf cuvette model are:

- The system is in steady state \( \left( \frac{dc_i}{dt} = 0 = g_s c_a + g_{m18} c_m - g_i c_i - g_{m18} c_i \right) \)
- The mixing in the cuvette is perfect (i.e. c_a = c_c)
- Boundary layer resistance can be neglected
- The conductance between intercellular space and mesophyll is assumed to be 3 times higher than the conductance between intercellular space and chloroplast (i.e. g_m18 = 3 \times g_{m13}).

Furthermore, we used a 100 ppm downdraw of CO_2 for each photosynthesis experiment. The mixing ratio of CO_2 entering the cuvette and leaving the cuvette is 500 ppm and 400 ppm, respectively. The leaf area, flowrate and assimilation rate used for the model are 30 cm^2, 0.7 L min^{-1} and 20.0 mol m^{-2}s^{-1}, respectively. The CO_2 mole fractions in all leaf reservoirs are calculated for each given c_m/c_a ratio, by assuming g_m18 = 0.3 mol m^{-2}s^{-1}).

Next, we assume a value for the C^{18}OO mole fraction inside the cuvette (c_{a,C^{18}OO}). From the mole fractions c_{a,C^{18}OO} and c_{m,C^{18}OO} and the conductance g_{s,C^{18}OO} and g_{m18,C^{18}OO} we can calculate the inflow of C^{18}OO into the intercellular air space. Since the system is in steady state, the inflow and outflow of C^{18}OO for the intercellular air space are equal and hence we can determine the mole fraction c_{i,C^{18}OO}. The ingoing C^{18}OO is known from the airflow rate and c_{c,C^{18}OO}, the outgoing C^{18}OO depends on the airflow rate and c_{a,C^{18}OO} and the plant uptake of C^{18}OO is A_{n,C^{18}OO} = g_{s,C^{18}OO} \times (c_{a,C^{18}OO} - c_{i,C^{18}OO}). By using an iterative procedure we can improve our estimate for c_{a,C^{18}OO}. Note that for each update in c_{a,C^{18}OO}, we also update the corresponding c_{i,C^{18}OO}

^{13}CO_2 balance

Next, we calculate the steady state ^{13}CO_2 mole fractions in the different model reservoirs and subsequently, we determine the discrimination Δ^{13}C. The additional assumptions used are:
For the fractionation factors we use $a_{13s} = 4.4$, $a_{18m} = 1.8\%\text{oo}$ and $b = 29\%\text{oo}$.

The uptake of CO$_2$ scales linearly with the CO$_2$ mole fraction in the chloroplast ($A_n = k \times c_c$) and similarly for $^{13}$CO$_2$ we have: $A_{n,^{13}CO_2} = k \times (1 - b) \times C_{c,^{13}CO_2}$.

We solve the steady state $^{13}$CO$_2$ balance by performing 2 nested iterations. First, we do an initial guess for the atmospheric $^{13}$CO$_2$ mole fraction ($C_{a,^{13}CO_2}$). Next, we do an initial guess for the photosynthetic uptake of $^{13}$CO$_2$ ($A_{n,^{13}CO_2}$). In the ‘inner’ iteration loop we use $c_{a,^{13}CO_2}$ and $A_{n,^{13}CO_2}$ to calculate the $^{13}$CO$_2$ mole fractions in all leaf reservoirs. From the $^{13}$CO$_2$ mole fraction in the chloroplast ($C_{c,^{13}CO_2}$) and the linear assimilation factor ($k \times (1 - b)$) we can calculate the corresponding $A_{n,^{13}CO_2}$ and compare this to our initial guess. Using an iterative procedure, we can find the $A_{n,^{13}CO_2}$ that corresponds to the assumed $C_{a,^{13}CO_2}$.

The ‘outer’ iteration loop is aimed at finding the steady state atmospheric $^{13}$CO$_2$ mole fraction ($C_{a,^{13}CO_2}$) using a mass balance for $^{13}$CO$_2$. We know the $^{13}$CO$_2$ mole fraction of the ingoing air ($C_{in,^{13}CO_2}$), we have assumed value for outgoing air ($C_{a,^{13}CO_2}$) and have calculated the corresponding photosynthetic uptake ($A_{n,^{13}CO_2}$). From the resulting imbalance, we can come up with a new guess for $C_{a,^{13}CO_2}$. After each update of our estimate for $C_{a,^{13}CO_2}$, we repeat the ‘inner’ iteration loop to update $A_{n,^{13}CO_2}$.

$^{18}$OO balance

To solve the $^{18}$OO balance, we assume on top of the earlier mentioned assumptions:

- For the fractionation of $^{18}$OO for diffusion through stomata and diffusion into the mesophyll cell, we used $a_{18s} = 8.8\%\text{oo}$ and $a_{18w} = 0.8\%\text{oo}$, respectively.
- The isotopic exchange between CO$_2$ and water in the mesophyll is fast enough to reach complete equilibration, which is a function of temperature.
- Isotopic equilibration between CO$_2$ and water does not occur in the intercellular air space.

We assume for the leaf water signature $\delta^{18}$O$_{\text{leaf}} = 10.467\%\text{oo}$ VSMOW and for the leaf water temperature $T_{\text{leaf}} = 22^\circ\text{C}$, from which we can calculate the $\delta^{18}$O signature and therefore (using $c_m$ from above) the $^{18}$OO mole fraction in the mesophyll ($c_{m,^{18}OO}$). Next, we assume a starting value for the $^{18}$OO mole fraction inside the cuvette ($c_{a,^{18}OO}$). From the steady state mass balance, we can then determine the mole fraction $c_{i,^{18}OO} = c_i = \frac{g_s c_a + g_m c_m}{g_s + g_m}$. The ingoing $^{18}$OO is known from the airflow rate and $c_{a,^{18}OO}$, the outgoing $^{18}$OO depends on the airflow rate and $c_{a,^{18}OO}$ and the plant uptake of $^{18}$OO is $A_{n,^{18}OO} = g_s c_{^{18}OO} \times (c_{a,^{18}OO} - c_{i,^{18}OO})$.

A similar mass balance is implemented for $^{17}$OO, the fractionation of $^{17}$OO for diffusion through stomata and diffusion into the mesophyll cell we used $a_{17s} = 4.4\%\text{oo}$ and $a_{18w} = 0.382\%\text{oo}$, respectively. We assume for the leaf water signature $\delta^{18}$O$_{\text{leaf}} = 5.39\%\text{oo}$ VSMOW.
def clumpy_e17O(key1, var1, Cin, d17Oin, d18Oin, An, Aleaf, key2, var2, gm13C, d17O_leaf, d18O_leaf, print_table=False):

    """
    Description:
    CLUMPy (Clumped isotopes Leaf Uptake Model in Python) calculates the outgoing D17O signature for a leaf in the cuvette experiment as described by Adnew et al.

    List of input variables:
    - Experimental conditions
      * airflow       rate of air entering cuvette [L/min]
      * drawdown     CO2 drawdown [ppm]
    - Ingoing air characteristics
      Ce              ingoing CO2 mixing ratio [ppm]
      d17Oe           ingoing d17O signature [permil VSMOW]
      d18Oe           ingoing d18O signature [permil VSMOW]
    - Leaf properties
      An              net assimilation rate [umol/(m2s)]
      Aleaf          leaf area [cm2]
      **gs            stomatal conductance [mol/(m2s)]
      **Ci_Ca        CO2-in-intercellular-space to CO2-in-atmosphere ratio [-]
      **Cm_Ca        CO2-in-mesophyll to CO2-in-atmosphere ratio [-]
      gm13C          conductance between intercellular space and chloroplast [mol/(m2s)]
    - Leaf water characteristics
      d17O_leaf       leaf water d17O signature [permil VSMOW]
      d18O_leaf       leaf water d18O signature [permil VSMOW]

    Notes:
    * The user can call the function with the value of airflow or drawdown for var1. The user is required to specify which input parameter is given by using the key1 variable.
    ** The user can call the function with the value of gs, Ci_Ca or Cm_Ca for var2. The user is required
to specify which input parameter is given by using the key2 variable.

```python
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""

#INITIALIZATION

# -- Import statement
import numpy as np

# -- Output settings
print_diag_d170a = False
print_diag_d180a = False

# -- Test key1
if (key1 == 'airflow'):
    airflow = var1  # L/min
elif (key1 == 'drawdown'):
    drawdown = var1  # ppm
else:
    print('Error: key1 not recognized')
    return

# -- Test key2
if (key2 == 'gs'):
    gs = var2  # mol/(m^2s)
else:
    print('Error: key2 not recognized')
    return
```

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""
# -- Isotope reference
R17_VSMOW = 0.0003799  # -
R18_VSMOW = 0.0020052  # -

# -- Reference line
RL = 0.528 #

# -- Physical constants
Vm = 24.5 # L/mol

# -- 17O fractionations
lambda_kinetic = 0.509 # (Young et al., 2002)
lambda_equilibration = 0.5229 # (Barkan and Luz, 2012)

# -- 18O fractionations
a18O_diff = 8.8 # permil (Farquhar et al., 1993)
a18O_liq = 0.8 # permil (Farquhar et al., 1993)
a18O_eq = 42.2 # permil (Brenninkmeijer et al., 1983)

# ==== CO2 MIXING RATIOS

# -- CO2 assimilation
CO2_assimilation = An*1.0e-6*Aleaf*1.0e-4 # molCO2/s

# -- Test key1
if (key1=='airflow'):
    # -- CO2 fluxes
    CO2_inflow = airflow/60.0/Vm*Ce*1.0e-6 # molCO2/s
    CO2_outflow = CO2_inflow-CO2_assimilation # molCO2/s

    # -- Cuvette condition
    Ca = CO2_outflow/(airflow/60.0)*Vm*1.0e6 # ppm

# -- Test key1
if (key1=='drawdown'):
    # -- Airflow
    airflow = CO2_assimilation*60.0*Vm/(drawdown*1.0e-6) # L/min

    # -- Cuvette condition
    Ca = Ce-drawdown # ppm

# -- Mesophyll conductance
gm18O = 3.0*gm13C # mol/(m2s)

# -- Test key2
if (key2=='gs'):
    # -- Intercellular CO2 mixing ratio
    Ci = Ca-An/gs # ppm
    # -- Mesophyll CO2 mixing ratio
    Cm = Ci-An/gm18O # ppm

# -- Test key2
if (key2=='Ci_Ca'):
    # -- Intercellular CO2 mixing ratio
    Ci = Ci_Ca*Ca # ppm
    # -- Mesophyll CO2 mixing ratio
    Cm = Ci-An/gm18O # ppm

# -- Stomatal conductance
gs = An/(Ca-Ci) # mol/(m2s)

# -- Test key2
if (key2=='Cm_Ca'):
    # -- Mesophyll CO2 mixing ratio
    Cm = Cm_Ca*Ca # ppm

    # -- Combined stomatal-mesophyll conductance
    gtot = An/(Ca-Cm) # mol/(m2s)

    # -- Stomatal conductance
    gs = 1.0/(1.0/gtot-1.0/gm18O) # mol/(m2s)

    # -- Intercellular CO2 mixing ratio
    Ci = Ca-An/gs # ppm

# ==== C17OO MIXING RATIOS

    # -- Mesophyll C17OO mixing ratio
    d17Om =
    1000.0*((d17O_leaf/1000.0+1)*((a18O_eq/1000.0+1.0)**lambda_equilibration)-1.0) # permil VSMOW
    R17m = (d17Om/1000.0+1.0)*R17_VSMOW # -
    Cm_17O = 2*R17m*Cm # ppm

    # -- C17OO conductances
    gs_17O = gs*((1.0-a18O_diff/1000.0)**lambda_kinetic) # mol/(m2s)
    gm18O_17O = gm18O*((1.0-a18O_liq/1000.0)**lambda_kinetic) # mol/(m2s)
# -- Ingoing C17OO mixing ratio
R17e = (d17Oin/1000.0+1.0)*R17_VSMOW # -
Ce_17O = 2*R17in*Ce # ppm

# -- C17OO signature
d17Oa = d17Oin # permil VSMOW
max_qq = 100 # -
relax_qq = 0.5 # -
for qq in np.arange(max_qq):
    # -- Atmospheric C17OO mixing ratio
    R17a = (d17Oa/1000.0+1.0)*R17_VSMOW # -
    Ca_17O = 2*R17a*Ca # ppm

    # -- Intercellular d17O signature
    Ce_17O = (Ca_17O*gs_17O+Cm_17O*gm18O_17O)/(gs_17O+gm18O_17O) # ppm
    R17i = 0.5*Ci_17O/Ci # -
    d17Oi = 1000.0*(R17i/R17_VSMOW-1.0) # permil VSMOW

    # -- Calculate C17OO fluxes
    inflow_C17OO = airflow/60.0/Vm*Ce_17O*1.0e-6 # molC17OO/s
    leafuptake_C17OO = (Ca_17O-Ci_17O)*gs_17O*1.0e-6*Aleaf*1.0e-4 # molC17OO/s
    outflow_C17OO = inflow_C17OO-leafuptake_C17OO # molC17OO/s

    # -- Copy previous d17Oa signature
    d17Oa_prev = d17Oa # permil VSMOW

    # -- Calculate new d17Oa signature
    Ca_17O = outflow_C17OO/(airflow/60.0)*Vm*1.0e6 # ppm
    R17a = 0.5*Ca_17O/Ca # -
    d17Oa_diff = 1000.0*(R17a/R17_VSMOW-1.0)-d17Oa # permil VSMOW
    d17Oa = d17Oa+relax_qq*d17Oa_diff # permil VSMOW

    # -- Print diagnostics
    if (print_diag_d17Oa):
        print ('---------------------------------------')
        print ('d17Oa iteration ', qq+1)
        print ('---------------------------------------')
        print ('Ca_17O ', Ca_17O)
        print ('Ci_17O ', Ci_17O)
        print ('Cm_17O ', Cm_17O)
        print ('R17a ', R17a)
        print ('R17i ', R17i)
        print ('R17m ', R17m)
        print ('d17Oin ', d17Oin)
        print ('d17Oa ', d17Oa)
        print ('d17Oi ', d17Oi)
        print ('d17Om ', d17Om)
        print ('---------------------------------------')
        print()
# -- Clear loop index
del qq

# -- Determine convergence
conv_d170a = np.abs((d170a-d170a_prev)/d170a_prev)  # -

# -- Test convergence
if (conv_d170a > 1.0e-6):
    print ('WARNING: Convergence in outgoing d170 signature less than 1.0e-6')
    print ('conv ', conv_d170a)
    print()

# ===== C180O MIXING RATIOS

# -- Mesophyll C180O mixing ratio
d18Om = 1000.0*((d180_leaf/1000.0+1.0)*(a18O_eq/1000.0+1.0)-1.0)  # permil VSMOW
R18m = (d18om/1000.0+1.0)*R18_VSMOW  # -
Cm_18O = 2*R18m*Cm  # ppm

# -- C180O conductances
gs_18O = gs*(1.0-a18O_diff/1000.0)  # mol/(m2s)
gm18O_18O = gm18O*(1.0-a18O_liq/1000.0)  # mol/(m2s)

# -- Ingoing C180O mixing ratio
R18e = (d180e/1000.0+1.0)*R18_VSMOW  # -
Ce_18O = 2*R18in*Ce  # ppm

# -- C180O signature
d180a = d180e  # permil VSMOW
max_pp = 100  # -
relax_pp = 0.5  # -
for pp in np.arange(max_pp):
    # -- Atmospheric C180O mixing ratio
    R18a = (d180a/1000.0+1.0)*R18_VSMOW  # -
    Ca_18O = 2*R18a*Ca  # ppm

    # -- Intercellular d180 signature
    Ci_18O = (Ca_18O*gs_18O+Cm_18O*gm18O_18O)/(gs_18O+gm18O_18O)  # ppm
    R18i = 0.5*Ci_18O/Ci  # -
    d180i = 1000.0*(R18i/R18_VSMOW-1.0)  # permil VSMOW

    # -- Calculate C180O fluxes
    inflow_C180O = airflow/60.0/Vm*Cin_18O*1.0e-6  # molC180O/s
    leafuptake_C180O = (Ca_18O-Ci_18O)*gs_18O*1.0e-6*Aleaf*1.0e-4  # molC180O/s
    outflow_C180O = inflow_C180O-leafuptake_C180O  # molC180O/s

    # -- Copy previous d180a signature
d180a_prev = d180a  # permil VSMOW

    # -- Calculate new d180a signature
Ca_{18O} = \text{outflow}_C18OO/(\text{airflow}/60.0)*Vm*1.0e6 \ # \ ppm
\text{R18a} = 0.5*\text{Ca}_{18O}/\text{Ca} \ # -
d18Oa_{diff} = 1000.0*(\text{R18a}/\text{R18}_{VSMOW}-1.0)-d18Oa \ # \ permil \ VSMOW
d18Oa = d18Oa+\text{relax}_pp^*d18Oa_{diff} \ # \ permil \ VSMOW

# -- Print diagnostics
if (print_diag_d18Oa):
    print ('---------------------------------------')
    print ('d18Oa iteration ', pp+1)
    print ('---------------------------------------')
    print ('Ca_{18O} ', Ca_{18O})
    print ('Ci_{18O} ', Ci_{18O})
    print ('Cm_{18O} ', Cm_{18O})
    print ('R18a ', R18a)
    print ('R18i ', R18i)
    print ('R18m ', R18m)
    print ('d18Oin ', d18Oe)
    print ('d18Oa ', d18Oa)
    print ('d18Oi ', d18Oi)
    print ('d18Om ', d18Om)
    print ('---------------------------------------')
    print ()

# -- Clear loop index
del pp

# -- Determine convergence
conv_d18Oa = np.abs((d18Oa-d18Oa_prev)/d18Oa_prev) # -

# -- Test convergence
if (conv_d18Oa > 1.0e-6):
    print ('WARNING: Convergence in outgoing d18O signature less than 1.0e-6')
    print ('conv ', conv_d18Oa)
    print ()

# ==== EXCESS -17O

# -- Calculate e17O in regions
e17Oe = 1.0e6*(np.log(d17Oe/1000.0+1.0)-RL*np.log(d18Oe/1000.0+1.0)) \ # \ per \ meg
e17Oa = 1.0e6*(np.log(d17Oa/1000.0+1.0)-RL*np.log(d18Oa/1000.0+1.0)) \ # \ per \ meg
e17Oi = 1.0e6*(np.log(d17Oi/1000.0+1.0)-RL*np.log(d18Oi/1000.0+1.0)) \ # \ per \ meg
e17Om = 1.0e6*(np.log(d17Om/1000.0+1.0)-RL*np.log(d18Om/1000.0+1.0)) \ # \ per \ meg

# RESULTS
# # #
# # #
# # #
# # #
# # #
# # #
# -- Test print settings
if print_table:
3. Derivation of the $^{18}$O-photosynthetic discrimination

Following (Farquhar and Lloyd, 1993), the assimilation rate for C$^{16}$O$^{16}$O is calculated as:

$$A = \frac{c_a - c_m}{r_m} \quad (S2)$$

where $A$, $r_m$, $c_a$, $c_m$ are the assimilation rate, resistance as CO$_2$ diffuses from the leaf surrounding to the CO$_2$-H$_2$O exchange site, the mole fraction of CO$_2$ in the air surrounding the leaf and at the CO$_2$-H$_2$O exchange site, respectively. The assimilation rate for C$^{18}$O$^{16}$O is calculated as:
\[ {^{18}}R_A \times A = \frac{{^{18}R_a \times c_a - ^{18}R_m \times c_m}}{{\alpha^{18}_m \times r_m}} \]  \hspace{1cm} (S3)

where \( \alpha^{18}_m \) is the fractionation factor as \( ^{18}O^{16}O \) diffuses from the leaf surrounding to the \( CO_2-H_2O \) exchange site. Dividing equation 2 by equation 1 leads to:

\[ \frac{{^{18}R_A}}{{^{18}R_a}} = \frac{{^{18}R_a \times c_a - ^{18}R_m \times c_m}}{{\alpha^{18}_m \times (c_a - c_m)}} \]  \hspace{1cm} (S4)

\[ \frac{{^{18}R_A}}{{^{18}R_a}} = \frac{{c_a - ^{18}R_a \times c_m}}{{\alpha^{18}_m \times (c_a - c_m)}} \]  \hspace{1cm} (S5)

\[ \frac{{^{18}R_A}}{{^{18}R_a}} = \frac{{\alpha^{18}_m \times (c_a - c_m)}}{{c_a - ^{18}R_m \times c_m}} \]  \hspace{1cm} (S6)

\[ \frac{{^{18}R_a}}{{^{18}R_a}} - 1 = \frac{{\alpha^{18}_m \times (c_a - c_m)}}{{c_a - ^{18}R_m \times c_m}} - 1 \]  \hspace{1cm} (S7)

Using the definitions

\[ \Delta_A^{^{18}}O_{FM} = \frac{{^{18}R_a}}{{^{18}R_A}} - 1 \]  \hspace{1cm} (S8)

And

\[ \delta^{^{18}}O_{ma} = \frac{{^{18}R_m}}{{^{18}R_a}} - 1 \]  \hspace{1cm} (S9)

This can be rewritten as

\[ \Delta_A^{^{18}}O_{FM} = \frac{{\alpha^{18}_m \times (c_a - c_m)}}{{c_a - c_m \times (\delta^{^{18}}O_{ma} + 1)}} - 1 \]  \hspace{1cm} (S10)
\[ \Delta A^{18}\text{O}_{\text{FM}} = \frac{\bar{a}_{18} + \frac{c_m}{c_a - c_m} \times \delta^{18}\text{O}_\text{ma}}{1 - \frac{c_m}{c_a - c_m} \times \delta^{18}\text{O}_\text{ma}} \]  

\( \Delta A^{17}\text{O}_{\text{FM}} \) can be calculated analogous to \( \Delta A^{18}\text{O}_{\text{FM}} \).

\[ \Delta A^{17}\text{O}_{\text{FM}} = \frac{\bar{a}_{17} + \frac{c_m}{c_a - c_m} \times \delta^{17}\text{O}_\text{ma}}{1 - \frac{c_m}{c_a - c_m} \times \delta^{17}\text{O}_\text{ma}} \]

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**Figure S1.** Calibration of the LGR water isotope analyser for the measurement of \( \delta^{18}\text{O} \) and \( \delta D \) of water vapor.  

a) \( \delta^{18}\text{O}_{\text{IAEA}} \) is the value assigned by IAEA while \( \delta^{18}\text{O}_{\text{LGR}} \) is the value reported by the LGR instrument.  

b) \( \delta D_{\text{IAEA}} \), is the value assigned by IAEA while \( \delta D_{\text{LGR}} \) is the value reported by the LGR instrument.
Figure S2 Dependency of isotope composition of water vapour on the water vapor concentration and the carrier gas used for the water vapor standard source for $\delta^{18}$O, $\delta^{17}$O, $\Delta^{17}$O and $\delta D$. 
Figure S3 Allan variance curves of $\delta D$, $\delta^{17}O$ and $\delta^{18}O$ for measurement at 20000 ppm water vapor concentration.
Figure S4 Schematic drawing of the setup used for the extraction of leaf water. The vial containing the leaf is heated to 60°C while the other vial is immersed in the liquid nitrogen to collect the water vapor.
Figure S5 Schematic diagram of the system used for the extraction of carbon dioxide from air samples. The moisture trap is cooled by a dry ice-ethanol mixture while the CO₂ trap is cooled by liquid nitrogen.
Figure S6 Schematic diagram of the conceptual leaf-cuvette model. $\delta$ and $c$ are the oxygen isotope composition and mixing ratio of CO$_2$. The subscript $c$, $a$, $i$, $m$, $e$ represents CO$_2$ entering the cuvette, leaving the cuvette, the intercellular air space, the mesophyll and the chloroplast, respectively.
Figure S7 The correlation between the observed $\Delta\Delta^{17}\text{O}$ (calculated using equation 12) and $\Delta\Delta^{17}\text{O}$ estimated from the Farquhar model ($\Delta\Delta^{17}\text{O}_{\text{FM}} = \ln(\Delta^{17}\text{O}_{\text{FM}} + 1) - 0.528 \times \ln (\Delta^{18}\text{O}_{\text{FM}} + 1)$).
Figure 8. $\Delta^{13}\text{C}$ of carbon dioxide during photosynthesis. a) The $\Delta^{13}\text{C}$ of the two C$_3$ plants sunflower (circles) and ivy (triangles) at two different irradiances. b) The $\Delta^{13}\text{C}$ of maize at two different irradiances. The measurement error in $\Delta^{13}\text{C}_{\text{obs}}$ is 0.15‰ (SD), calculated using error propagation.

Figure S9. $\Delta^{13}\text{C}$ of carbon dioxide during photosynthesis. The $\Delta^{13}\text{C}$ of the two C$_3$ plants sunflower (circle) and ivy (triangles) and a C$_4$ plant maize (stars).
Figure S10. The relative difference in δ^{17}O (a) and δ^{18}O (b) of CO₂ leaving (index a) and entering (index e) the leaf cuvette during experiments with two C₃ plants, sunflower (circles) and ivy (triangles) and a C₄ plant maize (stars) as a function of the cₘ/cₐ ratio. The experiments were performed at low light (300 µmol m⁻² s⁻¹, purple) and high light (1200 µmol m⁻² s⁻¹, yellow) conditions.
Figure S11 The difference in $\Delta^{17}O$ of the CO$_2$ leaving (index a) and entering (index e) the cuvette as a function of $c_m/c_a$ for sunflower (circles), ivy (triangles) and maize (stars).

Figure S12. 3D model result for the global average seasonal cycle of $\Delta^{17}O$ of atmospheric CO$_2$ for the years 2012 and 2013 (Koren et al., 2019). Note that $\Delta^{17}O$ value is reported here with $\lambda$ value of 0.5229. To convert to the $\lambda$ value of 0.528, $\Delta^{17}O(CO_2)_{\lambda=0.528}=\Delta^{17}O(CO_2)_{\lambda=0.5229}+(0.5229-0.528)\times\ln(\delta^{18}O(CO_2)+1)$. In the 3D global model, $\delta^{18}O(CO_2)$ is 41.5‰.
Figure S13. A 3D model result for the seasonal cycle of the global $\Delta^{17}O$ value of leaf water for the year 2012 (Koren et al., 2019). Note that $\Delta^{17}O$ value is reported here with $\lambda$ value of 0.5229. To convert to the $\lambda$ value of 0.528, $\Delta^{17}O(leaf)_{\lambda=0.528} = \Delta^{17}O(leaf)_{\lambda=0.5229} + (0.5229-0.528) \times \ln \alpha_{trans}$. $\alpha_{trans} = 1/0.9917$ (Koren et al., 2019; Hofmann et al., 2017).
Figure S14 Annual variability of $c_i/c_a$ ratio for C$_4$ and C$_3$ plants from SiBCASA model (Schaefer et al., 2008; Koren et al., 2019).

Table S1 Reproducibility of the extraction system and the CO$_2$-O$_2$ exchange system. Extraction reproducibility experiments were performed using compressed air. To establish the reproducibility of the CO$_2$-O$_2$ isotope exchange system we used pure CO$_2$ (SCOTT, Air Products, Germany). $SE$ and $SD$ is a standard error and standard deviation. All the isotope values given in the table are in per mill [%].

<table>
<thead>
<tr>
<th>Extraction date</th>
<th>$\delta^{18}$O</th>
<th>SE</th>
<th>$\delta^{13}$C</th>
<th>SE</th>
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<tbody>
<tr>
<td>03/03/2019</td>
<td>41.411</td>
<td>0.005</td>
<td>-8.636</td>
<td>0.004</td>
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<td>0.006</td>
<td>-8.642</td>
<td>0.003</td>
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<td>-8.648</td>
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<tr>
<td>05/03/2019</td>
<td>41.330</td>
<td>0.008</td>
<td>-8.652</td>
<td>0.004</td>
</tr>
<tr>
<td>Date</td>
<td>Pre-exchange O₂ (ppm)</td>
<td>Post-exchange O₂ (ppm)</td>
<td>Pre-exchange CO₂ (ppm)</td>
<td>Post-exchange CO₂ (ppm)</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------</td>
<td>------------------------</td>
<td>------------------------</td>
<td>------------------------</td>
</tr>
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<td>-8.653</td>
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<td>41.329</td>
<td>0.008</td>
<td>-8.652</td>
<td>0.003</td>
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<td>-8.646</td>
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<td>0.007</td>
<td>-8.643</td>
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<tr>
<td>Mean ± SD</td>
<td>41.351 ± 0.030</td>
<td>-8.647 ± 0.007</td>
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<table>
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<tr>
<th>EXP</th>
<th>Pre-exchange O₂</th>
<th>Post-exchange O₂</th>
<th>Pre-exchange CO₂</th>
<th>Post-exchange CO₂</th>
<th>Δ²¹⁷O₂</th>
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<td>9</td>
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<td>-4.210</td>
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<td>-20.85</td>
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<td>Mean ± SD</td>
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<td>-0.142±0.008</td>
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Table S2: The ratio of stomatal conductance to mesophyll conductance and weighted mean fractionation of $^{12}\text{C}^{18}\text{O}^{16}\text{O}$ as it diffuses from the CO$_2$-H$_2$O exchange site ($\bar{a}_{18}$) for numerous species determined in previous investigations.

<table>
<thead>
<tr>
<th>$g_s/g_m$</th>
<th>$\bar{a}_{18}$</th>
<th>Plant type</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>1.56</td>
<td>3.93</td>
<td><em>S. viridis</em> (C$_4$)</td>
<td>(Osborn et al., 2017)</td>
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<tr>
<td>1.26</td>
<td>4.33</td>
<td><em>S. viridis</em> (C$_4$)</td>
<td>(Osborn et al., 2017)</td>
</tr>
<tr>
<td>0.22</td>
<td>7.37</td>
<td>Tobacco (C$_3$)</td>
<td>(Gillon and Yakir, 2000)</td>
</tr>
<tr>
<td>0.16</td>
<td>7.71</td>
<td>Soya (C$_3$)</td>
<td>(Gillon and Yakir, 2000)</td>
</tr>
<tr>
<td>0.47</td>
<td>6.23</td>
<td>Oak (C$_3$)</td>
<td>(Gillon and Yakir, 2000)</td>
</tr>
<tr>
<td>0.17</td>
<td>7.76</td>
<td>Tobacco (C$_3$)</td>
<td>(Barbour et al., 2016)</td>
</tr>
<tr>
<td>0.06</td>
<td>8.32</td>
<td>Cotton (C$_3$)</td>
<td>(Barbour et al., 2016)</td>
</tr>
<tr>
<td>0.32</td>
<td>7.04</td>
<td>Wheat (C$_3$)</td>
<td>(Barbour et al., 2016)</td>
</tr>
<tr>
<td>0.06</td>
<td>8.27</td>
<td>Maize (C$_4$)</td>
<td>(Barbour et al., 2016)</td>
</tr>
<tr>
<td>0.24</td>
<td>7.29</td>
<td><em>S. viridis</em> (C$_4$)</td>
<td>(Barbour et al., 2016)</td>
</tr>
<tr>
<td>0.29</td>
<td>7.21</td>
<td><em>F. bindentis</em> (C$_4$)</td>
<td>(Barbour et al., 2016)</td>
</tr>
<tr>
<td>0.88</td>
<td>5.05</td>
<td>Sunflower (C$_3$)</td>
<td>This study</td>
</tr>
<tr>
<td>0.55</td>
<td>5.96</td>
<td>Ivy (C$_3$)</td>
<td>This study</td>
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<td>0.27</td>
<td>7.12</td>
<td>Maize (C$_4$)</td>
<td>This study</td>
</tr>
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<td>1.2</td>
<td>4.18</td>
<td><em>A. edulis</em> (C$_4$)</td>
<td>(Cousins et al., 2006)</td>
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<tr>
<td>2.1</td>
<td>3.74</td>
<td><em>A. edulis</em> (C$_4$)</td>
<td>(Cousins et al., 2006)</td>
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<td>0.13</td>
<td>7.90</td>
<td><em>A. edulis</em> (C$_4$)</td>
<td>(Cousins et al., 2007)</td>
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</table>
Table S3 List of symbols and equations used for $^{13}$C discrimination calculation

<table>
<thead>
<tr>
<th>Symbol</th>
<th>description</th>
<th>Unit/calculation/value</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t^{13}$</td>
<td>Ternary correction for $^{13}$C</td>
<td>$\frac{(1+a_{13b})E}{2E_{CO_2}}$</td>
<td>(Farquhar and Cernusak, 2012)</td>
</tr>
<tr>
<td>$eRd$</td>
<td>Dark respiration rate</td>
<td>0.8 µmol m$^{-2}$s$^{-1}$ [measured]</td>
<td></td>
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<tr>
<td>$Rd$</td>
<td>Day respiration rate</td>
<td>1.5 µmol m$^{-2}$s$^{-1}$</td>
<td>(Cernusak et al., 2004)</td>
</tr>
<tr>
<td>$a_{13b}$</td>
<td>Fractionation in $^{13}$CO$_2$ as CO$_2$ diffuses through the boundary layer</td>
<td>2.9‰</td>
<td>(Farquhar and Lloyd, 1993)</td>
</tr>
<tr>
<td>$a_{13s}$</td>
<td>Fractionation in $^{13}$CO$_2$ as CO$_2$ diffuses through the stomata</td>
<td>4.4‰</td>
<td>(Vogel J.C., 1980; Farquhar et al., 1982; Farquhar and Lloyd, 1993)</td>
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<tr>
<td>$a_m$</td>
<td>fractionation factor for dissolution and diffusion through water</td>
<td>1.8‰</td>
<td>(Farquhar et al., 1982; Wingate et al., 2007)</td>
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<td>$f$</td>
<td>a fractionation factor for photorespiration (decarboxylation of glycine)</td>
<td>16‰</td>
<td>(Evans and von Caemmerer, 2013)</td>
</tr>
<tr>
<td>$e$</td>
<td>The fractionation factor for day respiration</td>
<td>$eRd + e^\ast$, ‰</td>
<td>(Wingate et al., 2007; Bickford et al., 2009)</td>
</tr>
<tr>
<td>$e^\ast$</td>
<td>The apparent fractionation for day respiration</td>
<td>$\delta^{13}C_{at} - \Delta A^{13}C - \delta^{13}C_{subrate}$ ‰</td>
<td>(Wingate et al., 2007; Cernusak et al., 2013)</td>
</tr>
<tr>
<td>$b$</td>
<td>Fractionation factor for uptake by RubisCO</td>
<td>29‰</td>
<td>(Farquhar et al., 1982; Farquhar and Lloyd, 1993)</td>
</tr>
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<td>$\alpha_f$</td>
<td>a fractionation due to photorespiration (decarboxylation of glycine)</td>
<td>$1 + f$</td>
<td></td>
</tr>
<tr>
<td>$\alpha_e$</td>
<td>fractionation due to day respiration</td>
<td>$1 + e$</td>
<td></td>
</tr>
<tr>
<td>$\alpha_b$</td>
<td>Fractionation due to uptake by RubisCO</td>
<td>$1 + b$</td>
<td></td>
</tr>
<tr>
<td>$\delta^{13}C_{subt}$</td>
<td>Isotopic composition</td>
<td>$\delta^{13}C_a - \Delta A^{13}C - \delta^{13}C_{subrate}$ ‰</td>
<td>(Farquhar et al., 1989)</td>
</tr>
<tr>
<td>$\Delta A^{13}C$</td>
<td>13C-photosynthetic discrimination</td>
<td>$\frac{\zeta(\delta^{13}C_a - \delta^{13}C_e)}{1 + \delta^{13}C_a - \zeta(\delta^{13}C_a - \delta^{13}C_e)}$, ‰</td>
<td>(Evans et al., 1986)</td>
</tr>
</tbody>
</table>
| $A_{\Delta^{13}C_{ob}}$ | $^{13}$C-photosynthetic discrimination (Farquhar model) | \[
\frac{1}{1 - t} \left[ \alpha_{13bs} \frac{c_a - c_i}{c_a} \right] 
+ \frac{(1 + t)}{(1 - t)} \left[ a_m \frac{c_i - c_c}{c_a} + b \frac{c_c}{c_a} \right] 
- \alpha_b \frac{R_d}{\alpha_e R_d + A} \frac{c_c - \Gamma^*}{c_a} - \alpha_b \frac{\Gamma^*}{\alpha_f c_a} \right]
\] (Farquhar and Cernusak, 2012) |
| --- | --- | --- |
| $A_{\Delta^{13}C_i}$ | $^{13}$C-photosynthetic discrimination (assuming the no mesophyll conductance, i.e $c_i=c_c$) | \[
\frac{1}{1 - t} \left[ \frac{c_a - c_i}{c_a} \right] 
+ \frac{(1 + t)}{(1 - t)} \left[ \frac{b}{c_a} - \frac{\alpha_b}{\alpha_e} \frac{R_d}{R_d + A} \frac{c_i - \Gamma^*}{c_a} \right] 
- \frac{\alpha_b \Gamma^*}{\alpha_f c_a} \right]
\] (Farquhar and Cernusak, 2012) |
References

Barbour, M. M., Evans, J. R., Simonin, K. A., and Caemmerer, S. V.: Online CO\textsubscript{2} and H\textsubscript{2}O oxygen isotope fractionation allows estimation of mesophyll conductance in C\textsubscript{4} plants, and reveals that mesophyll conductance decreases as leaves age in both C\textsubscript{4} and C\textsubscript{3} plants, New Phytol., 14, 2016.


