



POTSDAM-INSTITUT FÜR
KLIMAFOLGENFORSCHUNG

Originally published as:

Werner, C., Schnyder, H., Cuntz, M., Keitel, C., Zeeman, M. J., Dawson, T. E., Badeck, F.-W., Brugnoli, E., Ghashghaie, J., Grams, T. E. E., Kayler, Z. E., Lakatos, M., Lee, X., Máguas, C., Ogée, J., Rascher, K. G., Siegwolf, R. T. W., Unger, S., Welker, J., Wingate, L., Gessler, A. (2012): Progress and challenges in using stable isotopes to trace plant carbon and water relations across scales. - Biogeosciences, 9, 8, 3083-3111

DOI: [10.5194/bg-9-3083-2012](https://doi.org/10.5194/bg-9-3083-2012)



Progress and challenges in using stable isotopes to trace plant carbon and water relations across scales

C. Werner^{1,19}, H. Schnyder², M. Cuntz³, C. Keitel⁴, M. J. Zeeman⁵, T. E. Dawson⁶, F.-W. Badeck⁷, E. Brugnoli⁸, J. Ghashghaie⁹, T. E. E. Grams¹⁰, Z. E. Kayler¹¹, M. Lakatos¹², X. Lee¹³, C. Máguas¹⁴, J. Ogée¹⁵, K. G. Rascher¹, R. T. W. Siegwolf¹⁶, S. Unger¹, J. Welker¹⁷, L. Wingate¹⁸, and A. Gessler¹¹

¹Experimental and Systems Ecology, University Bielefeld, 33615 Bielefeld, Germany

²Lehrstuhl für Grünlandlehre, Technische Universität München, 85350 Freising-Weißenstephan, Germany

³UFZ – Helmholtz Centre for Environmental Research, Permoserstr. 15, 04318 Leipzig, Germany

⁴University of Sydney, Faculty of Agriculture, Food and Natural Resources, 1 Central Avenue, Eveleigh, NSW 2015, Australia

⁵College of Oceanic and Atmospheric Sciences, Oregon State University, 104 COAS Admin Bldg, Corvallis (OR), USA

⁶Center for Isotope Biogeochemistry, Department of Integrative Biology, University of California, Berkeley, CA 94720, USA

⁷Potsdam Institute for Climate Impact Research (PIK) PF 60 12 03, 14412 Potsdam, Germany

⁸CNR – National Research Council of Italy – Institute of Agro-environmental and Forest Biology, via Marconi 2, 05010 Porano (TR), Italy

⁹Laboratoire d'Ecologie, Systématique et Evolution (ESE), CNRS AgroParisTech – UMR8079, Bâtiment 362, Université de Paris-Sud (XI), 91405 Orsay Cedex, France

¹⁰Ecophysiology of Plants, Department of Ecology and Ecosystem Management, Technische Universität München, Von-Carlowitz-Platz 2, 85354 Freising, Germany

¹¹Institute for Landscape Biogeochemistry Leibniz-Zentrum für Agrarlandschaftsforschung (ZALF) e.V., Eberswalderstr. 84, 15374 Müncheberg, Germany

¹²Experimental Ecology, University of Kaiserslautern, Erwin-Schroedinger Str. 13, 67663 Kaiserslautern, Germany

¹³School of Forestry and Environmental Studies, Yale University, New Haven, CT 06511, USA

¹⁴Centre for Environmental Biology, University of Lisbon, Campo Grande, 1749-016, Lisbon, Portugal

¹⁵UR 1263, INRA, Bordeaux, France

¹⁶Lab for Atmospheric Chemistry, Paul Scherrer Institute, 5232 Villigen-PSI, Switzerland

¹⁷Environment and Natural Resources Institute, University of Alaska Anchorage, 3211 Providence Dr. Anchorage, AK 99508-4614, USA

¹⁸Department of Plant Sciences, University of Cambridge, Cambridge, UK

¹⁹Agroecosystem, Functional Ecosystem Research, University Bayreuth, 95447 Bayreuth, Germany

Correspondence to: C. Werner (c.werner@uni-bayreuth.de)

Received: 18 February 2011 – Published in Biogeosciences Discuss.: 15 March 2011

Revised: 8 June 2012 – Accepted: 20 June 2012 – Published: 13 August 2012

Abstract. Stable isotope analysis is a powerful tool for assessing plant carbon and water relations and their impact on biogeochemical processes at different scales. Our process-based understanding of stable isotope signals, as well as technological developments, has progressed significantly, opening new frontiers in ecological and interdisciplinary research. This has promoted the broad utilisation of carbon, oxygen

and hydrogen isotope applications to gain insight into plant carbon and water cycling and their interaction with the atmosphere and pedosphere. Here, we highlight specific areas of recent progress and new research challenges in plant carbon and water relations, using selected examples covering scales from the leaf to the regional scale. Further, we discuss strengths and limitations of recent technological

developments and approaches and highlight new opportunities arising from unprecedented temporal and spatial resolution of stable isotope measurements.

1 Introduction

Stable isotopes are a powerful tool for tracing biogeochemical processes across spatio-temporal scales (Yakir and Sternberg, 2000). The stable isotope composition of plant material, animal tissues, sediments and trace gases can be used as indicators of ecological change (Dawson and Siegwolf, 2007). The assessment of the circulation of isotopes in the biosphere allows characterisation and quantification of biogeochemical cycles as well as exploration of food webs (Fry, 2006). Stable isotope studies give insights into key reactions of plant metabolism (Schmidt and Gleixner, 1998), can increase our understanding of water movement along the soil-plant-atmosphere continuum (Dawson et al., 1998), and allows palaeoclimatic/-physiological reconstructions (Beerling and Woodward, 1998). Moreover, the analysis of the isotopic composition of trace gases exchanged between ecosystems and the atmosphere gives insights in the underlying processes driving the source and sink strength of biomes for CO₂, CH₄ and/or N₂O (Flanagan et al., 2005). Stable carbon, oxygen and hydrogen isotope composition of organic matter and inorganic compounds such as CO₂ and H₂O is altered during vegetation–soil–atmosphere exchange processes, such as evapotranspiration, carbon assimilation and respiration. This leaves an isotopic imprint on soil, plant and atmospheric carbon and water pools and associated fluxes. These isotopic fingerprints can then be used to trace different processes involved in the transfer of carbon and water across the plant–soil–atmosphere continuum. Particularly the multiple-isotope approach, i.e. the simultaneous measurements of stable isotope composition of different elements ($\delta^2\text{H}$, $\delta^{18}\text{O}$ and/or $\delta^{13}\text{C}$, for definition see Tables 1 and 2), provides a unique way to investigate the interrelation between water and carbon fluxes (Ehleringer et al., 1993; Griffiths, 1998; Flanagan et al., 2005; Yakir and Sternberg, 2000). The use of biological archives may enable extrapolation of this information to longer time scales, such as the Anthropocene. Methodological advances allow isotopologue and compound-specific analyses at unprecedented resolution, providing new insight into isotope fractionation processes in metabolic pathways and in biogeochemical processes. Further, a more advanced mechanistic understanding of processes affecting the stable isotope composition in various ecosystem compartments allows modelling and prediction of water and carbon fluxes based on stable isotope information. In turn, new findings open new research frontiers and challenges. Here, we highlight recent progress and developments in carbon and oxygen isotope research and discuss the potential for further extending our

knowledge about water and carbon fluxes and cycling. We present advances and challenges on various scales from the leaf (Sect. 2.1), plant (Sect. 2.2), community (Sect. 2.3), ecosystem (Sect. 2.4), and regional scales (Sect. 2.5) as well as of different types of temporal (historical) isotopic archives (Sect. 2.6). At each scale, pertinent reviews are indicated which survey the published literature and pioneering work; thereafter, we focus on selected examples from the last decade. Finally, we highlight strengths and limitations of new technological developments (Sect. 3) and present an outlook (Sect. 4) on what we identify as main goals of the stable isotope research in carbon and water biogeochemistry.

2 Isotope effects across temporal and spatial scales

2.1 Leaf-level processes

2.1.1 CO₂ and H₂O exchange

Leaf CO₂ and H₂O fluxes have unique and distinct isotope signals that carry useful physiological and biogeochemical information. For example, environmental stresses, such as drought, cause systematic variation in carbon isotope discrimination during photosynthesis ($\Delta^{13}\text{C}$, see Table 1), shedding light on different steps of CO₂ transfer from the atmosphere to the chloroplasts (Evans et al., 1986; Farquhar et al., 1989a, b; Brugnoli and Farquhar, 2000). On the other hand, stomatal opening and, hence, transpiration, cause ¹⁸O and deuterium (²H) enrichment of water at the sites of evaporation (Δ_{ev} , see Table 2), which lead to the enrichment of the total leaf water (Dongmann et al., 1974; Farquhar and Lloyd, 1993). The oxygen of leaf-dissolved CO₂ exchanges with the ¹⁸O-enriched leaf water, entraining distinct ¹⁸O discrimination ($\Delta^{18}\text{O}$) during photosynthetic CO₂ exchange (Farquhar et al., 1993). The knowledge of $\Delta^{13}\text{C}$ and $\Delta^{18}\text{O}$ together can then be used to assess the limitations to CO₂ transfer between the intercellular air space and the chloroplasts (Gillon and Yakir, 2000b).

The theoretical understanding of the individual ¹³C and ¹⁸O fractionation phenomena (including transport/diffusion, transformations and exchange processes) in CO₂ and H₂O is well established for systems in steady-state (Dongmann et al., 1974; Farquhar et al., 1982; Evans et al., 1986; Farquhar et al., 1993). However, we are only at the beginning of gaining theoretical understanding for those in non-steady-state. On-line isotope discrimination studies, i.e. instantaneous measurements of leaf/plant gas exchange and the associated isotopic signals, during transient conditions and short-term dynamics bare the potential to expand our understanding beyond steady-state.

Even though our mechanistic understanding of photosynthetic carbon isotope discrimination and evaporative oxygen isotope enrichment has been increasing within the last decade, there are still open questions and methodological

Table 1. Introduction to terms and equations of carbon isotopes, photosynthetic discrimination and post-carboxylation fractionation.

Carbon isotopes, photosynthetic discrimination and post-carboxylation fractionation	Equations
<p>The delta notation for carbon isotopes</p> <p>Carbon has two stable isotopes, ^{12}C and ^{13}C, with natural abundances of 98.9 and 1.1 %, respectively. The relative abundance of ^{13}C in any sample is conventionally expressed in the δ notation (Eq. 1) which is defined as the relative deviation of the isotope ratio R ($R = ^{13}\text{C} / ^{12}\text{C}$) of a sample relative to that of an international standard (and is often expressed in ‰). The international standard is the R of CO_2 from a fossil belemnite in the Pee Dee formation of South Carolina. Today, ^{13}C standards are obtained from the IAEA in Vienna and are referred to as V-PDB (Coplen, 1995, 2011).</p>	$\delta^y X = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \quad (1)$ <p>where in the case of carbon isotopes, $^y X$ is replaced by ^{13}C, and R_{sample} and R_{standard} are the measured $^{13}\text{C} / ^{12}\text{C}$ ratios in the sample and standard, respectively</p>
<p>Carbon isotope discrimination</p> <p>The change in relative abundance of ^{13}C between an educt and product is called discrimination, often denoted with Δ. In the case of CO_2 as the source and the plant material as the product of photo- and biosynthesis, carbon isotope discrimination is described in Eq. (2).</p>	$\Delta^{13}\text{C} = (\delta^{13}\text{C}_a - \delta^{13}\text{C}_p) / (1 + \delta^{13}\text{C}_p) \quad (2)$ <p>where $\delta^{13}\text{C}_a$ and $\delta^{13}\text{C}_p$ are the $\delta^{13}\text{C}$ values of the CO_2 in air and the plant, respectively.</p>
<p>Photosynthetic carbon isotope discrimination</p> <p>Isotope discrimination during carbon assimilation has been modelled by Farquhar et al. (1982, 1989a) for C_3 plants by Eq. (3). This equation has been developed to describe leaf-level photosynthetic discrimination during the light period, where e denotes the fractionation of mitochondrial respiration in the light, i.e. day respiration (Tcherkez et al., 2010) and Γ_* the compensation point in the absence of day respiration. When Eq. (3) is applied to analyse $\Delta^{13}\text{C}$ of bulk tissue as an integrative parameter for preceding photosynthetic discrimination during formation of this material, e denotes the integrated respiratory discrimination both during light and dark-respiration. However, additional factors such as fractionation during carbon allocation, tissue turnover or carbon partitioning into different plant organs may affect the observed discrimination. To date, we still lack a quantitative description of these processes (see Sects. 2.1.2 and 2.2).</p>	$\Delta^{13}\text{C} = a_b \frac{p_a - p_s}{p_a} + a \frac{p_s - p_i}{p_a} + (e_s + a_1) \frac{p_i - p_c}{p_a} + b \frac{p_c}{p_a} - \frac{e \frac{R_d}{k} + f \Gamma_*}{p_a} \quad (3)$ <p>where a_b is the fractionation during diffusion in the boundary layer (2.9 ‰); a is fractionation during binary diffusion in air (4.4 ‰); e_s is discrimination during CO_2 dissolution (1.1 ‰ at 25 °C); a_1 is fractionation during diffusion in the liquid phase (0.7 ‰); b is fractionation during carboxylation in C_3 plants (≈ 29.5 ‰); p_a is atmospheric CO_2 partial pressure; p_s is CO_2 partial pressure at the leaf surface; p_i is sub-stomatal CO_2 partial pressure; p_c is CO_2 partial pressure at the site of carboxylation; e is fractionation during mitochondrial respiration; f is fractionation during photorespiration; k is carboxylation efficiency; and Γ_* is compensation point in the absence of mitochondrial respiration (R_d).</p>
<p>Simplified model of C_3 photosynthetic isotope discrimination</p> <p>Few empirical/experimental studies have used Eq. (3), partly due to lack of needed input data. Instead, a simplified version (Farquhar et al., 1982) has been used extensively (Eq. 4). This equation is valid on the condition that effects of boundary layer, internal conductance, photorespiration, day respiration and allocation are negligible. In strict terms, these conditions are met if boundary layer and internal conductance are infinitely high, photorespiration and respiration are infinitely low or non-discriminating, and isotope discrimination during allocation and partitioning does not happen. To account for effects of the neglected terms in Eq. (4), the value of b is often slightly reduced (≈ 28 ‰) (Brugnoli and Farquhar, 2000).</p>	$\Delta^{13}\text{C} = a + (b - a) \frac{p_i}{p_a} \quad (4)$ <p>where a is the fractionation during binary diffusion in air (4.4 ‰); b is fractionation during carboxylation in C_3 plants; and p_a and p_i are the atmospheric and sub-stomatal CO_2 partial pressures, respectively.</p>
<p>Intrinsic water use efficiency (WUE_i)</p> <p>In Eq. (4), $\Delta^{13}\text{C}$ is directly proportional to p_i/p_a, which is determined by the relationship between photosynthetic assimilation (A) and stomatal conductance (g_s). Therefore, $\Delta^{13}\text{C}$ is a measure of intrinsic water use efficiency WUE_i (or transpiration efficiency), the ratio of assimilation to transpiration, which can be estimated as $\text{WUE}_i / \text{VPD}$ (Farquhar and Richards, 1984; Farquhar et al., 1989b).</p>	$\text{WUE}_i = \frac{A}{g_s} = \frac{p_a (1 - \frac{p_i}{p_a})}{1.6} = \frac{p_a (1 - \frac{\Delta^{13}\text{C} - a}{b - a})}{1.6} \quad (5)$ <p>where A is photosynthetic assimilation, g_s is stomatal conductance; a is the fractionation during binary diffusion in air (4.4 ‰); b is fractionation during carboxylation in C_3 plants; and p_a and p_i are the atmospheric and sub-stomatal CO_2 partial pressures, respectively. The factor 1.6 denotes the ratio of diffusivities of water vapour and CO_2 in air.</p>

Table 2. Introduction to terms and equations of oxygen and hydrogen isotopes and evaporative enrichment.

Oxygen and hydrogen isotopes and evaporative enrichment	Equations
<p>The delta notation for oxygen and hydrogen isotopes</p> <p>Whereas hydrogen has two stable isotopes, ^1H and ^2H (or D; deuterium), oxygen possesses the isotopes ^{16}O, ^{17}O and ^{18}O. Since the natural abundance of ^{17}O is very low (approx. 0.038 atom %), mostly the ratio between ^{18}O and ^{16}O and thus the relative abundance expressed as $\delta^{18}\text{O}$ (calculated as shown in Eq. (1) with the standard of Vienna standard mean ocean water (VSMOW) in case of water and VPDB-CO_2 in case of CO_2) is considered.</p>	$\delta^y X = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \quad (1)$ <p>where $^y X$ is replaced by ^{18}O or ^2H in the case of oxygen and hydrogen isotopes and R_{sample} and R_{standard} are the measured $^{18}\text{O}/^{16}\text{O}$ or $^2\text{H}/^1\text{H}$ ratios in the sample and standard, respectively.</p>
<p>Evaporative enrichment</p> <p>The (evaporative) oxygen ($\Delta^{18}\text{O}_{\text{ev}}$) or hydrogen ($\Delta^2\text{H}_{\text{ev}}$) isotope enrichment of leaf water or plant organic matter is expressed as enrichment above source water (often assumed to be soil or xylem water) by Eq. (6).</p>	$\Delta^{18}\text{O}_{\text{ev}} = \frac{\delta^{18}\text{O}_p - \delta^{18}\text{O}_{\text{sw}}}{1 + \delta^{18}\text{O}_{\text{sw}}} \quad \text{and} \quad \Delta^2\text{H}_{\text{ev}} = \frac{\delta^2\text{H}_p - \delta^2\text{H}_{\text{sw}}}{1 + \delta^2\text{H}_{\text{sw}}} \quad (6)$ <p>where $\delta^{18}\text{O}_p$ and $\delta^2\text{H}_p$ is the oxygen and hydrogen isotopic composition, respectively, of leaf water or plant organic matter and $\delta^{18}\text{O}_{\text{sw}}$ and $\delta^2\text{H}_{\text{sw}}$ are the respective isotope compositions of the source water.</p>
<p>Leaf water enrichment</p> <p>The enrichment of the leaf water has been modelled with approaches of increasing complexity (e.g. Cuntz et al., 2007). Steady-state isotopic enrichment of oxygen or hydrogen over source water at the site of evaporation in the leaf (Δ_e) can be calculated by the Craig & Gordon model (Craig and Gordon, 1965; Dongmann et al., 1974) by Eq. (7). In steady state conditions (i.e. source water isotopic composition is equal to the one of transpired water), the isotopic enrichment of water vapour relative to the source water taken up by the plant (Δ_v) can be approximated by $-\varepsilon^+$. This model was developed for open water surfaces and only applies to the water composition at the site of evaporation, and not the whole leaf (mean lamina mesophyll water).</p>	$\Delta_e = \varepsilon^+ + \varepsilon_k + (\Delta_v - \varepsilon_k) \frac{e_a}{e_i} \quad (7)$ <p>ε^+ is the equilibrium fractionation between liquid water and water vapour; ε_k is the kinetic fractionation as vapour diffuses from leaf intercellular spaces to the atmosphere (Farquhar et al., 1989a), Δ_v is the isotopic enrichment of water vapour relative to the source water taken up by the plant, and e_a/e_i is the ratio of ambient to intercellular vapour pressures.</p>
<p>Steady-state isotopic enrichment of leaf water</p> <p>The steady-state isotopic enrichment of mean lamina mesophyll water (Δ_{LSP}) can be described by correcting Eq. (7) for the so-called <i>Péclet</i> effect (Farquhar and Lloyd, 1993), as shown in Eq. (8). The <i>Péclet</i> effect is the net effect of the convection of unenriched source water to the leaf evaporative sites via the transpiration stream as opposed by the diffusion of evaporatively enriched water away from the sites of evaporation.</p>	$\Delta_{\text{LSP}} = \Delta_e \frac{1 - e^{-\varphi}}{\varphi} \quad \text{with} \quad \varphi = \frac{E \cdot L}{C \cdot D} \quad (8)$ <p>where φ is the <i>Péclet</i> number, E the leaf transpiration rate ($\text{mol m}^{-2} \text{s}^{-1}$), L is the scaled effective path length (m) for water movement from the xylem to the site of evaporation, C the molar concentration of water (mol m^{-3}), and D the tracer-diffusivity ($\text{m}^2 \text{s}^{-1}$) of heavy water isotopologues (either H_2^{18}O or $^2\text{H}^1\text{HO}$) in “normal” water. L is a fitted parameter (using Eq. 8; Flanagan et al., 1993) as it cannot be measured directly.</p>
<p>Non-steady-state isotopic enrichment of leaf water</p> <p>Non-steady-state effects in lamina mesophyll water enrichment (Δ_{LNP}) have been added by Farquhar and Cernusak (2005) by Eq. (9). This equation has an analytical solution and can be calculated with the “Solver” function in Excel.</p>	$\Delta_{\text{LNP}} = \Delta_{\text{LSP}} - \frac{\alpha^+ \alpha_k}{g_t w_i} \frac{1 - e^{-\varphi}}{\varphi} \frac{d(V_m \Delta_{\text{LNP}})}{dt} \quad (9)$ <p>where $\alpha = 1 + \varepsilon$, (α^+ and α_k are corresponding to ε^+ and ε_k, respectively), V_m is lamina leaf water molar concentration (mol m^{-2}), t is time (s), g_t is the combined conductance of stomata and boundary layer for water vapour ($\text{mol m}^{-2} \text{s}^{-1}$), and w_i is the mole fraction of water vapour in the leaf intercellular air spaces (mol mol^{-1}).</p>
<p>Advection-diffusion model of leaf water enrichment</p> <p>The non-steady-state model of leaf water enrichment as given by Eq. (9) is a simplification of the advection-diffusion description of leaf water enrichment (Δ_{LAD}), as given by Cuntz et al. (2007) and Ogée et al. (2007) in Eq. (10).</p> <p>Steady-state approaches often accurately describe leaf water isotopic enrichment (e.g. Welp et al., 2008), especially for longer times (weeks, months or years) or spatial scales (ecosystem studies). If shorter times and spatial scales are considered (diel measurements or gradients across a leaf), non steady-state approaches are more suitable, especially for modelling leaf water enrichment during the night (Cernusak et al., 2005).</p>	$\frac{\partial \Delta_{\text{LAD}}}{\partial t} = -\frac{v_r}{\Theta_m} \frac{\partial \Delta_{\text{LAD}}}{\partial r} + \frac{D_r}{\Theta_m} \frac{\partial^2 \Delta_{\text{LAD}}}{\partial r^2} \quad (10)$ <p>where r denotes the distance from the xylem to the evaporating site (m), v_r is the advection speed of water in the mesophyll (m s^{-1}), Θ_m the volumetric water content of the mesophyll, and $D_r = \Theta_m \kappa_m D$ the effective diffusivity of the water isotopologues ($\text{m}^2 \text{s}^{-1}$), with κ_m (< 1) the tortuosity factor of the water path through the mesophyll. The volumetric water content in the leaf mesophyll Θ_m is related to the water volume V_m (per unit leaf area) and the mesophyll thickness r_m through $\Theta_m = V_m / (C r_m)$ (Cuntz et al., 2007).</p>
<p>Enrichment of organic matter</p> <p>Newly produced assimilates are assumed to obtain an imprint of the signature of the average bulk mesophyll leaf water at the time when they were produced. For oxygen, an equilibrium fractionation factor (ε_{wc}) results in carbonyl oxygen being ca. 27 ‰ more enriched than water (Sternberg and DeNiro, 1983), which has been confirmed for cellulose (e.g. Yakir and DeNiro, 1990), leaf soluble organic matter (e.g. Barnard et al., 2007) and phloem sap sucrose (e.g. Cernusak et al., 2003b, Gessler et al., 2007a).</p>	

issues which need to be addressed to better understand the physiological information imprinted on plant material.

Progress and challenges

Mesophyll conductance

Mesophyll or leaf internal conductance (often referred to as g_m or g_i) has emerged as a significant (co-)limitation for CO_2 transport to the chloroplast, with large variation between species and environmental scenarios of light, temperature, drought and salinity (Warren and Adams, 2006; Flexas et al., 2008). On-line measurements of $\Delta^{13}\text{C}$ in conjunction with gas exchange have been instrumental in detecting these variations of g_i . Variation in g_i is related to developmental changes and morphological/structural features of leaves, such as cell wall thickness, chloroplast arrangement, and leaf porosity (Flexas et al., 2008; Evans et al., 2009). Moreover, g_i may be regulated via the expression of particular aquaporins capable of transporting CO_2 across plasma membranes (cooporins) (Hanba et al., 2004; Flexas et al., 2007). Strong dynamic responses of g_i to various environmental factors at the scale of minutes to days have been reported (Flexas et al., 2008; Bickford et al., 2009), and such variation has also been observed at the canopy-scale (Schäufele et al., 2011). So far, the metabolic basis of these short-term adjustments of g_i is unknown.

Contribution of day respiration to $\Delta^{13}\text{C}$ dynamics

Recent high-resolution on-line $\Delta^{13}\text{C}$ measurements (Bickford et al., 2009) as well as labelling and modelling approaches (Tcherkez et al., 2010) indicate that the isotopic composition of day respiration is not the same as that of concurrently fixed carbon dioxide. In part, the respiratory carbon isotope fractionation during daytime (Tcherkez et al., 2010) is related to fuelling of respiration by old carbon pools (Nogués et al., 2004). This calls for further experimental studies, a more detailed theoretical description of whole-leaf $\Delta^{13}\text{C}$ during daytime gas exchange (Wingate et al., 2007; Tcherkez et al., 2004), and consideration of this effect in carbon isotope-based estimations of g_i .

Water isotope enrichment in leaves

Isotopic enrichment in leaf water is reasonably well understood (Craig and Gordon, 1965; Dongmann et al., 1974; Farquhar and Lloyd, 1993; Cuntz et al., 2007; Ogée et al., 2007), except for the parameter that characterises the effective path length for water movement from the xylem to the site of evaporation (see *Péclet* effect, Table 2). This parameter is especially important for modelling leaf water enrichment in non-steady state. Understanding how the effective length is adjusted by environmental conditions requires knowledge of how water transport inside the leaf is changing, for example, with the leaf's water status (Barbour et al., 2004, 2007;

Ferrio et al., 2009; Kahmen et al., 2009; Ferrio et al., 2012). It is likely that in leaves, all water pools are involved during water transport (Yakir, 1998); however, the leaf water pools might not be considered as perfectly mixed (e.g. Helliker and Ehleringer, 2000). Gan et al. (2002) compared different leaf water evaporative enrichment models (i.e. the two-pool model, the *Péclet* effect model and the string-of-lakes model), which assume different water isotopic gradients and different mixing of leaf water pools. The different models all described large parts of the observed dynamics of leaf water enrichment but not all facets were captured by a single model. The non-steady state *Péclet* model of Farquhar and Cernusak (2005; see Table 2, Eq. 9) is the simplification of the diffusion-advection model of Cuntz et al. (2007; Eq. 10), which assumes that the leaf represents a continuum of un-enriched (source) and enriched (evaporative sites) water. The latter, more complex model is less sensitive to noise in the input data and gives smoother results. Cuntz et al. (2007), however, state that comparably good results could also be achieved with different well mixed metabolic pools of water. For epiphytic and non-vascular plants which lack permanent access to soil water, it has been shown that a description of water isotope dynamics requires consideration of distinct water pools as well as water potentials (Helliker and Griffiths, 2007; Hartard et al., 2009; Helliker, 2011).

Exchange of H_2O and CO_2 oxygen isotopes

The enzyme carbonic anhydrase (CA) catalyzes oxygen exchange between water and CO_2 via (reversible) interconversions of CO_2 and H_2O to bicarbonate (HCO_3^-) and protons (Badger and Price, 1994). This exchange underlies ^{18}O discrimination during CO_2 exchange ($\Delta^{18}\text{O}$), and retroflux to the atmosphere of CO_2 that has previously equilibrated with leaf water, which has a strong effect on the ^{18}O content of atmospheric CO_2 (Farquhar et al., 1993). Also, this signal provides a measure of photosynthetic activity of the terrestrial biosphere (Farquhar et al., 1993). At the leaf level, measurements of gas exchange, CA, $\Delta^{18}\text{O}$ and $\Delta^{13}\text{C}$ can help to partition mesophyll conductance into a cell wall and a chloroplast component (Gillon and Yakir, 2000a). However, work by Cousins et al. (2008) indicates that CA activity may not be a good predictor for CO_2 - H_2O isotopic exchange, endorsing the view that more work is needed to fully understand the control of $\Delta^{18}\text{O}$ and its physiological implications.

Ternary effects on CO_2 isotopes during gas exchange

Ternary effects, i.e. effects of concurrent water vapor diffusion on CO_2 diffusion through stomata, are taken into account for CO_2 exchange (von Caemmerer and Farquhar, 1981) but not for isotopes. Mesophyll conductance is a parameter greatly influenced by ternary effects. Farquhar and Cernusak (2012) recently showed that by applying the ternary correction, oxygen isotope composition of CO_2 in the

chloroplast and mitochondria better match the oxygen isotopic composition of water at the sites of evaporation. The ternary effect has been observed to be greatest when the leaf-to-air water vapor pressure deficit is large. Farquhar and Cernusak (2012) also observed that a large impact of ternary corrections occurred when the difference in the isotopic composition of CO₂ between the leaf interior and the ambient air was large. The precision of current isotope fractionation models can be improved by applying the ternary correction equations for isotope fractionation and isotope exchange during gas exchange measurements.

2.1.2 Post-carboxylation and respiratory fractionation

The carbon isotope signal, imprinted through photosynthetic ¹³C discrimination (the sum of terms one to four on the right hand side of Eq. 3, Table 1), can be altered by multiple processes in down-stream metabolic pathways (termed post-carboxylation fractionation), which will be reflected in different carbon pools and respired CO₂. Despite early evidence by Park and Epstein (1961), carbon isotope fractionation during dark respiration has long been considered negligible (Lin and Ehleringer, 1997). Systematic studies by Duranceau et al. (1999) and Ghashghaie et al. (2001) with a range of C₃ species again provided clear evidence for substantial and systematic variation in carbon isotope ratios of leaf dark respiration (see review by Ghashghaie et al., 2003). These authors introduced the term “apparent respiratory fractionation” to describe the manifested differences between carbon isotope compositions of leaf dark-respired CO₂ and its putative substrates (mainly carbohydrates), caused by multiple processes in the respiratory pathways (see below). The work of Ghashghaie and coworkers promoted a significant number of studies on post-carboxylation fractionations in down-stream metabolic processes (Klump et al., 2005; Badeck et al., 2005; Cernusak et al., 2009; Tcherkez et al., 2011; Werner and Gessler, 2011).

Progress and challenges

Post-carboxylation fractionation

Already within the Calvin cycle, isotopic fractionation occurs mainly due to metabolic branching points and the use of triose phosphates that can either be exported to the cytosol or continue to be used within the Calvin cycle. The triose phosphates that are not exported are subject to certain enzyme catalyzed reactions (aldolisation and transketolisation) which involve position-specific discrimination during C–C bond making. As a result, the C-3 and C-4 positions within glucose are enriched in ¹³C and thus a non-homogeneous intra-molecular distribution of ¹³C within carbohydrates is established (Rossmann et al., 1991; Tcherkez et al., 2004; Gilbert et al., 2009). Subsequently, photorespiration and starch–sucrose partitioning result in diel changes in

isotopic signatures of phloem sugars, with day sucrose being ¹³C-depleted, while night exported sucrose is ¹³C-enriched (Tcherkez et al., 2004; Gessler et al., 2008). Analyses of sugar δ¹³C and its diurnal variations offer potential for improved tracing of these changes in these metabolic activities.

Apparent respiratory fractionation

During the last decade the knowledge of relevant apparent fractionation in the respiratory pathways has significantly advanced, demonstrating substantial variability in respiratory fractionation among species, organs and functional groups, as well as with environmental conditions (see reviews by Ghashghaie et al., 2003; Werner and Gessler, 2011). The observed apparent respiratory fractionation and its variability are mainly attributed to (i) non-homogeneous ¹³C-distribution within hexose molecules reported by Rossmann et al. (1991) and modelled by Tcherkez et al. (2004), (ii) relative contributions of different pathways to respiration (reviewed by Ghashghaie et al., 2003), as well as (iii) enzymatic isotope effects during decarboxylation reactions (recently reviewed by Tcherkez et al., 2011).

Fragmentation fractionation

The non-homogeneous intra-molecular distribution of ¹³C in carbohydrates, results in so-called “fragmentation fractionation” (Tcherkez et al., 2004), leaving its imprint on synthesized metabolites. If one of these products is decarboxylated, then respired CO₂ will carry an isotopic signature different from the average sugar signature. New data indicate that the heterogeneous ¹³C distribution in carbohydrates may vary among species and with environmental conditions (Gilbert et al., 2011). Moreover, switches between substrates (Tcherkez et al., 2003) during light–dark transition of leaves (i.e. light enhanced dark respiration due to decarboxylation of light-accumulated malate, Barbour et al., 2007) and the oxidative pentosephosphate pathway (PPP) were shown to markedly change δ¹³C, of respired CO₂ (Bathellier et al., 2008, 2009). The implication of these processes still needs to be explored.

Temporal dynamics and apparent respiratory fractionation

So far, a full quantitative understanding of apparent respiratory fractionation has not yet been achieved. However, measurements with a high temporal resolution indicated remarkable diel dynamics in leaf respiratory δ¹³CO₂, which differed between functional plant groups (Priault et al., 2009; Werner et al., 2009; Werner and Gessler, 2011). Feeding experiments with positionally labelled glucose or pyruvate can trace changes in carbon partitioning in the metabolic branching points of the respiratory pathways (Tcherkez et al., 2004), which has been used at the organ level as evidence for an important contribution of PPP to root respiration (Bathellier et al., 2009) as well as to identify differences between

functional groups (Priault et al., 2009; Wegener et al., 2010). One challenge of labelling experiments is to find methods for channelling additional labelled metabolites into plant organs *in vivo* to shed further light on potential involvement of these metabolites and their metabolic pathways. Furthermore, the commitment of metabolites to alternative pathways at metabolic branching points needs to be quantified. This is particularly relevant where metabolic channelling evokes compartmentation in organelles with membranes, which are impermeable for intermediate products, as shown recently for the Krebs cycle (Werner et al., 2011).

Intra-molecular site-specific isotope fractionation

Recent results of Gilbert et al. (2011) demonstrated that access to site-specific isotope fractionation is now possible using ^{13}C NMR (see Sect. 3) to directly determine intramolecular ^{13}C distributions at natural abundance. The challenge in the application of this new technology is to ensure sufficient sample quantities from metabolic pools of interest. Of special interest is that obtained data can be interpreted directly in terms of isotope effects associated with specific enzymes.

Environmental effects

More studies of the sensitivity of respiratory $\delta^{13}\text{C}$ to changes in environmental conditions and between organs are needed, which will allow for a better understanding of temporal variability in post-photosynthetic fractionation (Dubbert et al., 2012) and could provide a basis for the use of respiratory $\delta^{13}\text{CO}_2$ as an indicator of physiological activity (e.g. Barbour et al., 2011a, b).

2.1.3 Bulk leaf tissue $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ and water use efficiency

The $\Delta^{13}\text{C}$ model by Farquhar et al. (1982; Eq. 3 in Table 1) predicts a linear relationship between $\Delta^{13}\text{C}$ and intrinsic water use efficiency (WUE_i ; the ratio of net assimilation, A , to stomatal conductance, g_s), for conditions where mesophyll conductance is very high and (photo)respiratory ^{13}C discrimination is negligible (Eq. 4). Empirical studies in controlled conditions confirmed this linear relationship between $\Delta^{13}\text{C}$, estimated from bulk biomass carbon isotope composition ($\Delta^{13}\text{C}_b$), and WUE_i (Farquhar et al., 1982, 1989b; Ehleringer et al., 1993; Griffiths, 1998; Brugnoli and Farquhar, 2000). In the following three decades, this linear (simplified) model of $\Delta^{13}\text{C}$ (Eq. 4) was used widely as an indicator of water use efficiency at the leaf, plant and ecosystem scale (e.g. Bonal et al., 2000; Lauteri et al., 2004; Saurer et al., 2004; Ponton et al., 2006) in retrospective studies of carbon–water relations based on biological archives (see Sect. 2.6), and in breeding crop varieties for improved yield under water-limited conditions (Condon et al., 2002).

Progress and challenges

The dual-isotope approach

Combined analyses of the carbon and oxygen isotopic composition of bulk leaf biomass provide a means to distinguish the separate effects of stomatal conductance and net photosynthesis on WUE_i (Scheidegger et al., 2000). Preferably, however, carbon isotope discrimination $\Delta^{13}\text{C}_b$, and bulk biomass oxygen isotope discrimination, $\Delta^{18}\text{O}_b$, should be used in such an approach to account for effects of differences in $\delta^{13}\text{C}$ of assimilated CO_2 and variations of $\delta^{18}\text{O}$ of source water. A distinction between stomatal and photosynthetic influences cannot be made from analysis of $\Delta^{13}\text{C}_b$ alone. The conceptual model of Scheidegger et al. (2000) was successfully applied in the field (Keitel et al., 2003, Sullivan and Welker, 2007) and further adapted for air pollution studies evaluating the effect of NO_x on plant metabolism (Siegwolf et al., 2001; Guerrieri et al., 2009, 2010; Savard, 2010). Grams et al. (2007) extended the model to estimate stomatal aperture directly for interpreting physiological changes. $\delta^{18}\text{O}$ of bulk organic matter has also been used to determine whether a change in WUE_i results from the increase in atmospheric CO_2 (Saurer and Siegwolf, 2007). Also, the effects of changes in vapor pressure deficit (VPD) resulting from increasing temperature or decreasing precipitation have been assessed along a Siberian transect (Sidorova et al., 2009; Knorre et al., 2010). The dual-isotope approach has proven a valuable concept for ecological applications. However, the interpretation of $\Delta^{13}\text{C}_b$ in terms of WUE_i under natural changing environments is complex (e.g. Seibt et al., 2008), as it provides a time-integrated record of photosynthetic discrimination over the period that the carbon forming the leaf was fixed, which can be derived from multiple sources, e.g. fresh assimilates, carbon exported from mature leaves or even older storage pools. Moreover, different leaf carbon pools have different residence and turnover times (Nogués et al., 2004; Lehmeier et al., 2008, 2010b). Thus, during leaf formation, growth and maintenance leaf bulk material integrates isotopic information from different time periods and sources, which is weighted by the amount of carbon incorporated from each source/period. Therefore, interpretation of $\Delta^{13}\text{C}_b$ in terms of WUE_i under natural changing environments requires several precautions, as described below.

Interpretation of $\Delta^{13}\text{C}_b$ and $\Delta^{18}\text{O}_b$ in relation to leaf traits

A comparison of WUE_i between different species based on $\Delta^{13}\text{C}$ in bulk leaf material is nontrivial. Differences in not only leaf structural, anatomical, but also physiological traits can modulate $\Delta^{13}\text{C}_b$ (Ehleringer, 1993; Werner and Máguas, 2010), as well as $\Delta^{18}\text{O}$ in lamina leaf water (Barbour and Farquhar, 2003; Kahmen et al., 2008) and $\Delta^{18}\text{O}_b$. Different leaf structures may affect mesophyll conductance (e.g.

Kogami et al., 2001; Hanba et al., 2003) and thus $\Delta^{13}\text{C}_b$ (see Sect. 2.1.1). Mesophyll conductance is generally neglected when calculating WUE_i from stable isotope discrimination (see Eq. 5). If there are varying influences of mesophyll conductance on $\Delta^{13}\text{C}$ among species, WUE_i calculated from Eq. (5) will be not directly comparable. Leaf traits may also affect the scaled effective path length for water movement from the xylem to the site of evaporation (Wang and Yakir, 1995) and thus influence $\Delta^{18}\text{O}_b$ (cf. Eq. 8, Table 2). The conceptual model of Scheidegger (2000) does not account for such effects but strictly assumes oxygen isotope enrichment to be only affected by the ratio of ambient to intracellular water pressure (e_a/e_i ; cf. Eq. 7). Any other factor varying leaf water evaporative enrichment and thus $\Delta^{18}\text{O}_b$ will thus constrain the interpretation of the impact of stomatal conductance versus net photosynthesis on WUE_i . Moreover, due to differences in phenological phases and length of growing period leaf $\Delta^{13}\text{C}_b$ and $\Delta^{18}\text{O}_b$ of co-occurring species might provide an integrated signal over diverging environmental conditions (e.g. Werner and Máguas, 2010). Thus, species-specific differences in phenology, growth pattern and leaf structures might constrain a direct comparison of bulk leaf $\Delta^{13}\text{C}$ and $\Delta^{18}\text{O}$ between different species (Hanba et al., 2003; Warren and Adams, 2006). Moreover, ontogeny can markedly alter the isotopic signature (Terwilliger et al., 2001; Bathellier et al., 2008; Salmon et al., 2011). Repetitive sampling and isotope analysis of tissues and compounds which are known to integrate shorter and more defined time periods such as phloem sugars (e.g. Keitel et al., 2003; Dubbert et al., 2012) during the growing season, together with bulk leaf assessments, might help to better constrain the physiological meaning of $^{13}\text{C}_b$ and $\Delta^{18}\text{O}_b$. For community scale stable isotope approaches (see Sect. 2.3), the potential limitations of using the bulk isotope signature need to be kept in mind and sampling strategies need to be adapted. For trees, the intra-annual analysis of tree ring, whole wood or cellulose can provide a tool to study periods during the growing season when the isotopic signature in this archive is directly coupled to leaf physiology (Helle and Schleser, 2004; Offermann et al., 2011).

Interpretation of leaf $\Delta^{13}\text{C}_b$ and $\Delta^{18}\text{O}_b$ in relation to storage and remobilization

A part of the leaf structural organic matter of deciduous trees is made from remobilized starch (or other non-structural compounds) from overwinter storage pools in stems and roots (Kozłowski and Pallardy, 1997). That material was derived from the photosynthetic activity of previous year leaves, with different morpho-physiological characteristics in other environmental conditions, producing a previous year isotopic signal. Since starch can be ^{13}C -enriched by up to 4‰ as compared to newly assimilated sugars (Gleixner et al., 1998), growing leaves supplied from storage pools are often strongly ^{13}C enriched in spring (e.g. Terwilliger, 1997; Helle

and Schleser, 2004). Moreover, during starch breakdown, carbonyl oxygen atoms are exchanged with unenriched water in stems, causing these incorporated starch-derived sugars to be ^{18}O depleted as compared to sugars formed in transpiring leaves (Gessler et al., 2007b). This “isotopic starch imprint” in the newly developed leaves is thought to be diluted during the growing season by carbon turnover and the incorporation of new assimilates into bulk leaf organic matter (see e.g. the seasonal course of bulk leaf $\delta^{13}\text{C}$ of beech shown by Helle and Schleser, 2004). For interpreting the isotopic composition of a deciduous leaf, it is thus important to consider when the leaf was harvested during the season and that there might be species-specific differences in the extent to which the starch imprint or the influence of the assimilates incorporated during the current growing season dominate the bulk isotope signal. In turn, coniferous needles can accumulate large amounts of starch in spring, followed by mobilization towards the summer, and starch contents are generally low during the winter (e.g. Ericsson, 1979). As a consequence, at least part of the $\Delta^{13}\text{C}_b$ during the growing season is related to the variation of starch content and isotopic composition (Jäggi et al., 2002), a fact that also needs to be taken into account when calculating WUE_i from $\Delta^{13}\text{C}_b$ and comparing it among species.

Thus, the complexity of processes influencing $\Delta^{13}\text{C}_b$ may constrain its use in ecological field studies. Carbon pools with shorter turnover times and thus a better-defined origin such as leaf soluble sugars (Brugnoli and Farquhar, 1988), phloem allocated carbon (e.g. Gessler et al., 2004; Scartazza et al., 2004) or even dark-respired $\delta^{13}\text{CO}_2$ of recent photosynthates (Barbour et al., 2011b) are therefore better indicators for recent changes in WUE_{ia} , as outlined below (Sect. 2.2).

2.2 ^{13}C and ^{18}O isotopes to trace plant integrated processes and plant-soil coupling

With their pioneering work on the phloem carbon isotopic composition of grasses (Yoneyama et al., 1997) and trees (Pate and Arthur, 1998), two groups paved the way to getting temporally and spatially (whole canopy) integrated information on leaf physiology. Pate and Arthur (1998) applied natural abundance stable isotope approaches to investigate transport and allocation of assimilates by combining sampling of phloem sap organic matter and source and sink organs. This work focused on transport directions and patterns of source-to-sink transport, while tracing of carbon allocation with high temporal resolution in plants required the use of labelling experiments (e.g. Hansen and Beck, 1990).

Within the last ten years it was, however, shown that the transport of newly assimilated carbon within the plant and from the plant to the rhizosphere can also be followed by natural abundance stable isotope techniques (e.g. Scartazza et al., 2004; Brandes et al., 2006; Wingate et al., 2010b).

Progress and challenges

Plant integrating information and phloem transport

The $\delta^{13}\text{C}$ of phloem organic matter is increasingly being used to derive information on carbon allocation, canopy integrated WUE and canopy integrated mesophyll conductance in plants affected by environmental conditions (e.g. Keitel et al., 2003; Gessler et al., 2004; Scartazza et al., 2004; Barbour et al., 2005; Rascher et al., 2010; Ubierna and Marshall, 2011; Dubbert et al., 2012). A dual-isotope approach ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ see Sect. 2.1.3) can also be successfully applied to phloem sugars to distinguish whether net assimilation and/or stomatal conductance is changing as a result of environmental conditions (Keitel et al., 2003; Cernusak et al., 2003, 2005; Brandes et al., 2006; Keitel et al., 2006). Even though the carbon and oxygen isotope composition of phloem organic matter can, in principle, integrate leaf physiology over the whole canopy and track transport of assimilates within the plant, it is now clear that several uncertainties constrain the interpretation of phloem isotopic information. These are related to (i) the temporal integration of the isotope signal in the phloem, (ii) potential changes of the isotope composition of phloem sugars in basipetal direction, and (iii) the chemical composition of phloem transported organic matter.

Phloem sugars and temporal integration

Short-term variations in the isotopic composition of leaf sugars – induced by either an environmental signal or plant internal processes – might or might not be reflected in the isotopic composition of phloem organic matter. Twig phloem organic matter of trees (e.g. Gessler et al., 2007a) and the stem phloem of herbaceous species (e.g. Gessler et al., 2008) can be applied to monitor diel variation of evaporative ^{18}O and ^2H enrichment or carbon isotope fractionation. In the trunks of adult trees, however, the mixing of sugars of different metabolic origins can dampen the short-term variations and the isotope signatures provide time-integrated information on canopy processes instead (Keitel et al., 2006; Rascher et al., 2010).

2.2.1 Change of the original isotope signal in phloem sugars

The original isotope signal imprinted on sugars in the leaf may be altered during basipetal transport in the phloem of trees (e.g. Rascher et al., 2010). The transport of sugar molecules itself does not fractionate to a measurable extent. However, carbon fixation by PEPC in the bark and oxygen atom exchange with stem water during metabolic processes in the stem tissue together with the continuous unloading and loading of sugars from and to the phloem might contribute to the observed isotope patterns (Barnard et al., 2007; Gessler et al., 2009). The change in $\delta^{13}\text{C}$ along the transport path, however, varies strongly among species ranging from

^{13}C enrichment (Brandes et al., 2006; Wingate et al., 2010a) and no change in $\delta^{13}\text{C}$ (Pate and Arthur, 1998; Gessler et al., 2007a) to ^{13}C depletion (Rascher et al., 2010). The nature of these species-specific differences remains to be clarified and might shed new light on mechanisms controlling assimilate partitioning in trees.

Chemical composition of phloem sugars

It is often assumed that only one major sugar, namely sucrose, is present in the phloem. However, besides sucrose, there are other transport carbohydrates – depending on species and phloem loading mechanisms – such as myo-inositol and raffinose family sugars (Karner et al., 2004). In addition, it is still a matter of debate if hexoses are transported in the phloem or not (van Bel and Hess, 2008; Liu et al., 2012). Phloem sugar composition can vary with environmental conditions, which could be one factor for changes in phloem $\delta^{13}\text{C}$ (Merchant et al., 2010), independent of the original leaf-borne isotope signal, since $\delta^{13}\text{C}$ differs between different carbohydrate molecules (Schmidt, 2003; Devaux et al., 2009). Compound-specific analysis, provided by modern LC- and GC-IRMS techniques (Sect. 3), will help to differentiate between changes in phloem $\delta^{13}\text{C}$ that result from either changes in the chemical composition or changes in leaf level fractionation. In addition, comparable methods should be used to characterise the compound-specific oxygen isotope composition of phloem organic matter.

Only recently, the natural abundance stable isotope information in soil and ecosystem respired CO_2 cross-correlated to photosynthesis (or its proxies) has been used systematically to characterise the speed of link between canopy and soil processes (see review by Kuzyakov and Gavrichkova, 2010; Wingate et al., 2010b). Even though such approaches have significant potential, there is still debate about the physiological information conveyed by the isotope signal and of the processes involved (see review by Brüggemann et al., 2011).

The link between above- and belowground processes

In their review, Kuzyakov and Gavrichkova (2010) postulated that approaches which quantify time lags between proxies of photosynthetic activity and natural abundance $\delta^{13}\text{C}$ in soil respiration are (besides other techniques) appropriate to study the link between above- and belowground processes. Mencuccini and Holttä (2010) reviewed different approaches to assess the speed of link between assimilation and soil respiration and concluded, in contrast, “that isotopic approaches are not well suited to document whether changes in photosynthesis of tall trees can rapidly affect soil respiration”. These different opinions may be related to uncertainties on the mechanisms involved, as described by Kayler et al. (2010a, b): on the one hand, there is evidence that pressure–concentration waves (Thompson and

Holbrook, 2004), which travel rapidly through the phloem of plants (and not the supply of new assimilates transported via the phloem to the roots and the rhizosphere), are responsible for the fast response of soil respiration to changes in photosynthesis. On the other hand, the time-lag between the fixation of a carbon molecule during photosynthesis and its respiration belowground may contain real and important information about plant physiology and carbon use as well as the degree to which plant and soil are coupled (Kayler et al., 2010a). This information may be obtained by the assessment of $\delta^{13}\text{C}$ in respired CO_2 but also in respiratory substrates when points listed above are taken into account. To unravel the importance of the different relevant processes, we need novel, pertinent experiments which combine (a) continuous measurements of the natural abundance stable isotope composition of soil respired CO_2 , as done by Wingate et al. (2010b), with (b) appropriate statistical approaches that are able to track time lags between photosynthesis and soil CO_2 efflux, as applied by Vargas et al. (2011). Whereas (a) indicates how long it takes until a molecule with a given isotope composition imprinted during photosynthesis is transported from the leaves via the phloem to the roots where it is respired, (b) allows to detect the response time(s) of soil respiration towards changes in carbon assimilation, which might or might not be faster than the transport of a given molecule from the canopy to belowground.

2.3 Community-scale processes

Because different species living within the same habitat show marked differences in the isotopic composition of their leaf tissues, characterising community-wide variation in $\delta^{13}\text{C}$ and/or $\delta^{18}\text{O}$, can provide potentially powerful tools for investigating the physiological basis for niche partitioning among community members (Dawson et al., 2002). Good examples are utilization of different water sources and redistribution (Caldwell et al., 1998; Ryel et al., 2003), which can in turn be linked to community composition (Ehleringer et al., 1991), niche partitioning and spatial and temporal variations in plant distributions (e.g. Dawson et al., 2002; Snyder and Williams, 2000; Stratton et al., 2000; Drake and Franks, 2003; Rose et al., 2003; Grams and Matyssek, 2010). As stated above (Sect. 2.1.3), it must be kept in mind when comparing different species that the isotopic composition of bulk leaf material might be influenced by multiple factors such as structural, anatomical and physiological traits but also phenology. There are only very few community-wide investigations on this topic (see Smedley et al., 1991; Guehl et al., 2004; Kahmen and Buchmann, 2007). This scarcity may be in part related to difficulties in assigning cause and effect to observed variation from either physical (e.g. water availability, light) or biological (e.g. resource competition) factors.

Progress and challenges

Adding dual-isotope approaches to community ecology

^{13}C and ^{18}O signals can trace biotic and abiotic interactions within the plant community and may contribute to identifying what shapes community-scale processes. However, individual plants will not necessarily respond to environmental perturbations as “a community”, but may respond according to species-specific traits and requirements and additionally depend on the interactions with the surrounding environment and other present species (e.g. Roscher et al., 2004; Gubsch et al., 2011). Competition and/or facilitation interactions between species, e.g. through depletion of a particular resource, may also be a source of isotopic variation, as shown for plant–plant competition for above- and belowground resources by combining $\delta^{13}\text{CO}_2$ and $\delta^{18}\text{H}_2\text{O}$ analyses (Ramírez et al., 2009; Grams and Matyssek, 2010). Moreover, changes in community functioning, for example by alterations in nutrient, carbon and hydrological cycles after exotic plant invasion, can also be traced through stable isotopes (e.g. Rascher et al., 2011).

Tracing spatial interaction between species within plant communities

Spatio-temporal variations in isotope ratios (i.e. *isoscares*) contain a potential wealth of information regarding ecological processes (West et al., 2008; Bowen et al., 2009), which have, so far been applied at larger spatial scales (see Sect. 2.5). At the community scale, spatial heterogeneity in resource availability, differential resource utilisation by neighbouring species and their interactions (competition and facilitation) occur in a spatially explicit dimension, which may contain crucial information regarding community functioning. For example, hydraulic redistribution of water sources is a key process which can shape plant communities (see review by Prieto et al., 2012). Recently it has been shown that downscaling isoscares to the community level allowed tracing the spatial impact of an invasive species on community functioning (Rascher et al., 2012), and may therefore open new possibilities in resolving the spatial component of within-community interactions.

Tracing functional groups/community composition

During the last decade, the functional group approach has proved to be an efficient way to analyse plant functioning at the community scale. Leaf bulk $\Delta^{13}\text{C}$ allows the distinction of broad plant functional types, differing in structural, phenological and physiological leaf traits (Brooks et al., 1997; Bonal et al., 2000; Werner and Máguas, 2010). Functional traits such as water or nutrient use strategies, carbon acquisition, growth behaviours, and phenological cycles contribute significantly to the observed variation in isotope composition (e.g. Warren and Adams, 2006; Gubsch et al., 2011; Salmon

et al., 2011; Ramírez et al., 2012). However, the responsiveness of leaf $\Delta^{13}\text{C}$ as a functional tracer has to be verified for different communities and may differ with the predominant environmental constraints for plant growth and survival (e.g. Caldeira et al., 2001). For example, in a tropical rainforest, $\Delta^{13}\text{C}$ was associated with differences in shade tolerance (Bonal et al., 2000; Guehl et al., 2004), whereas in an upland water-limited grassland of Greece, a semi-arid Inner Mongolian steppe, and a Portuguese mediterranean macchia grouping according to $\Delta^{13}\text{C}$ was associated with species' competitive ability related to WUE_i , nitrogen use efficiency, and structural adaptations to drought (Tsialtas et al., 2001; Gong et al., 2010; Werner and Máguas, 2010).

The role of water source partitioning on community functioning

Several mixing models have been used to determine the contribution of different water sources to plant and ecosystem evapotranspiration: Linear mixing models can be applied if the differences of $\delta^{18}\text{O}$ among the water sources and xylem plant water are large enough; δD - $\delta^{18}\text{O}$ plots can be used if the difference between water sources and xylem water is small (Ogle and Reynolds, 2004; Dawson and Simonin, 2011). The use of multiple source mass balance analyses can improve the capacity to quantitatively and objectively evaluate complex patterns in stable isotope data for determining possible contributions of different sources to total plant water uptake (see review by Hu et al., 2009). Furthermore, combining water source partitioning with indicators of species functional responses (e.g. changes in leaf water potential and carbon isotope discrimination) lent insight regarding the degree of plasticity among individual members of a given plant community (Máguas et al., 2011). However, there is increasing awareness that the utilisation of simple linear mixing models to infer plant water uptake by comparing δD and $\delta^{18}\text{O}$ of xylem or root crown, on the one hand, and soil water, on the other hand, does not adequately reflect the high heterogeneity of water sources that may be available for a plant. Given the importance of resource variability at the community level, the utilisation of more complex mixing models (for example, by Phillips, 2001; Phillips and Gregg, 2001, 2003; Parnell et al., 2010) as well as Bayesian models (Ogle et al., 2004) may be fruitful.

2.4 Use of stable isotopes to disentangle ecosystem exchange processes

At the ecosystem scale, stable isotopes can provide insight into the complex interaction between vegetation, soil and atmosphere exchange of carbon and water fluxes, including their responses and feed-backs to environmental drivers (e.g. Flanagan and Ehleringer, 1998; Dawson et al., 2002; Yakir and Sternberg, 2000; Yakir, 2003; Hemming et al., 2005; please see Bowling et al., 2008 for review of pioneer and

recent literature). The core of the variation behind patterns in $\delta^{13}\text{C}$ of ecosystem respiration ($\delta^{13}\text{C}_R$) lies in photosynthetic discrimination, the magnitude of metabolic fluxes and several post-carboxylation fractionation processes that differ between autotrophic and heterotrophic organs (see Sect. 2.1.2 and references therein). How these components manifest into integrative measures such as ecosystem respiration is fundamental to understanding ecosystem physiology and biogeochemistry. It is clear that ecosystem responses to climate and land use change, or perturbations, such as drought or fire, are an integrative signal from a network of carbon pools and organisms linking legacy conditions to current observations (e.g. Buchmann et al., 1997a, b, 1998; Ehleringer et al., 2000). Thus, to properly account for ecosystem trace gas exchange and partitioning by stable isotopes, a detailed knowledge of the physical and biological basis of the isotopic signals for each of the fluxes and their dynamics across spatial and temporal scales in soil–biosphere–atmosphere interactions is required.

Progress and challenges

Recent findings on component sources and fluxes

Previous ecosystem ^{13}C and ^{18}O isotope research primarily focused on partitioning of soil and canopy sources, which are now a mainstay of ecosystem isotopic investigations (e.g. Buchmann et al., 1998; Kaplan et al., 2002; Yakir and Sternberg, 2000 and literature therein). The inherent complexity behind ecosystem respiration lies behind the many contributing sources. Nowadays, studies of these components have expanded to include stem CO_2 flux, mycorrhizal and microbial contributions (Esperschütz et al., 2009), litter decomposition (Bird et al., 2008; Rubino et al., 2010), dissolved organic carbon (Sanderman and Amundson, 2008; Müller et al., 2009), erosion (Schaub and Alewell, 2009), soil organic matter dynamics (Klumpp et al., 2007; Kayler et al., 2011) and CO_2 storage in soil air and solution (Gammitzer et al., 2011). Labelling has also played a central role in achieving a higher level of certainty in observing single source temporal patterns (Ubierna et al., 2009; Powers and Marshall, 2011). Similarly, the water oxygen and hydrogen isotope composition has been used as natural or artificial tracer of the ecosystem and component water fluxes and to partition evaporation and transpiration (e.g. Yakir and Wang, 1996; Yopez et al., 2005, 2007; Williams et al., 2004; Lai et al., 2006; Rothfuss et al., 2010; Wang et al., 2010; Kim and Lee, 2011) to assess ecosystem water use efficiency (WUE) (Ponton et al., 2006) and, e.g. the effects of hydraulic redistribution by roots and mycorrhiza (e.g. Ludwig et al., 2004; Kurz-Besson et al., 2006; see Sect. 2.3.2). These detailed studies are important because inferences can be drawn concerning carbon and water dynamics at larger time scales (e.g. erosion, soil organic matter transformations), and spatial variability across the ecosystem can be better described. The

advantage of these studies is two-fold: (1) underlying connections between ecosystem carbon pools and fluxes and the influence of changes in environmental drivers can be characterised, and (2) results can be used in models designed to partition ecosystem respiration.

Canopy labelling

Whole ecosystem dynamics studied in situ using isotopes, at first pioneered through girdling (Högberg et al., 2001), have increased in number through whole canopy tracer application. Advances in our understanding of ecosystem processes through canopy labelling include assessing photosynthetic-soil-respiration coupling strength (Steinmann et al., 2004; Högberg et al., 2008; Bahn et al., 2009; Gamnitzer et al., 2009, 2011), carbon allocation patterns (Kuptz et al., 2011; Epron et al., 2011), and shading impacts (Warren et al., 2012), to name a few. Quantitative methods of canopy labelling in connection with on-line tracer measurement techniques (Sect. 3) and modelling of the tracer distribution data (e.g. by compartmental analysis), holds the promise of testing hypotheses of ecosystem physiology, aboveground-belowground response to a changing climate, and the turnover times of seasonally dynamic carbon pools (Epron et al., 2012), studies that were previously limited to laboratory studies (e.g. Schnyder et al., 2003; Lehmeier et al., 2008, 2010a, b) or inferred from annual changes in biomass measured in the field. Recent findings have illustrated the complexity of dynamic processes that interact at the ecosystem scale. This calls into question the applicability of simple two-end member mixing models in complex systems with multiple sources (Kayler et al., 2010a) and poses a significant challenge for ecosystem studies, as outlined below.

Heterogeneous flux sources

Ecosystem respiration is a complex mixture of isofluxes from a range of biotic and abiotic sources that span the soil to vegetation canopy continuum (see Badeck et al., 2005 and Bowling et al., 2008 and literature therein). These sources contribute with distinct isotopic signatures at time scales from daily (Bowling et al., 2003; Mortazavi et al., 2006; Werner et al., 2006; Kodama et al., 2008; Unger et al., 2010a; Wingate et al., 2010a) to seasonal cycles (Griffis et al., 2004; McDowell et al., 2004; Ponton et al., 2006; Alstad et al., 2007; Schaeffer et al., 2008). These are based partly on phenology and disturbance regimes and all exhibit different effects on component fluxes. The challenge to advance our understanding of $\delta^{13}\text{C}_\text{R}$ lies in identifying and quantifying these fluxes and isotopic signatures of important ecosystem components (e.g. Unger et al., 2010a; Epron et al., 2011; Barbour et al., 2011a). This is especially important to test hypotheses about temporal $\delta^{13}\text{C}_\text{R}$ patterns, for example, if $\delta^{13}\text{C}_\text{R}$ dynamics are heavily influenced by a sole component flux, resulting in a poorly mixed ecosystem source signal. Similarly, species-specific

transport times of recent assimilates (Epron et al., 2011) can potentially delay the photosynthetic response signal in $\delta^{13}\text{C}_\text{R}$, or abiotic phenomena (following section) can obscure component iso-fluxes (e.g. Ekblad et al., 2005; Knohl et al., 2005). In these cases, deciphering the drivers behind $\delta^{13}\text{C}_\text{R}$ may become increasingly difficult.

Abiotic influences

Analyses of $\delta^{13}\text{C}_\text{R}$ may lead to the identification of drivers and mechanisms underlying the dynamics of ecosystem metabolism; yet, other abiotic processes that are also affected by biological drivers (e.g. temperature) may amplify, dampen or time-lag responses in $\delta^{13}\text{C}_\text{R}$, obfuscating the signal of biological respiration (Brüggemann et al., 2011). Soil respiration, which can represent 20 to 70 % of total ecosystem respiration, is an integrative signal driven by many abiotic and biological processes. Recent studies have shown that factors such as diffusivity of soil CO_2 , dissolution of CO_2 from bicarbonates, and advection of soil gas may be responsible for strong ^{13}C -isotopic disequilibria between the CO_2 efflux at the soil surface and concurrent soil respiration (Crow et al., 2006; Kayler et al., 2008, 2010a; Nickerson and Risk, 2009; Ohlsson, 2009; Gamnitzer et al., 2011). Likewise, the oxygen isotope composition of soil respired CO_2 ($\delta^{18}\text{O}_\text{S}$) not only carries the isotopic signature of the soil water it interacted with, but also is influenced by the carbonic anhydrase in soil microorganisms that accelerate isotopic equilibration between CO_2 and soil water (Wingate et al., 2009, 2010b). Despite their potential to propagate uncertainties in isotopic information through the soil–canopy continuum, such processes merit inclusion in isotope ecosystem models, enhancing the interpretation of patterns and drivers of $\delta^{13}\text{C}_\text{R}$.

Flux partitioning

Conventional partitioning methods based on eddy covariance methods typically require several days or weeks of data to cover key phenological periods in order to obtain robust regression parameters (e.g. Reichstein et al., 2005), neglecting ecosystem responses at shorter time scales. These are, for example, “switches” of ecosystem states (Baldocchi et al., 2006; Lee et al., 2007) or the pulse-like response of soil respiration to strong rain events, occurring at time scales from minutes to hours (e.g. Xu et al., 2004; Unger et al., 2010b, 2012). It would be helpful if the partitioning scheme could resolve episodic responses of this kind, because it is the transient, non-equilibrium responses that provide a rigorous test of model performance and validity. Assimilating continuous measurements of CO_2 and H_2O fluxes and their isotopic compositions (e.g. $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, $\delta^2\text{H}$) into process-based models should therefore provide a better-constrained solution. Similarly, assimilating chamber-based flux measurements of these isotopic fluxes should help to explain and constrain our model predictions during metabolic switches, especially

when photosynthetic products may become limiting such as during drought (Unger et al., 2010a), rainy periods (Wingate et al., 2010a) or when post-photosynthetic fractionation processes dominate the isofluxes, e.g. at dawn (Barbour et al., 2011a).

2.5 Regional scale isotope variation in precipitation and linkages to carbon cycling

High frequency, spatially dense precipitation isoscapes (i.e. spatial distribution maps of isotope records) over long time periods are continuing to assist our understanding of plant water relations, water sources and the extent to which they are driven by seasonally varying water sources and how these sources vary at the regional, inter-annual to inter-decadal scales (Rozanski et al., 1993; Welker, 2000; Vachon et al., 2007). Knowledge on the spatial distribution of the isotope signatures of the source water taken up by plants is also prerequisite to disentangle the climatic and physiological information laid down as ^{18}O signal in plant organic matter and isotopic archives (Augusti and Schleucher, 2007) on larger scales. At the regional scale, we now fully appreciate that seasonally snow covered systems provide meltwater to soils and river systems that reflect the highly depleted values of winter precipitation (Dutton et al., 2005; Vachon et al., 2010), and that this snow meltwater allows high rates of stomatal conductance and high rates of carbon fixation (Alstad et al., 1999). The duration and extent to which snowmelt and summer precipitation sources are available to the vegetation may be critical to supporting higher plant water use, thus affecting stomatal conductance as well as carbon fixation and gross ecosystem production. The complexity of seasonal patterns of snow meltwater availability at the regional scale is reflected in the vegetation at higher latitudes where Arctic and North Atlantic Oscillation phase changes are recorded in the carbon and oxygen isotope composition (Welker et al., 2005). Our emerging understanding of the temporal patterns of $\delta^{18}\text{O}$ and δD during swings in the major climate oscillations provides a modern basis for calibrating storm track, climate oscillations and the source water of vegetation in conjunction with carbon fixation rates and variability.

Progress and challenges

Tracing climate phase variation

Understanding the role of moisture sourced from multiple regions (i.e. different storm tracks), and how those sources vary with climate phases (i.e. climate oscillations and modes, such as El Niño) as it affects vegetation carbon fixation is unknown (Holmgren et al., 2001; Birks and Edwards, 2009; Sjoström and Welker, 2009). We continue to recognize that tree rings may be recorders of the general isotopic history of source water (Briffa, 2000; Csank et al., 2011) regardless of geologic time period. However, understanding the

extent to which these moisture sources and climate phases are recorded and how plant physiology alters the source water signal in the long-term growth record of trees is one of the great challenges today.

Isotope tracers in back trajectory analysis

Back trajectory analysis of weather and thus precipitation (Draxler and Hess, 2004; Sjoström and Welker, 2009) is a modelling tool that has been used extensively to quantify long-distance transport of pollutants, and more recently for studies of isotopic characteristics of precipitation (Burnett et al., 2004). Combining this tool with isotopic measurements of continental precipitation and water vapour (e.g. networks such as MIBA and GNIP) and carbon and water fluxes (e.g. networks such as Fluxnet) may be means by which almost real-time linkages between climate phases, moisture sources, plant water relations, carbon exchange and continental carbon cycling may be possible.

2.6 Isotopic archives and relevant aspects of spatio-temporal integration

Over the past decades, the use of stable isotope ratios in a wide range of materials – from tree, sediment and ice cores to corals, hair, cactus spines, the baleen of whales and fish otoliths – has provided some of the most important and novel insights into the patterns of past environmental changes and organismal response to these changes of almost any type of recorder (Dawson and Siegwolf, 2007). Such archives not only provide a way to look back in time but more recent examples show that one can also assign causes to responses to environmental changes on a mechanistic basis (e.g. Ogée et al., 2009). Stable isotope analysis of biological or abiotic archives can thus provide excellent tracers for spatial- and temporal-integration over different scales. Here we discuss progress and challenges of a few selected examples of biological archives.

Progress and challenges

Isotopic archives in trees

Tree rings enable retrospective analyses of intra- and interannual variation of carbon and oxygen isotope composition and the related ecophysiological drivers over many centuries (Sidorova et al., 2009; Nock et al., 2010; Knorre et al., 2010; Andreu-Hayles et al., 2011; Peñuelas et al., 2011). The advantages of tree rings are that they (i) can be reliably dated with a high temporal and spatial resolution; (ii) contain several proxies (stable C, H, O and N isotopes, tree ring width and tree ring density) in the same matrix (tree ring wood/cellulose), which was formed at the same time, location, and environmental conditions; and (iii) mostly the inclusion of a limited number of trees and species may provide a strong signal. However, single tree ring chronologies provide

only limited spatial and community integration and report only local signals (ca. 10^{-1} to 10^2 m). Signal strength is further reduced by species-specific responses to environmental impacts. Furthermore, the tree response is strongly affected by ontogeny (e.g. Monserud and Marshall, 2001) and site specific properties such as competition, soil type, water and nutrient availability, resulting in a considerable variability of the signal expression, even within the same species (Saurer et al., 1997). Thus, constructions of ecosystem chronologies depend on the combination of several tree ring records from trees of different locations within the same site. This requires additional information, such as knowledge of past species dynamics.

Isotopic archives of herbaceous vegetation

The life span of herbaceous vegetation is much shorter than that of trees. However, isotopic reconstructions of climate change in herbaceous vegetation (crops and grassland) are possible if plants were sampled and preserved during the epoch. Such archives are relatively rare and are mainly represented by herbaria (e.g. Penuelas and Azcón-Bieto, 1992). In general, herbarium specimens have been sampled at different locations, so that long-term isotopic records from these involve a spatially disperse representation of a species' changing isotopic composition. Because of site differences, such isotopic records display relatively high variation. Rare opportunities for community-scale isotopic reconstructions are presented by long-term (agro-) ecological experiments with crops and grassland where biomass samples have been stored in dedicated archives (Zhao et al., 2001; Köhler et al., 2010).

Grazer tissues as isotopic archives

For grassland, an analogy to tree rings is given by the yearly rings (annuli) of horns (or hoofs) of obligate grazers (Barbosa et al., 2009, 2010). These can yield carbon isotopic records over many years, which reflect that of grassland vegetation (Schnyder et al., 2006). The spatial integrations of tree and horn ring isotope compositions are quite contrasting: local and stationary for the tree, and vast and cyclic for horns, reflecting visits of the different parts of the year-round grazing grounds. Still, the use of grazer tissue for reconstructions of grassland isotopic chronologies usually rests on a number of assumptions, e.g. concerning the selectivity of grazing, the constancy of the relationships between isotopic composition of grazer tissues, and contributions of diet components of differential digestibility (Wittmer et al., 2010). Such assumptions can be and should be validated (Wittmer et al., 2010). A significant advantage of keratinous tissue (horn, hair/wool and hoofs) is given by its homogenous chemical composition, which reduces variation associated with metabolic isotope fractionation that can be significant in chemically heterogeneous tissue.

Micro-scale environmental record

A particular case of small-scale environmental records are carbon and oxygen isotope ratios of non-vascular plants (NVP). Cyanobacteria, algae, lichens, and bryophytes integrate local changes of CO_2 (e.g. Máguas and Brugnoli, 1996) and water over long time periods due to their passive exchange with environmental conditions, low growth rates (ca. $0.02\text{--}30\text{ mm a}^{-1}$) and long life spans (hundreds to thousands of years). Therefore, NVP can be used, for example, for geochronologic aging (e.g. lichenometry), particularly dating deposited surfaces over the past 500 years with an accuracy of 10 % error (Armstrong 2004). The $\delta^{13}\text{C}$ of NVP archives environmental impacts over the whole life span in bulk organic material, and over several years if a chronosequence is sampled from the thallus margins or young shoots. Short-term and online records can be obtained from analysing respired CO_2 and extracted bulk water. $\delta^{13}\text{C}$ of NVP can be used to trace environmental CO_2 gradients (Flanagan et al., 1999; Lakatos et al., 2007; Meyer et al., 2008), whereas fossil bryophytes record ancient CO_2 levels (Fletcher et al., 2005, 2006). Additionally, epiphytic plants function as atmospheric water traps (Helliker and Griffiths, 2007; Helliker 2011). Because epiphytic NVP are commonly in equilibrium with water vapour, it is inferred that $\delta^{18}\text{O}$ of bulk water and organic material might serve as a short and long-term recorder for atmospheric vapour, respectively (Helliker and Griffiths, 2007; Hartard et al., 2008, 2009). In the same line, peat mosses serve as proxies for palaeoenvironmental changes (Loader et al., 2007; Lamentowicz et al., 2008; Moschen et al., 2009; Loisel et al., 2010). However, approaches that use oxygen isotopes as long-term recorder of environmental conditions need to account for the contributions of the different water signals from rain, dew and vapour, as well as physiological offsets which add considerable uncertainties (Moschen et al., 2009).

3 New technical and methodological developments in stable isotope research

The past decade has seen tremendous progress in the development of new techniques that complement or rival traditional Isotope Ratio Mass Spectrometry (IRMS) for the determination of stable isotope abundances. This has led to new dimensions in measurement speed, number of quantifiable isotopologues and sensitivity, increased the repeatability, precision and sample turn-over considerably, and offered new opportunities for in situ observations at natural abundance and in tracer experiments. Most important for carbon and water cycle research was the introduction of instruments using light absorption properties of small molecules for determination of stable isotope abundances, as well as the introduction of innovative techniques for compound-specific sample extraction.

Laser absorption spectroscopy (LAS)

The development of absorption spectroscopy instrumentation (LAS) provided new dimensions of measurement speed and number of quantifiable isotopologues offering data richness that had never been possible to achieve in field-deployable instrumentation (e.g. Bowling et al., 2003, Kammer et al., 2011, Sturm et al., 2012; but see Schnyder et al., 2004). The laser absorption spectroscopy is based on analysis of absorption of light in selected wavelengths in the near and mid-infrared to determine the mole fractions of individual isotopologues (Kerstel, 2004; Kerstel and Gianfrani, 2008; Fried and Richter, 2006). Optical measurement methods based on Fourier Transform Infrared Spectroscopy (FTIR), Cavity Ringdown Spectroscopy (CRDS), Integrated Cavity Output Spectroscopy (ICOS) and Tunable Diode Laser Absorption Spectroscopy (TDLAS) now approach levels of detection of small-molecule isotopologues comparable to laboratory-based isotope ratio mass spectrometers (IRMS). Measurement by absorption spectroscopy is non-destructive and can therefore be repeated to increase measurement precision (Werle et al., 2004). Furthermore, LAS enables a high temporal resolution of accurate isotope ratios, an ideal property for the visualisation of processes and temporal variability (e.g. Bowling et al., 2003; Lee et al., 2005; Tuzson, 2008). Further, new multi-species instruments that are becoming available enable so-called “clumped isotope” measurements (Eiler, 2007), wherein the occurrence of two heavy isotopes in the same molecule can serve as a unique stable isotope tracer itself.

Compound Specific Isotope Analysis (CSIA)

The IRMS has experienced technological and methodological development, particularly Compound Specific Isotope Analysis (CSIA), which includes IRMS coupled to Gas Chromatography-Combustion (GC-C-IRMS; Maier-Augenstein, 1999) or Liquid Chromatography (LC-IRMS; Godin et al., 2007). This facilitates the analysis of different compounds such as structural and labile carbohydrates extracted from plant organs, leaf wax alkanes, phloem sap and soil fractions (see review by Sachse et al., 2012). For compound specific isotope analysis, the extraction method is crucial and might strongly affect the results obtained (Richter et al., 2009). Moreover, the need for derivatization of polar metabolites for GC-MS (gas chromatography-mass spectrometry) analysis and thus the introduction of additional carbon and oxygen via the derivatization agent to the analyte complicates the measurement of natural abundance stable isotope composition of these compound classes (e.g. Gross and Glaser, 2004). This problem does not occur with LC-IRMS systems, which are currently, however, restricted to carbon isotope analyses.

Nuclear Magnetic Resonance (NMR)

At the advent of development of new techniques for nuclear magnetic resonance spectroscopy (NMR), new options arise for studies of, e.g. starch-sugar partitioning and complementary information on (photo-)respiration by analyses of non-homogeneous distribution of ^{13}C within carbohydrate molecules (e.g. Gilbert et al., 2009, 2011). Analogously, options to distinguish between different water pools within the plant arise from new techniques for ^{18}O positional analyses by novel derivatization approaches (Sternberg et al., 2006).

Nano-scale secondary ion mass spectrometers (NanoSIMS)

Linking isotopic analysis with high resolution microscopy has provided significant progress of spatially resolved information on the molecular and isotopic compositions of (biological) materials. New Nano-scale Secondary Ion Mass Spectrometers (NanoSIMS) represent a significant improvement in sensitivity and spatial resolution (down to 50 nm). In a destructive manner, NanoSIMS analysis involves continuous bombardment of the sample surface with an ion beam and subsequent analysis of the released secondary ions according to their mass-to-charge ratios (Herrmann et al., 2007). Although adequate sample preparation remains challenging, imaging mass spectrometry via NanoSIMS represents a promising avenue for mapping the spatial organisation, metabolic pathways and resource fluxes within cells, plants and at the root–fungus–soil interface, in particular in labelling studies (e.g. Clode et al., 2009).

Progress and challenges

The pool of various new and improved techniques currently available for application of stable isotopes in environmental, physiological and ecological research is large. However, from the user's perspective, particularly for laser absorption spectroscopy, some general issues, as described below, should be resolved.

Instrument accuracy and calibration

Calibration biases for water vapour isotope laser spectrometers can result, for instance, from evaporation efficiency of the reference water, instrument nonlinearity and impurity of the carrier gas. Calibration of water vapour analysers is done, for example, using a liquid water injector (“dripper”) into a flow of dry air (Lee et al., 2005; Wen et al., 2008; Baker and Griffis, 2010; Griffis et al., 2010; Sturm and Knoch, 2010). In addition, a heated vaporisation system is used wherein the liquid standard is completely vaporized without fractionation. Nevertheless, any concentration dependence in the analyser itself can bias the overall calibration of the instrument, especially when measuring ambient water vapour of widely varying mixing ratios (Lee et al., 2005; Wen et al., 2008;

Schmidt et al., 2010; Sturm and Knohl, 2010). For CO₂, calibration against two or more mixtures of CO₂ and dry air, which are tied to international reference standards, are critical. Impurities in the water sample to be analysed can cause a spectral interference with organic contaminants and have been observed in analysis of liquid samples extracted from biological sources, e.g. leaf water (West et al., 2010; Schultz et al., 2011).

High instrument precision at short detection intervals

Free of sample preparation and processing, new optical techniques can achieve much faster detection than IRMS. In-situ measurements of CO₂ and H₂O isotope ratios in ambient air, especially if made on a long-term basis and calibrated precisely, can provide a powerful tool for atmospheric inverse analysis of the terrestrial carbon sink and tracking of water transport in the atmosphere. However, to measure the source/sink signature properly near the land surface, one should interface the isotopic analyser with plant (Barbour et al., 2007; Barthel et al., 2011) and soil chambers (e.g. Wingate et al., 2010a, b) and deploy it in the gradient-diffusion mode either over the vegetation (Griffis et al., 2004) or over the soil surface inside the canopy (Santos et al., 2010), or combine it with a sonic anemometer for direct eddy covariance measurement of isotopic fluxes (Lee et al., 2005; Griffis et al., 2008, 2010) or landscape scale measurements in high elevation or airborne conditions (e.g. Tuszon et al., 2010). In all these configurations, suitable interfaces between the analyser and the sample and calibration periphery are useful. The system as a whole must be robust and designed and tuned for minimal interference, memory effects or signal drifts. Fast detection is particularly critical for eddy covariance applications, which require an instrument response to be faster than 10 Hz and relies on continuous-flow sampling. However, fast detection is also desired for chamber based measurements in studies of short-term events, such as water vapour and CO₂ flux pulses after rain (Santos et al., 2010; Unger et al., 2012). Maximizing precision at short integration times and maintaining accuracy for long periods should be a high priority in future instrument development.

Instrument and infrastructure cost

High instrument and maintenance costs limit the broad adoption of new technologies in field research. It is highly desirable that the costs are brought down to a level comparable to that of a broadband infrared gas analyser, which is now an indispensable tool for ecosystem carbon and water flux monitoring worldwide (Baldocchi et al., 2001). We envision the development of a network with real-time observations of isotopic fluxes of CO₂ and H₂O to help diagnose changes in biospheric processes. This can become a realistic goal if instrumentation costs are lower.

Multi-isotopologue instruments

New instruments are becoming available and enable so-called “clumped isotope” measurements (Eiler, 2007), wherein the occurrence of two heavy isotopes in the same molecule can serve as a unique stable isotope tracer itself. Currently its applicability as a paleo-thermometer is being tested. The basic assumption is based on the observation that the heavier molecules and atoms are not randomly distributed within the same matrix, but rather form a clumped aggregate of substrates with the heavier isotope. For the distinction of such clumped isotopes highly sensitive instruments are needed.

4 Outlook

New research opportunities at all scales of isotope biogeochemistry of carbon and water are arising from deepened process-based understanding and improved analysis tools, together with the development of mechanistic models. Especially combinations of multiple isotope and non-isotope variables have the potential to stimulate our understanding across a wide range of scales, including leaves, plants, mesocosms, natural ecosystems, and the atmosphere. The scale-spanning assessment of carbon and water fluxes is, on the one hand, a great opportunity offered by stable isotope approaches. On the other hand, deeper insights into the multitude of processes affecting carbon and oxygen isotope discrimination during photosynthesis and transpiration, as well as during downstream metabolic processes, are challenging a generalisation of the information contained in the isotopic signature and a transfer to higher temporal and/or spatial scale. One example is the knowledge that plant phenology or growth patterns (Sect. 2.1.3) might complicate the comparison of the isotopic composition of bulk material between species. However, we can apply appropriate techniques such as the assessment of organic matter pools with a well defined turnover time and chemical composition to avoid misinterpretation. Moreover, experimental designs focussing on changes in environmental conditions or species interactions and on the effect of such changes on the isotopic composition can often overcome the problem. While the isotopic composition might not be directly comparable between species, the direction and magnitude of change can give quantitative information on physiological reactions within and between species, communities and ecosystems.

At the leaf-level (see Sect. 2.1), combined analyses of different isotopes might lead to a better understanding of mesophyll conductance and related components, including diffusion through intercellular airspaces and transport through barriers in cells such as the cell wall, membranes, or stroma. It might also help to assess the possible role of cooporins (membrane proteins acting as pores for CO₂) in facilitating and controlling transport of CO₂. Combined measurements

of the isotopologues of CO₂ and H₂O will further allow quantifying the extent of equilibration between dissolved CO₂ and leaf water, and thus can provide a non-invasive reconstruction of leaf water dynamics. These are critical aspects for validation and further development of carbon and water isotope approaches and models. Information on different species and ecotypes will in turn enhance our understanding of the different morpho-physiological factors controlling carbon and water fluxes and, hence, water use efficiency of leaves.

Although the last ten years have seen a large increase in knowledge of post-carboxylation fractionation phenomena (see Sect. 2.1.2), we expect no slowdown in the development of this field. In part, empirical progress will be facilitated by improvements in NMR technology as well as in derivatisation techniques (see Sect. 3) which permit measurements of natural intramolecular isotope distribution patterns in intermediates of primary and secondary metabolism, and respiratory substrates. Dynamic labelling experiments with ¹³C-enriched or depleted CO₂ or with (intra-molecular) position-labelled metabolites will permit better assessment of metabolic networks and turnover times of different carbon pools. Such work will also enhance our understanding for the metabolic causes of variations in post-carboxylation fractionation. Temporal dynamics of apparent fractionation during dark respiration may vary, depending on the identity of the different metabolic intermediates, their synthesis pathways and metabolic functions as well as on the demand for substrates in the respiratory pathways.

Investigations of natural intra-molecular ¹³C and ¹⁸O distribution patterns might also be key to quantify isotope fractionation phenomena during loading, phloem transport and unloading of different organic compounds (see Sect. 2.2). These include assessments of isotopic exchange reactions along the path from leaves to sites of assimilate use, and fractionation or isotopic exchange during biosynthetic processes such as cellulose synthesis. Such approaches may elucidate the mechanisms underlying spatio-temporal variation of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ during transfer from the chloroplast to heterotrophic tissues, the rhizosphere/soil and atmosphere. The mechanistic understanding, on the other hand, will strengthen climatological and physiological interpretation of tree ring cellulose and similar isotope archives such as grass, sediments, hair, horn, or tooth enamel of herbivores (see Sect. 2.6). We are of the strong opinion that a deeper knowledge of fractionation during photosynthesis, transport and post-carboxylation metabolism is an important basis for understanding ecosystem-scale isotope discrimination and for linking the carbon balance with water relations at different scales. Whereas the mechanistic understanding of photosynthetic carbon isotope fractionation and evaporative ¹⁸O enrichment of water in leaves is relatively advanced, equivalent understanding of fractionation phenomena in the downstream metabolism – as expressed in quantitative models – is still in its infancy.

It is, therefore, not surprising that the interpretation of ecosystem scale fractionation remains challenging (see Sect. 2.4). We expect that significant steps for resolving this complexity will include similar approaches as advocated for leaf- and plant-level studies: (i) joint flux measurements of the different isotopologues of CO₂ and H₂O in natural systems – which will enable a better distinction of the CO₂ and H₂O flux components and pools, (ii) tracing metabolite and intramolecular labelling patterns between and within system components in artificial setups as well as in field labelling experiments – shedding light on allocation, turnover of different carbon pools as well as plant-soil-atmosphere interaction, and (iii) hypothesis-testing mesocosm-scale experiments – testing our system-scale understanding. Insights from these approaches may then help to improve and test stable-isotope-enabled models of carbon and water fluxes at the ecosystem scale.

Regional-scale studies (see Sect. 2.5) of the water isotope cycle are becoming more important to our understanding of synoptic climates, ecosystem processes, the role of abiotic processes (e.g. temperature of condensation), moisture sources, and storm tracks on the ecohydrology of entire landscapes and continents. However, isotope fractionations are quite uncertain on global and continental scales and it is therefore important to identify robust features that can be constrained by large-scale isotope observations. C₄-plant distribution is one such feature that might become well constrained by ¹³C isotopes. But isotope studies will benefit greatly from the combination with other non-isotope tracers also on landscape, regional and continental scales. It might be the tapping into the above-mentioned multitude of information that will advance the usage of isotope signals on the global scale.

In conclusion, we are in the midst of a rapid growth in process-based understanding of the behaviour of carbon and oxygen stable isotopes in organisms and in the environment. On the one hand, we are increasingly recognising the complexity of ¹³C and ¹⁸O fractionation processes and their spatial and temporal variation. On the other hand, new technologies (see Sect. 3) can deliver high resolution records of short- and long-term variability in isotope signatures, overcoming the constraints of earlier laborious procedures. New analytical tools and process-based understanding will allow further development of isotope-enabled biogeochemical models for investigations of the complex interplay of soil, plant, ecosystem and atmosphere processes in the carbon and water cycles.

Acknowledgements. We gratefully acknowledge helpful comment from anonymous referees and Nina Buchmann. This review resulted from a workshop during the SIBAE (Stable Isotopes and Biogeochemical Cycles in Terrestrial Ecosystems) meeting in Monte Verità, Switzerland. We gratefully acknowledge financial support from SIBAE COST action for workshop organisation and publication. Further, financial support to AG from DFG and the EU project FundivEurope; CM from Portuguese FCT; MJZ from Swiss NSF (SNSF; grant PBEZP2-127819), Marie Curie Excellence Grant (MEXT-CT-2006-042268) and BASIN; JW from US NSF (J Welker-AGS-0080952, DBI-0923571); and CW, MC and AG from German DFG (WE 2681/3-1, 5-1, 6-1, CU 173/2-1, GE 1090/7-1, 8-1, 9-1, 10-1, 11-1) is acknowledged.

Edited by: N. Buchmann

References

- Alstad, K. P., Welker, J. M., Williams, S., and Trilica, M. J.: Carbon and water relations of *Salix monticola* in response to winter browsing and changes in surface water hydrology: an isotopic study using $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, *Oecologia*, 120, 375–385, 1999.
- Alstad, K. P., Lai, C.-T., Flanagan, L. B., and Ehleringer, J. R.: Environmental controls on the carbon isotope composition of ecosystem respired CO_2 in contrasting forest ecosystems in Canada and the USA, *Tree Physiol.*, 27, 1361–1374, 2007.
- Andreu-Hayles, L., Gutiérrez, E., Muntan, E., Helle, G., Anchukaitis, K. J., and Schleser, G. H.: Long tree-ring chronologies reveal 20th century increases in water-use efficiency but no enhancement of tree growth at five Iberian pine forests, *Glob. Change Biol.*, 17, 2095–2112, doi:10.1111/j.1365-2486.2010.02373.x, 2011.
- Armstrong, R. A.: Lichens, lichenometry and global warming, *Microbiologist*, 5, 32–35, 2004.
- Augusti, A. and Schleucher, J.: The ins and outs of stable isotopes in plants, *New Phytol.*, 174, 473–475, 2007.
- Badeck, F. W., Tcherkez, G., Nogués, S., Piel, C., and Ghashghaie, J.: Post-photosynthetic fractionation of stable carbon isotopes between plant organs – a widespread phenomenon, *Rapid Commun. Mass Sp.*, 19, 1381–1391, 2005.
- Badger, M. R. and Price, G. D.: The role of carbonic-anhydrase in photosynthesis, *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, 45, 369–392, 1994.
- Bahn, M., Schmitt, M., Siegwolf, R., Richter, A., and Brüggemann, N.: Does photosynthesis affect grassland soil-respired CO_2 and its carbon isotope composition on a diurnal timescale?, *New Phytol.*, 182, 451–460, 2009.
- Baker, J. M. and Griffis, T. J.: A simple, accurate, field-portable mixing ratio generator and rayleigh distillation device, *Agr. Forest Meteorol.*, 150, 1607–1611, 2010.
- Baldocchi, D., Falge, E., Gu, L., Olson, R., Hollinger, D., Running, S., Anthoni, P., Bernhofer, C., Davis, K., Evans, R., Fuentes, J., Goldstein, A., Katul, G., Law, B., Lee, X., Malhi, Y., Meyers, T., Munger, W., Oechel, W., Paw, U., Pilegaard, K., Schmid, H., Valentini, R., Verma, S., Vesala, T., Wilson, K., and Wofsy, S.: Fluxnet: A new tool to study the temporal and spatial variability of ecosystem-scale carbon dioxide, water vapor and energy flux densities, *B. Am. Meteorol. Soc.*, 82, 2415–2434, 2001.
- Baldocchi, D., Tang, J., and Xu, L.: How switches and lags in biophysical regulators affect spatial-temporal variation of soil respiration in an oak-grass savannah, *J. Geophys. Res.*, 111, G02008, doi:10.1029/2005JG000063, 2006.
- Barbosa, I. C. R., Köhler, I., Auerswald, K., Lüps, P. and Schnyder, H.: Last-century changes of alpine grassland water-use efficiency – a reconstruction through carbon isotope analysis of a time-series of *Capra ibex* horns, *Glob. Change Biol.*, 16, 1171–1180, 2010.
- Barbosa, I. C. R., Kley, M., Schäufele, R., Auerswald, K., Schröder, W., Filli, F., Hertwig, S., and Schnyder, H.: Analysing the isotopic life history of the alpine ungulates *Capra ibex* and *Rupicapra rupicapra rupicapra* through their horns, *Rapid Commun. Mass Sp.*, 23, 2347–2356, 2009.
- Barbour, M. M.: Stable oxygen isotope composition of plant tissue: a review, *Funct. Plant Biol.*, 34, 83–94, 2007.
- Barbour, M. and Farquhar, G.: Do pathways of water movement and leaf anatomical dimensions allow development of gradients in $(\text{H}_2\text{O})\text{-O-18}$ between veins and the sites of evaporation within leaves?, *Plant Cell Environ.*, 27, 107–121, 2003.
- Barbour, M. M., Roden, J. S., Farquhar, G. D., and Ehleringer, J. R.: Expressing leaf water and cellulose oxygen isotope ratios as enrichment above source water reveals evidence of a Péclet effect, *Oecologia*, 138, 426–435, 2004.
- Barbour, M. M., Hunt, J. E., Dungan, R. J., Turnbull, M. H., Brailsford, G. W., Farquhar, G. D., and Whitehead, D.: Variation in the degree of coupling between $\delta^{13}\text{C}$ of phloem sap and ecosystem respiration in two mature *Nothofagus* forests, *New Phytol.*, 166, 497–512, 2005.
- Barbour, M. M., Farquhar, G. D., Hanson, D. T., Bickford, C. P., Powers, H., and McDowell, N. G.: A new measurement technique reveals temporal variation in $\delta^{18}\text{O}$ of leaf-respired CO_2 , *Plant Cell Environ.*, 30, 456–468, 2007.
- Barbour, M. M., Hunt, J. E., Kodama, N., Laubach, J., McSeveny, T., Rogers, N. D., Tcherkez, G., and Wingate, L.: Rapid changes in $\delta^{13}\text{C}$ of ecosystem-respired CO_2 after sunset are consistent with transient ^{13}C enrichment of leaf respired, *New Phytol.*, 190, 990–1002, doi:10.1111/j.1469-8137.2010.03635.x, 2011a.
- Barbour, M. M., Tcherkez, G., Bickford, C. P., Mauve, C., Lamothe, M., Sinton, S., and Brown, H.: $\delta^{13}\text{C}$ of leaf-respired CO_2 reflects intrinsic water-use efficiency in barley, *Plant Cell Environ.*, 34, 792–799, 2011b.
- Barnard, R. L., Salmon, Y., Kodama, N., Sörgel, K., Holst, J., Rennerberg, H., Gessler, A., and Buchmann, N.: Evaporative enrichment and time lags between $\delta^{18}\text{O}$ of leaf water and organic pools in a pine stand, *Plant Cell Environ.*, 30, 539–550, 2007.
- Barthel, M., Hammerle, A., Sturm, P., Baur, T., Gentsch, L., and Knohl, A.: The diel imprint of leaf metabolism on the $\delta^{13}\text{C}$ signal of soil respiration under control and drought conditions, *New Phytol.*, 192, 925–938, doi:10.1111/j.1469-8137.2011.03848.x, 2011.
- Bathellier, C., Badeck, F. W., Couzi, P., Harscoet, S., Mauve, C., and Ghashghaie, J.: Divergence in $\delta^{13}\text{C}$ of dark respired CO_2 and bulk organic matter occurs during the transition between heterotrophy and autotrophy in *Phaseolus vulgaris* plants, *New Phytol.*, 177, 406–418, 2008.
- Bathellier, C., Tcherkez, G., Bligny, R., Gout, E., Cornic, G., and Ghashghaie, J.: Metabolic origin of the $\delta^{13}\text{C}$ of respired CO_2 in roots of *Phaseolus vulgaris*, *New Phytol.*, 181, 387–399, 2009.

- Beerling, D. J. and Woodward, F. I.: Modelling changes in plant functions over the Phanerozoic, in: *Stable Isotopes: Integration of Biological and Geochemical Processes*, edited by: Griffiths, H., BIOS Scientific Publishers, Oxford, 347–361, 1998.
- Bickford, C. P., McDowell, N. G., Erhardt, E. B., and Hanson, D. T.: High-frequency field measurements of diurnal carbon isotope discrimination and internal conductance in a semi-arid species, *Juniperus monosperma*, *Plant Cell Environ.*, 32, 7, 796–819, doi:10.1111/j.1365-3040.2009.01959.x, 2009.
- Bird, J. A., Kleber, M., and Torn, M. S.: ^{13}C and ^{15}N stabilization dynamics in soil organic matter fractions during needle and fine root decomposition, *Org. Geochem.*, 39, 465–477, 2008.
- Birks, S. J. and Edwards, T. W. D.: Atmospheric circulation controls on precipitation isotope-climate relations in western Canada, *Tellus*, 61B, 566–576, 2009.
- Bonal, D., Sabatier, D., Montpied, P., Tremeaux, D., and Guehl, J. M.: Interspecific variability of $\Delta^{13}\text{C}$ among trees in rainforests of French Guiana: functional groups and canopy integration, *Oecologia*, 124, 454–468, 2000.
- Bowen, G. J., West, J. B., Vaughn, B. H., Dawson, T. E., Ehleringer, J. R., Fogel, M. L., Hobson, K., Hoogewerff, J., Kendall, C., Lai, C.-T., Miller, C. C., Noone, D., Schwarcz, H., and Still, C. J.: Isoscapes to address large-scale earth science challenges, *EOS*, 90, 109–116, 2009.
- Bowling, D. R., Sargent, S. D., Tanner, B. D., and Ehleringer, J. R.: Tunable diode laser absorption spectroscopy for stable isotope studies of ecosystem-atmosphere CO_2 exchange, *Agr. Forest Meteorol.*, 118, 1–19, 2003.
- Bowling, D. R., Pataki, D. E., and Randerson, J. T.: Carbon isotopes in terrestrial ecosystem pools and CO_2 fluxes, *New Phytol.*, 178, 24–40, 2008.
- Brandes, E., Kodama, N., Whittaker, K., Weston, C., Rennenberg, H., Keitel, C., Adams, M. A., and Gessler A.: Short-term variation in the isotopic composition of organic matter allocated from the leaves to the stem of *Pinus sylvestris*: effects of photosynthetic and postphotosynthetic carbon isotope fractionation, *Glob. Change Biol.*, 12, 1922–1939, 2006.
- Briffa, K. R.: Annual climate variability in the Holocene: interpreting the message of ancient trees, *Quaternary Sci. Rev.*, 19, 87–105, 2000.
- Brooks, J. R., Flanagan, L. B., Buchmann, N., and Ehleringer, J. R.: Carbon isotope composition of boreal plants: functional grouping of life forms, *Oecologia*, 110, 301–311, 1997.
- Brüggemann, N., Gessler, A., Kayler, Z., Keel, S. G., Badeck, F., Barthel, M., Boeckx, P., Buchmann, N., Brugnoli, E., Espereschütz, J., Gavrichkova, O., Ghashghaie, J., Gomez-Casanovas, N., Keitel, C., Knohl, A., Kuptz, D., Palacio, S., Salmon, Y., Uchida, Y., and Bahn, M.: Carbon allocation and carbon isotope fluxes in the plant-soil-atmosphere continuum: a review, *Biogeosciences*, 8, 3457–3489, doi:10.5194/bg-8-3457-2011, 2011.
- Brugnoli, E. and Farquhar, G. D.: Correlation between the carbon isotope discrimination in leaf starch and sugars of C-3 plants and the ratio of intercellular and atmospheric partial pressures of carbon dioxide, edited by: Brugnoli, E., Hubick, K. T., von Caemmerer, S., Wong, C. S., and Farquhar, G. D., *Plant Physiol.*, 88, 1418–1424, 1988.
- Brugnoli, E. and Farquhar, G. D.: Photosynthetic fractionation of carbon isotopes, in: *Photosynthesis: Physiology and Metabolism*, edited by: Leegood, R. C., Sharkey, T. D., and von Caemmerer, S., Kluwer Academic Publisher, The Netherlands, 399–434, 2000.
- Buchmann, N., Guehl, J. M., Barigah, T. S., and Ehleringer, J. R.: Interseasonal comparison of CO_2 concentrations, isotopic composition, and carbon dynamics in an Amazonian rainforest (French Guiana), *Oecologia*, 110, 120–131, 1997a.
- Buchmann, N., Kao, W. Y., and Ehleringer, J. R.: Influence of stand structure on carbon-13 of vegetation, soils, and canopy air within deciduous and evergreen forests in Utah (USA), *Oecologia*, 110, 109–119, 1997b.
- Buchmann, N., Hinckley, T. M., and Ehleringer, J. R.: Carbon isotope dynamics in *Abies amabilis* stands in the Cascades, *Can. J. Forest Res.*, 28, 808–819, 1998.
- Burnett, A. W., Mullins, H. T., and Patterson, W. P.: Relationship between atmospheric circulation and winter precipitation $\delta^{18}\text{O}$ in central New York State, *Geophys. Res. Lett.*, 31, L22209, doi:10.1029/2004GL021089, 2004.
- Caldeira, M. C., Ryel, R. J., Lawton, J. H., and Pereira, J. P.: Mechanisms of positive biodiversity-production relationships: insights provided by $\delta^{13}\text{C}$ analysis in experimental Mediterranean grassland plots, *Ecol. Letters*, 4, 439–443, 2001.
- Caldwell, M. M., Dawson, T. E., Richards, J. H.: Hydraulic lift: consequences of water efflux from the roots of plants, *Oecologia*, 113, 151–161, 1998.
- Cernusak, L. A., Arthur, D. J., Pate, J. S., and Farquhar, G. D.: Water relations link carbon and oxygen isotope discrimination to phloem sap sugar concentration in *Eucalyptus globules*, *Plant Physiol.*, 131, 1544–1554, 2003a.
- Cernusak, L. A., Wong, S. C., and Farquhar, G. D.: Oxygen isotope composition of phloem sap in relation to leaf water in *Ricinus communis*, *Funct. Plant Biol.*, 30, 1059–1070, 2003b.
- Cernusak, L. A., Farquhar, G. D., and Pate, J. S.: Environmental and physiological controls over oxygen and carbon isotope composition of Tasmanian blue gum, *Eucalyptus globules*, *Tree Physiol.*, 25, 129–146, 2005.
- Cernusak, L. A., Tcherkez, G., Keitel, C., Cornwell, W. K., Santiago, L. S., Knohl, A., Barbour, M. M., Williams, D. G., Reich, P. B., Ellsworth, D. S., Dawson, T. E., Griffiths, H. G., Farquhar, G. D., and Wright, I. J.: Viewpoint: Why are non-photosynthetic tissues generally ^{13}C enriched compared with leaves in C₃ plants?, Review and synthesis of current hypotheses, *Funct. Plant Biol.*, 36, 199–213, 2009.
- Clode, P. L., Kilburn, M. R., Jones, D. L., Stockdale, E. A., Cliff, J. B., Herrmann, A. M., and Murphy, D. V.: In situ mapping of nutrient uptake in the rhizosphere using nanoscale secondary ion mass spectrometry, *Plant Physiol.*, 151, 1751–1757, 2009.
- Condon, A. G., Richards, R. A., Rebetzke, G. J., and Farquhar, G. D.: Improving intrinsic water-use efficiency and crop yield, *Crop Sci.*, 42, 122–131, 2002.
- Coplen, T. B.: Reporting of stable carbon, hydrogen, and oxygen isotopic abundances, in: *Reference and intercomparison materials for stable isotopes of light elements*, IAEA-Tecdoc 825, IAEA, Vienna, 31–34, 1995.
- Coplen, T. B.: Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results, *Rapid Comm. Mass Spectrometry*, 25, 2538–2560, 2011.
- Cousins, A. B., Badger, M. R., and von Caemmerer, S.: C₄ photosynthetic isotope exchange in NAD-ME- and NADP-ME-type grasses, *J. Exp. Bot.*, 59, 1695–1703, 2008.

- Craig, H. and Gordon, L. I.: Deuterium and oxygen-18 variations in the ocean and the marine atmosphere, in: Proceedings of a Conference on Stable Isotopes in Oceanographic Studies and Paleotemperatures, edited by: Tongiorgi, E., CNR, Laboratorie Geologia Nuclear, Pisa, Italy, 9–130, 1965.
- Crow, S. E., Sulzman, E. W., Rugh, W. D., Bowden, R. D., and Lajtha, K.: Isotopic analysis of respired CO₂ during decomposition of separated soil organic matter pools, *Soil Biol. Biochem.*, 38, 3279–3291, 2006.
- Csank, A. Z., Patterson, W. P., Eglinton, B. M., Rybczynski, N., and Basinger, J. F.: Climate variability in the Early Pliocene Arctic: Annually resolved evidence from stable isotope values of sub-fossil wood, Ellesmere Island, Canada, *Palaeogeogr. Palaeoclimatol.*, 308, 339–349, doi:10.1016/j.palaeo.2011.05.038, 2011.
- Cuntz, M., Ogee, J., Farquhar, G. D., Peylin, P., and Cernusak, L. A.: Modelling advection and diffusion of water isotopologues in leaves, *Plant Cell Environ.*, 30, 892–909, 2007.
- Dawson, T. E. and Siegwolf, R. T. W. (Eds.): *Stable Isotopes as Indicators of Ecological Change*, Academic Press-Elsevier, San Diego, 2007.
- Dawson, T. E. and Simonin, K. S.: The roles of stable isotopes in forest hydrology and biogeochemistry, in: *Forest Hydrology and Biogeochemistry: Synthesis of Research and Future Directions*, Ecological Studies Series, edited by: Levia, D. F., Carlyle-Moses, D., and Tanaka, T., No. 216, Springer-Verlag, Heidelberg, Germany, 137–161, 2011.
- Dawson, T. E., Pausch, R. C., and Parker, H. M.: The role of hydrogen and oxygen isotopes in understanding water movement along the soil-plant-atmosphere continuum, in: *Stable Isotopes: Integration of Biological and Geochemical Processes*, edited by: Griffiths, H., BIOS Scientific Publishers, Oxford, 169–183, 1998.
- Dawson, T. E., Mambelli, S., Plamboeck, A. H., Templer, P. H., and Tu, K. P.: Stable isotopes in plant ecology, *Annu. Rev. Ecol. Syst.*, 33, 507–559, 2002.
- De Groot, P. A. (Ed.): *Handbook of Stable Isotope Analytical Techniques*, Elsevier B.V., Amsterdam, 2004.
- Devaux, M., Ghashghaie, J., Bert, D., Lambrot, C., Gessler, A., Bathellier, C., Ogee, J., and Loustau, D.: Carbon stable isotope ratio of phloem sugars in mature pine trees throughout the growing season: comparison of two extraction methods, *Rapid Commun. Mass Sp.*, 23, 2511–2518, 2009.
- Dongmann, G., Nürnberg, H. W., Förstel, H., and Wagener, K.: On the enrichment of H₂¹⁸O in the leaves of transpiring plants, *Radiat. Environ. Bioph.*, 11, 41–52, 1974.
- Drake, P. and Franks, P. J.: Water resource partitioning, stem xylem hydraulic properties and water use strategies in a seasonally dry riparian tropical rainforest, *Oecologia*, 137, 321–329, 2003.
- Draxler, R. R. and Hess, G. D.: Description of the HYSPLIT 4 modeling system, NOAA Technical Memorandum ERL ARL, 224–225, 2004.
- Dubbert, M., Rascher, K. G., and Werner, C.: Temporal and spatial variations in carbon isotope fractionation of dark-respired CO₂ and different plant carbon pools in three Mediterranean species under different climatic conditions, *Photosynth. Res.*, doi:10.1007/s11120-012-9748-3, 2012.
- Duranceau, M., Ghashghaie, J., Badeck, F., Deléens, E., and Cornic, G.: $\delta^{13}\text{C}$ of CO₂ respired in the dark in relation to $\delta^{13}\text{C}$ of leaf carbohydrates in *Phaseolus vulgaris* L. under progressive drought, *Plant Cell Environ.*, 22, 515–523, 1999.
- Dutton, A. L., Wilkinson, B. H., Welker, J. M., and Lohmann, K. C.: Comparison of river water and precipitation $\delta^{18}\text{O}$ across the 48 contiguous United States, *Hydrol. Process.*, 19, 3551–3572, 2005.
- Ehleringer, J. R.: Variation in leaf carbon isotope discrimination in *Encelia farinosa*: implications for growth, competition, and drought survival, *Oecologia*, 95, 340–346, 1993.
- Ehleringer, J. R., Sage, R. F., Flanagan, L. B., and Pearcy, R. W.: Climate change and the evolution of C₄ photosynthesis, *Trends Ecol. Evol.*, 6, 95–99, 1991.
- Ehleringer, J. R., Hall, A. E., and Farquhar, G. D. (Eds.): *Stable isotopes and plant carbon-water relations*, Academic Press, San Diego, 1993.
- Ehleringer, J. R., Buchmann, N., and Flanagan, L. B.: Carbon and oxygen isotope ratios in below-ground carbon-cycle processes, *Ecol. Appl.*, 10, 412–422, 2000.
- Eiler, J. M.: “Clumped-isotope” geochemistry—The study of naturally-occurring, multiply-substituted isotopologues, *Earth Planet. Sc. Lett.*, 262, 309–327, 2007.
- Ekblad, A., Bostrom, B., Holm, A., and Comstedt, D.: Forest soil respiration rate and $\delta^{13}\text{C}$ is regulated by recent above ground weather conditions, *Oecologia*, 143, 136–142, 2005.
- Epron, D., Ngao, J., Dannoura, M., Bakker, M. R., Zeller, B., Bazot, S., Bosc, A., Plain, C., Lata, J. C., Priault, P., Barthes, L., and Loustau, D.: Seasonal variations of belowground carbon transfer assessed by in situ ¹³C₂ pulse labelling of trees, *Biogeochemistry*, 8, 1153–1168, doi:10.5194/bg-8-1153-2011, 2011.
- Epron, D., Bahn, M., Derrien, D., Lattanzi, F., Pumpanen, J., Gessler, A., Höglberg, P., Dannoura, M., Gerant, D., Maillard, P., and Buchmann, N.: Pulse-labelling trees to study carbon allocation dynamics: a review of methods, current knowledge and future prospects, *Tree Phys.*, 32, 776–798, 2012.
- Ericsson, A.: Effects of fertilization and irrigation on the seasonal changes of carbohydrate reserves in different age-classes of needle on 20-year-old Scots pine trees (*Pinus sylvestris*), *Physiol. Plantarum*, 45, 270–280, 1979.
- Esperschütz, J., Buegger, F., Winkler, J. B., Munch, J. C., Schlöter, M., and Gatterer, A.: Microbial response to exudates in the rhizosphere of young beech trees (*Fagus sylvatica* L.) after dormancy, *Soil Biol. Biochem.*, 41, 1976–1985, 2009.
- Evans, J. R., Sharkey, T. D., Berry, J. A., and Farquhar, G.: Carbon isotope discrimination measured concurrently with gas exchange to investigate CO₂ diffusion in leaves of higher plants, *Aust. J. Plant Physiol.*, 13, 281, 281–292, 1986.
- Evans, J. R., Kaldenhoff, R., Genty, B., and Terashima, I.: Resistances along the CO₂ diffusion pathway inside leaves, *J. Exp. Bot.*, 60, 2235–2248, 2009.
- Farquhar, G. D. and Cernusak, L. A.: On the isotopic composition of leaf water in the non-steady state, *Funct. Plant Biol.*, 32, 293–303, 2005.
- Farquhar, G. D. and Cernusak, L. A.: Ternary effects on the gas exchange of isotopologues of carbon dioxide, *Plant Cell Environ.*, 35, 1221–1231, doi:10.1111/j.1365-3040.2012.02484.x, 2012.
- Farquhar, G. D. and Lloyd, J.: Carbon and oxygen isotope effects in the exchange of carbon dioxide between terrestrial plants and the atmosphere, in: *Stable isotopes and plant carbon-water relations*, edited by: Ehleringer, J. R., Hall, A. E., and Farquhar, G. D.,

- Academic Press, San Diego, 47–70, 1993.
- Farquhar, G. D. and Richards, R. A.: Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes, *Aust. J. Plant Physiol.*, 11, 539–552, 1984.
- Farquhar, G. D., Ehleringer, J. R., and Hubick, K. T.: Carbon isotope discrimination and photosynthesis, *Annu. Rev. Plant Phys.*, 40, 503–537, 1989a.
- Farquhar, G. D., Hubick, K. T., Condon, A. G., and Richards, R. A.: Carbon isotope fractionation and plant water-use-efficiency, in: *Stable Isotopes in Ecological Research*, edited by: Rundel, P. W., Ehleringer, J. R., and Nagy, K. H., Springer-Verlag, Berlin, 21–40, 1989b.
- Farquhar, G. D., O’Leary, M. H., and Berry, J. A.: On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves, *Aust. J. Plant Physiol.*, 9, 121–137, 1982.
- Farquhar, G. D., Lloyd, J., Taylor, J. A., Flanagan, L. B., Syvertsen, J. P., Hubick, K. T., Wong, S. C., and Ehleringer, E. R.: Vegetation effects on the isotope composition of oxygen in atmospheric CO₂, *Nature*, 363, 439–443, 1993.
- Ferrio, J. P., Cuntz, M., Offermann, C., Siegwolf, R., Saurer, M., and Gessler, A.: Effect of water availability on leaf water isotopic enrichment in beech seedlings shows limitations of current fractionation models, *Plant Cell Environ.*, 32, 1285–1296, 2009.
- Ferrio, J. P., Pou, A., Florez-Sarasa, I., Gessler, A., Kodama, N., Flexas, J., and Ribas-Carbo, M.: The Péclet effect on leaf water enrichment correlates with leaf hydraulic conductance and mesophyll conductance for CO₂, *Plant Cell Environ.*, 35, 611–625, 2012.
- Flanagan, L. B. and Ehleringer, J.: Ecosystem-atmosphere CO₂ exchange: interpreting signals of change using stable isotope ratios, *Trends Ecol. Evol.*, 13, 10–14, 1998.
- Flanagan, L. B., Marshall, J. D., and Ehleringer, J. R.: Photosynthetic gas exchange and the stable isotope composition of leaf water: comparison of a xylem-tapping mistletoe and its host, *Plant Cell Environ.*, 16, 623–631, 1993.
- Flanagan, L. B., Kubien, D. S., and Ehleringer, J. R.: Spatial and temporal variation in the carbon and oxygen stable isotope ratio of respired CO₂ in a boreal forest ecosystem, *Tellus B*, 51, 367–384, 1999.
- Flanagan, L. B., Ehleringer, J. R., and Pataki D. E. (Eds.): *Stable isotopes and biosphere-atmosphere interactions*, Elsevier Academic Press, San Diego, US, 318 pp., 2005.
- Fletcher, B. J., Beerling, D. J., Brentnall, S. J., and Royer, D. L.: Fossil bryophytes as recorders of ancient CO₂ levels: Experimental evidence and a cretaceous case study, *Global Biogeochem. Cy.*, 19, GB3012, doi:10.1029/2005GB002495, 2005.
- Fletcher, B. J., Brentnall, S. J., Quick, W. P., and Beerling, D. J.: Bryocarb: A process-based model of thallose liverwort carbon isotope fractionation in response to CO₂, O₂, light and temperature, *Geochim. Cosmochim. Ac.*, 70, 5676–5691, 2006.
- Flexas, J., Dias-Espejo, A., Galmes, J., Kaldenhoff, R., Medrano, H., and Ribas-Carbo, M.: Rapid variations in mesophyll conductance in response to changes in CO₂ concentration around leaves, *Plant Cell Environ.*, 30, 1284–1298, 2007.
- Flexas, J., Ribas-Carbo, M., Diaz-Espejo, A., Galmes, J., and Medrano, H.: Mesophyll conductance to CO₂: current knowledge and future prospects, *Plant Cell Environ.*, 31, 602–621, 2008.
- Fried, A. and Richter, D.: Infrared absorption Spectroscopy, in: *Analytical Techniques for Atmospheric Measurement*, edited by: Heard, D. E., Blackwell Publishing, Oxford, UK, 72–146, 2006.
- Fry, B.: *Stable Isotope Ecology*, Springer, New York, US, 308 pp., 2006.
- Gammitzer, U., Schäufele, R., and Schnyder, H.: Observing ¹³C labelling kinetics in CO₂ respired by a temperate grassland ecosystem, *New Phytol.*, 184, 376–386, 2009.
- Gammitzer, U., Moyes, A. B., Bowling, D. R., and Schnyder, H.: Measuring and modelling the isotopic composition of soil respiration: insights from a grassland tracer experiment, *Biogeosciences*, 8, 1333–1350, doi:10.5194/bg-8-1333-2011, 2011.
- Gan, K. S., Wong, S. C., Yong, J. W. H., and Farquhar, G. D.: O-18 spatial patterns of vein xylem water, leaf water, and dry matter in cotton leaves, *Plant Physiol.*, 130, 1008–1021, 2002.
- Gessler, A., Keitel, C., Kodama, N., Weston, C., Winters, A. J., Keith, H., Grice, K., Leuning, R. and Farquhar, G. D.: Delta C-13 of organic matter transported from the leaves to the roots in *Eucalyptus delegatensis*: short-term variations and relation to respired CO₂, *Funct. Plant Biol.*, 34, 692–706, 2007a.
- Gessler, A., Peuke, A. D., Keitel, C., and Farquhar, G. D.: Oxygen isotope enrichment of organic matter in *Ricinus communis* during the diel course and as affected by assimilate transport, *New Phytol.*, 174, 600–613, 2007b.
- Gessler, A., Rennenberg, H., and Keitel, C.: Stable isotope composition of organic compounds transported in the phloem of European beech – Evaluation of different methods of phloem sap collection and assessment of gradients in carbon isotope composition during leaf-to-stem transport, *Plant Biol.*, 6, 721–729, 2004.
- Gessler, A., Tcherkez, G., Peuke, A. D., Ghashghaie, J., and Farquhar, G. D.: Experimental evidence for diel variations of the carbon isotope composition in leaf, stem and phloem sap organic matter in *Ricinus communis*, *Plant Cell Environ.*, 31, 941–953, 2008.
- Gessler, A., Löw, M., Heerdt, C., Op de Beeck, M., Schumacher, J., Grams, T. E. E., Bahnweg, G., Ceulemans, R., Werner, H., Matyssek, R., Rennenberg, H., and Haberer, K.: Within-canopy and ozone fumigation effects on δ¹³C and Δ¹⁸O in adult beech (*Fagus sylvatica*) trees: relation to meteorological and gas exchange parameters, *Tree Physiol.*, 11, 1349–1365, 2009.
- Ghashghaie, J., Duranceau, M., Badeck, F. W., Cornic, G., Adeline, M. T., and Deleens, E.: δ¹³C of CO₂ respired in the dark in relation to δ¹³C of leaf metabolites: comparison between *Nicotiana sylvestris* and *Helianthus annuus* under drought, *Plant Cell Environ.*, 24, 505–515, 2001.
- Ghashghaie, J., Badeck, F. W., Lanigan, G., Nogués, S., Tcherkez, G., Deléens, E., Cornic, G., and Griffiths, H.: Carbon isotope fractionation during dark respiration and photorespiration in C3 plants, *Phytochem. Rev.*, 2, 145–161, 2003.
- Gilbert, A., Silvestre, V., Robins, R., and Remaud, G.: Accurate quantitative isotopic ¹³C NMR spectroscopy for the determination of the intramolecular distribution of ¹³C in glucose at natural abundance, *Anal. Chem.*, 81, 8978–8985, 2009.
- Gilbert, A., Silvestre, V., Segebarth, N., Tcherkez, G., Guillou, C., Robins, R. J., Akoka, S., and Remaud, G.: The intramolecular ¹³C-distribution in ethanol reveals the influence of the CO₂-fixation pathway and environmental conditions on the site-specific ¹³C variation in glucose, *Plant, Cell Environ.*, 34, 1104–

- 1112, 2011.
- Gillon, J. S. and Yakir, D.: Internal conductance to CO₂ diffusion and C¹⁸O discrimination in C₃ leaves, *Plant Physiol.*, 123, 201–213, 2000a.
- Gillon, J. S. and Yakir, D.: Naturally low carbonic anhydrase activity in C₄ and C₃ plants limits discrimination against C¹⁸O during photosynthesis, *Plant Cell Environ.*, 23, 903–915, 2000b.
- Gleixner, G., Scrimgeour, C., Schmidt, H.-L., and Viola, R.: Stable isotope distribution in the major metabolites of source and sink organs of *Solanum tuberosum* L.: a powerful tool in the study of metabolic partitioning in intact plants, *Planta*, 207, 241–245, 1998.
- Godin, J.-P., Fay, L.-B., and Hopfgartner, G.: Liquid chromatography combined with mass spectrometry for C-13 isotopic analysis in life science research, *Mass Spectrom. Rev.*, 26, 751–774, 2007.
- Gong, Y. X., Chen, Q., Lin, S., Brueck, H., Dittert, K., Taube, F., and Schnyder, H.: Tradeoffs between nitrogen- and water-use efficiency in dominant species of the semiarid steppe of Inner Mongolia, *Plant Soil*, 340, 227–238, 2010.
- Grams, T. E. E. and Matyssek, R.: Stable isotope signatures reflect competitiveness between trees under changed CO₂/O₃ regimes, *Environ. Pollut.*, 158, 1036–1042, doi:10.1016/j.envpol.2009.08.037, 2010.
- Grams, T. E. E., Kozovits, A. R., Häberle, K.-H., Matyssek, R., and Dawson, T. E.: Combining δ¹³C and δ¹⁸O analyses to unravel competition, CO₂ and O₃ effects on the physiological performance of different-aged trees, *Plant Cell Environ.*, 30, 1023–1034, 2007.
- Griffis, T. J., Black, T. A., Gaumont-Guay, D., Drewitt, G. B., Nestic, Z., Barr, A. G., Morgenstern, K., and Kljun, N.: Seasonal variation and partitioning of ecosystem respiration in a southern boreal aspen forest, *Agr. Forest Meteorol.*, 125, 207–223, 2004.
- Griffis, T. J., Sargent, S. D., Baker, J. M., Lee, X., Tanner, B. D., Greene, J., Swiatek, E., and Billmark, K.: Direct measurement of biosphere-atmosphere isotopic CO₂ exchange using the eddy covariance technique, *J. Geophys. Res.-Atmos.*, 113, D08304, doi:10.1029/2007JD009297, 2008.
- Griffis, T., Sargent, S., Lee, X., Baker, J., Greene, J., Erickson, M., Zhang, X., Billmark, K., Schultz, N., Xiao, W., and Hu, N.: Determining the oxygen isotope composition of evapotranspiration using eddy covariance, *Bound.-Lay. Meteorol.*, 137, 307–326, 2010.
- Griffiths, H. (Ed.): *Stable Isotopes – integration of biochemical, ecological and geochemical processes*, BIOS Scientific Publishers Ltd, Oxford, 1998.
- Gross, S. and Glaser, B.: Minimization of carbon addition during derivatization of monosaccharides for compound-specific delta C-13 analysis in environmental research, *Rapid Comm. Mass Sp.*, 18, 2753–2764, 2004.
- Gubsch, M., Roscher, C., Gleixner, G., Habekost, M., Lipowsky, A., Schmid, B., Schulze, E. D., Steinbeiss, S., and Buchmann, N.: Foliar and soil delta ¹⁵N values reveal increased nitrogen partitioning among species in diverse grassland communities, *Plant Cell Environ.*, 34, 895–908, 2011.
- Guehl, J. M., Bonal, D., Ferhi, A., Barigah, T. S., Farquhar, G., and Granier, A.: Community-level diversity of carbon-water relations in rainforest trees, in: *Ecology and Management of a Neotropical Rainforest*, edited by: Gourlet-Fleury, S., Laroussinie, O., and Guehl, J. M., Elsevier, Paris, 65–84, 2004.
- Guerrieri, M. R., Siegwolf, R. T. W., Saurer, M., Jäggi, M., Cherubini, P., Ripullone, F., and Borghetti, M.: Impact of different nitrogen emission sources on tree physiology: a triple stable isotope approach, *Atmos. Environ.*, 43, 410–418, doi:10.1016/j.atmosenv.2008.08.042, 2009.
- Guerrieri, R., Siegwolf, R., Saurer, M., Ripullone, F., Mencuccini, M. and Borghetti, M.: Anthropogenic NO_x emissions alter the intrinsic water-use efficiency (WUEi) for *Quercus cerris* stands under Mediterranean climate conditions, *Environ. Pollut.*, 158, 2841–2847, 2010.
- Hanba, Y. T., Kogami, H., and Terashima, I.: The effect of internal CO₂ conductance on leaf carbon isotope ratio, *Isot. Environ. Healt. S.*, 39, 5–13, 2003.
- Hanba, Y. T., Shibasaki, M., Hayashi, Y., Hayakawa, T., Kasamo, K., Terashima, I., and Katsuhara, M.: Overexpression of the barley aquaporin HvPIP2;1 increases internal CO₂ conductance and CO₂ assimilation in the leaves of transgenic rice plants, *Plant Cell Physiol.*, 45, 521–529, 2004.
- Hansen, J. and Beck, E.: The fate and path of assimilation products in the stem of 8-year-old Scots pine (*Pinus sylvestris* L.) trees, *Trees-Struct. Funct.*, 4, 16–21, 1990.
- Hartard, B., Máguas, C., and Lakatos, M.: δ¹⁸O characteristics of lichens and their effects on evaporative processes of the subjacent soil, *Isot. Environ. Healt. S.*, 44, 111–125, 2008.
- Hartard, B., Cuntz, M., Máguas, C., and Lakatos, M.: Water isotopes in desiccating lichens, *Planta*, 231, 179–193, 2009.
- Helle, G. and Schleser, G.: Beyond CO₂-fixation by Rubisco – an interpretation of ¹³C/¹²C variations in tree rings from novel intra-seasonal studies on broad-leaf trees, *Plant Cell Environ.*, 27, 367–380, 2004.
- Helliker, B. R.: On the controls of leaf-water oxygen isotope ratios in the atmospheric Crassulacean Acid Metabolism epiphyte *Tillandsia usneoides*, *Plant Physiol.*, 155, 2096–2107, 2011.
- Helliker, B. R. and Ehleringer, J. R.: Establishing a grassland signature in veins: ¹⁸O in the leaf water of C₃ and C₄ grasses, *P. Natl. Acad. Sci.*, 97, 7894–7898, 2000.
- Helliker, B. R. and Griffiths, H.: Toward a plant-based proxy for the isotope ratio of atmospheric water vapor, *Glob. Change Biol.*, 13, 723–733, 2007.
- Hemming, D., Yakir, D., Ambus, P., Aurela, M., Besson, C., Black, K., Buchmann, N., Burlett, R., Cescatti, A., Clement, R., Gross, P., Granier, A., Grunwald, T., Havrankova, K., Janous, D., Janssens, I. A., Knohl, A., Ostner, B. K., Kowalski, A., Laurila, T., Mata, C., Marcolla, B., Matteucci, G., Moncrieff, J., Moors, E. J., Osborne, B., Pereira, J. S., Pihlatie, M., Pilegaard, K., Ponti, F., Rosova, Z., Rossi, F., Scartazza, A., and Vesala, T.: Pan-European delta C-13 values of air and organic matter from forest ecosystems, *Glob. Change Biol.*, 11, 1065–1093, 2005.
- Herrmann, A. M., Ritz, K., Nunan, N., Clode, P. L., Pett-Ridge, J., Kilburn, M. R., Murphy, D. V., O’Donnell, A. G., and Stockdale, E. A.: Nano-scale secondary ion mass spectrometry – A new analytical tool in biogeochemistry and soil ecology: A review article, *Soil Biol. Biochem.*, 39, 1835–1850, 2007.
- Högberg, P., Nordgren, A., Buchmann, N., Taylor, A. F. S., Ekblad, A., Högberg, M. N., Nyberg, G., Ottosson-Lofvenius, M., and Read, D. J.: Large-scale forest girdling shows that current photosynthesis drives soil respiration, *Nature*, 411, 789–792, 2001.

- Högberg, P., Högberg, M. N., Göttlicher, S. G., Betson, N. R., Keel, S. G., Metcalfe, D. B., Campbell, C., Schindlbacher, A., Hurry, V., Lundmark, T., Linder, S., and Nasholm, T.: High temporal resolution tracing of photosynthate carbon from the tree canopy to forest soil microorganisms, *New Phytol.*, 177, 220–228, 2008.
- Holmgren, M., Scheffer, M., Ezcurra, E., Gutierrez, J., and Mohren, G.: El Niño effects on the dynamics of terrestrial ecosystems, *Trends Ecol. Evol.*, 16, 89–94, 2001.
- Hu, Z. M., Yu, G. R., Zhou, Y. L., Sun, X. M., Li, Y. N., Shi, P. L., Wang, Y. F., Song, X., Zheng, Z. M., Zhang, L., and Li, S. G.: Partitioning of evapotranspiration and its controls in four grassland ecosystems: Application of a two-source model, *Agr. Forest Meteorol.*, 149, 1410–1420, 2009.
- Jäggi, M., Saurer, M., Fuhrer, J., and Siegwolf, R.: The relationship between the stable carbon isotope composition of needle bulk material, starch, and tree rings in *Picea abies*, *Oecologia*, 131, 325–332, 2002.
- Kahmen, A. and Buchmann, N.: Addressing the functional value of biodiversity for ecosystem functioning using stable isotopes, in: *Stable isotopes as indicators for ecological change*, edited by: Dawson, T. E. and Siegwolf, R. T. W., Academic Press-Elsevier, San Diego, 2007, 347–359, 2007.
- Kahmen, A., Simonin, K., Tu, K. P., Merchant, A., Callister, A., Dawson, T. E., and Arndt, S. K.: Physiological and morphological effects on leaf water $\delta^{18}\text{O}$ enrichment in different Eucalyptus species, *Plant Cell Environ.*, 31, 738–751, 2008.
- Kahmen, A., Simonin, K. S., Tu, K. P., Goldsmith, G., and Dawson, T. E.: The influence of species and growing conditions on oxygen isotope leaf water enrichment and its impact on “effective path length”, *New Phytol.*, 184, 619–630, 2009.
- Kammer, A., Tuzson, B., Emmenegger, L., Knohl, A., Mohn, J., and Hagedorn, F.: Application of a quantum cascade laser-based spectrometer in a closed chamber system for real-time $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ measurements of soil-respired CO_2 , *Agr. Forest Meteorol.*, 151, 39–48, 2011.
- Kaplan, J. O., Prentice, I. C., and Buchmann, N.: The stable carbon isotope composition of the terrestrial biosphere: Modeling at scales from the leaf to the globe, *Global Biogeochem. Cy.*, 16, 1060–1071, 2002.
- Karner, U., Peterbauer, T., Raboy, V., Jones, D. A., Hedley, C. L., and Richter, A.: myo-Inositol and sucrose concentrations affect the accumulation of raffinose family oligosaccharides in seeds, *J. Exp. Bot.*, 55, 1981–1987, 2004.
- Kayler, Z. E., Sulzman, E. W., Marshall, J. D., Mix, A., Rugh, W. D., and Bond, B. J.: A laboratory comparison of two methods used to estimate the isotopic composition of soil $\delta^{13}\text{CO}_2$ efflux at steady state, *Rapid Commun. Mass Sp.*, 22, 2533–2538, 2008.
- Kayler, Z. E., Ganio, L., Hauck, M., Pypker, T., Sulzman, E., Mix, A., and Bond, B.: Bias and uncertainty of $\delta^{13}\text{CO}_2$ isotopic mixing models, *Oecologia*, 163, 227–234, 2010a.
- Kayler, Z. E., Sulzman, E. W., Rugh, W. D., Mix, A. C., and Bond, B. J.: Characterizing the impact of diffusive and advective soil gas transport on the measurement and interpretation of the isotopic signal of soil respiration, *Soil Biol. Biochem.*, 42, 435–444, 2010b.
- Kayler, Z. E., Kaiser, M., Gessler, A., Ellerbrock, R. H., and Sommer, M.: Application of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures of organic matter fractions sequentially separated from adjacent arable and forest soils to identify carbon stabilization mechanisms, *Biogeosciences*, 8, 2895–2906, doi:10.5194/bg-8-2895-2011, 2011.
- Keitel, C., Adams, M. A., Holst, T., Matzarakis, A., Mayer, H., Rennenberg, H., and Gessler, A.: Carbon and oxygen isotope composition of organic compounds in the phloem sap provides a short-term measure for stomatal conductance of European beech (*Fagus sylvatica* L.), *Plant Cell Environ.*, 26, 1157–1168, 2003.
- Keitel, C., Matzarakis, A., Rennenberg, H., and Gessler, A.: Carbon isotopic composition and oxygen isotopic enrichment in phloem and total leaf organic matter of European beech (*Fagus sylvatica* L.) along a climate gradient, *Plant Cell Environ.*, 29, 1492–1507, 2006.
- Kerstel, E.: Isotope ratio infrared spectrometry, in: *Handbook of Stable Isotope Analytical Techniques*, edited by: de Groot, P. A., Elsevier B.V., Amsterdam, Chapter 34, 759–787, 2004.
- Kerstel, E. and Gianfrani, L.: Advances in laser-based isotope ratio measurements: selected applications, *Appl. Phys. B-Lasers O.*, 92, 439–449, 2008.
- Kim, K. and Lee, X.: Isotopic enrichment of liquid water during evaporation from water surfaces, *J. Hydrol.*, 399, 364–375, 2011.
- Klumpp, K., Schäufele, R., Lötscher, M., Lattanzi, F. A., Feneis, W., and Schnyder, H.: C-isotope composition of CO_2 respired by shoots and roots: fractionation during dark respiration?, *Plant Cell Environ.*, 28, 241–250, 2005.
- Klumpp, K., Soussana, J. F., and Falcimagne, R.: Long-term steady state ^{13}C labelling to investigate soil carbon turnover in grasslands, *Biogeosciences*, 4, 385–394, doi:10.5194/bg-4-385-2007, 2007.
- Knohl, A., Werner, R. A., Brand, W. A., and Buchmann, N.: Short-term variations in $\delta^{13}\text{C}$ of ecosystem respiration reveals link between assimilation and respiration in a deciduous forest, *Oecologia*, 142, 70–82, 2005.
- Knorre, A. A., Siegwolf, R. T. W., Saurer, M., Sidorova, O. V., Vaganov, E. A., and Kiryanov, A. V.: Twentieth century trends in tree ring stable isotopes ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) of *Larix sibirica* under dry conditions in the forest steppe in Siberia, *J. Geophys. Res.-Bio.*, 115, G03002, doi:10.1029/2009JG000930, 2010.
- Kodama, N., Barnard, R., Salmon, Y., Weston, C., Ferrio, J. P., Holst, J., Werner, R. A., Saurer, M., Rennenberg, H., Buchmann, N., and Gessler, A.: Temporal dynamics of the carbon isotope composition in a *Pinus sylvestris* stand: from newly assimilated organic carbon to respired carbon dioxide, *Oecologia*, 156, 737–750, 2008.
- Kogami, H., Hanba, Y. T., Kibe, T., Terashima, I., and Masuzawa, T.: CO_2 transfer conductance, leaf structure and carbon isotope composition of *Polygonum cuspidatum* leaves from low and high altitudes, *Plant Cell Environ.*, 24, 529–538, 2001.
- Köhler, I. H., Poulton, P. R., Auerswald, K., and Schnyder, H.: Intrinsic water-use efficiency of temperate semi-natural grassland has increased since 1857: an analysis of carbon isotope discrimination of herbage from the Park Grass Experiment, *Glob. Change Biol.*, 16, 1531–1541, 2010.
- Kozłowski, T. T. and Pallardy, S. G. (Eds.): *Physiology of woody plants*, 2nd Edn., Academic Press, San Diego, CA, USA, 1997.
- Kuptz, D., Fleischmann, F., Matyssek, R., and Grams, T. E. E.: Seasonal patterns of carbon allocation to respiratory pools in 60-year-old deciduous (*Fagus sylvatica*) and evergreen (*Picea abies*) trees assessed via whole-tree stable carbon isotope labeling, *New Phytol.*, 191, 160–172, 2011.

- Kurz-Besson, C., Otieno, D., do Vale, R. L., Siegwolf, R., Schmidt, M., Herd, A., Nogueira, C., David, T. S., David, J. S., Tenhunen, J., Pereira, J. S., and Chaves, M.: Hydraulic lift in cork oak trees in a savannah-type Mediterranean ecosystem and its contribution to the local water balance, *Plant Soil*, 282, 361–378, 2006.
- Kuzyakov, Y. and Gavrichkova, O.: Review: Time lag between photosynthesis and carbon dioxide efflux from soil: a review of mechanisms and controls, *Glob. Change Biol.*, 16, 3386–3406, 2010.
- Lai, C. T., Ehleringer, J. R., Bond, B. J., and Paw, U. K. T.: Contributions of evaporation, isotopic non-steady state transpiration and atmospheric mixing on the $\delta^{18}\text{O}$ of water vapour in Pacific Northwest coniferous forests, *Plant Cell Environ.*, 29, 77–94, 2006.
- Lakatos, M., Hartard, B., and Máguas, C.: The stable isotopes $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of lichens can be used as tracers of microenvironmental carbon and water sources, in: *Stable isotopes as indicators of ecological change*, edited by: Dawson, T. E. and Siegwolf, R. T. W., Elsevier, Oxford, 73–88, 2007.
- Lamentowicz, M., Cedro, A., Galka, M., Goslar, T., Miotk-Szpiganowicz, G., Mitchell, E. A. D., and Pawlyta, J.: Last millennium palaeoenvironmental changes from a baltic bog (poland) inferred from stable isotopes, pollen, plant macrofossils and testate amoebae, *Palaeogeogr. Palaeoclimatol.*, 265, 93–106, 2008.
- Lauteri, M., Pliura, A., Monteverdi, M. C., Brugnoli, E., Villani, F., and Eriksson, G.: Genetic variation in carbon isotope discrimination in six European populations of *Castanea sativa* Mill. originating from contrasting localities, *J. Evolution. Biol.*, 17, 1286–1296, 2004.
- Lee, X., Sargent, S., Smith, R., and Tanner, B.: In-situ measurement of water vapor $^{18}\text{O}/^{16}\text{O}$ isotope ratio for atmospheric and ecological applications, *J. Atmos. Ocean. Tech.*, 22, 555–565, 2005.
- Lee, X., Kim, K., and Smith, R.: Temporal variations of the isotopic signal of the whole-canopy transpiration in a temperate forest, *Global Biogeochem. Cy.*, 21, GB3013, doi:10.1029/2006GB002871, 2007.
- Lehmeier, C. A., Lattanzi, F. A., Schäufole, R., Wild, M., and Schnyder, H.: Root and shoot respiration of perennial ryegrass are supplied by the same substrate pools: assessment by dynamic ^{13}C labeling and compartmental analysis of tracer kinetics, *Plant Physiol.*, 148, 1148–1158, 2008.
- Lehmeier, C. A., Lattanzi, F. A., Schäufole, R., and Schnyder, H.: Nitrogen deficiency increases the residence time of respiratory carbon in the respiratory substrate supply system of perennial ryegrass, *Plant Cell Environ.*, 33, 76–87, 2010a.
- Lehmeier, C. A., Lattanzi, F. A., Gammitzer, U., Schäufole, R., and Schnyder, H.: Day-length effects on carbon stores for respiration of perennial ryegrass, *New Phytol.*, 188, 719–725, 2010b.
- Lin, G. and Ehleringer, J. E.: Carbon isotopic fractionation does not occur during dark respiration in C3 and C4 plants, *Plant Physiol.*, 114, 391–394, 1997.
- Liu, D. D., Chao, W. M., and Turgeon, R.: Transport of sucrose, not hexose, in the phloem *J. Exp. Bot.*, 63, 4315–4320, doi:10.1093/jxb/ers127, 2012.
- Loader, N. J., McCarroll, D., van der Knaap, W. O., Robertson, I., and Gagen, M.: Characterizing carbon isotopic variability in sphagnum, *The Holocene*, 17, 403–410, 2007.
- Loisel, J., Garneau, M., and Hélie, J.-F.: Sphagnum $\delta^{13}\text{C}$ values as indicators of palaeohydrological changes in a peat bog, *The Holocene*, 20, 285–291, 2010.
- Ludwig, F., Dawson, T. E., Prins, H. H. T., Berendse, F., and de Kroon, H.: Below-ground competition between trees and grasses may overwhelm the facilitative effects of hydraulic lift, *Ecol. Lett.*, 7, 623–631, 2004.
- Máguas, C. and Brugnoli, E.: Spatial variation in carbon isotope discrimination across the thalli of several lichen species, *Plant Cell Environ.*, 19, 437–446, 1996.
- Máguas, C., Rascher, K. G., Martins-Loução, A., Carvalho, P., Pinho, P., Ramos, M., Correia, O., and Werner, C.: Responses of woody species to spatial and temporal ground water changes in coastal sand dune systems, *Biogeosciences*, 8, 3823–3832, doi:10.5194/bg-8-3823-2011, 2011.
- McDowell, N. G., Bowling, D. R., Bond, B. J., Irvine, J., Law, B. E., Anthoni, P., and Ehleringer, J. R.: Response of the carbon isotopic content of ecosystem, leaf, and soil respiration to meteorological driving factors in a *Pinus ponderosa* ecosystem, *Global Biogeochem. Cy.*, 18, GB1013, doi:10.1029/2003GB002049, 2004.
- Meier-Augenstein, W.: Applied gas chromatography coupled to isotope ratio mass spectrometry, *J. Chromatogr. A.*, 842, 351–371, 1999.
- Mencuccini, M. and Hölttä, T.: The significance of phloem transport for the speed with which canopy photosynthesis and below-ground respiration are linked, *New Phytol.*, 185, 189–203, 2010.
- Merchant, A., Peuke, A. D., Keitel, C., Macfarlane, C., Warren, C. R., and Adams, M. A.: Phloem sap and leaf $\delta^{13}\text{C}$, carbohydrates, and amino acid concentrations in *Eucalyptus globulus* change systematically according to flooding and water deficit treatment, *J. Exp. Bot.*, 61, 1785–1793, 2010.
- Meyer, M., Seibt, U., and Griffiths, H.: To concentrate or ventilate? Carbon acquisition, isotope discrimination and physiological ecology of early land plant life forms, *Philos. T. Roy. Soc. B.*, 363, 2767–2778, 2008.
- Monserud, R. A. and Marshall, J. D.: Time-series analysis of $\delta^{13}\text{C}$ from tree rings, I. Time trends and autocorrelation, *Tree Physiol.*, 21, 1087–1102, 2001.
- Mortazavi, B., Chanton, J. P., and Smith, M. C.: Influence of ^{13}C -enriched foliage respired CO_2 on $\delta^{13}\text{C}$ of ecosystem-respired CO_2 , *Global Biogeochem. Cy.*, 20, 3029–3038, 2006.
- Moschen, R., Kühl, N., Rehberger, I., and Lücke, A.: Stable carbon and oxygen isotopes in sub-fossil sphagnum: Assessment of their applicability for palaeoclimatology, *Chem. Geol.*, 259, 262–272, 2009.
- Müller, M., Alewell, C., and Hagedorn, F.: Effective retention of litter-derived dissolved organic carbon in organic layers, *Soil Biol. Biochem.*, 41, 1066–1074, 2009.
- Nickerson, N. and Risk, D.: A numerical evaluation of chamber methodologies used in measuring the $\delta^{13}\text{C}$ of soil respiration, *Rapid Commun. Mass Sp.*, 23, 2802–2810, 2009.
- Nock, C. A., Baker, P. J., Wanek, W., Leis, A., Grabner, M., Bunyavejchevin, S., and Hietz, P.: Long-term increases in intrinsic water-use efficiency do not lead to increased stem growth in a tropical monsoon forest in western Thailand, *Glob. Change Biol.*, 17, 1049–1063, doi: 10.1111/j.1365-2486.2010.02222.x, 2010.
- Nogués, S., Tcherkez, G., Cornic, G., and Ghashghaie, J.: Respiratory carbon metabolism following illumination in intact french bean leaves using $^{13}\text{C}/^{12}\text{C}$ isotope labelling, *Plant Physiol.*, 136, 3245–3254, 2004.

- Offermann, C., Ferrio, J. P., Holst, J., Grote, R., Siegwolf, R., Kayler, Z., and Gessler, A.: The long way down—are carbon and oxygen isotope signals in the tree ring uncoupled from canopy physiological processes?, *Tree Physiol.*, 31, 1088–1102, 2011.
- Ogée, J., Cuntz, M., Peylin, P., and Bariac, T.: Non-steady-state, non-uniform transpiration rate and leaf anatomy effects on the progressive stable isotope enrichment of leaf water along monocot leaves, *Plant Cell Environ.*, 30, 367–387, 2007.
- Ogée, J., Barbour, M., Wingate, L., Bert, D., Bosc, A., Stievenard, M., Lambrot, C., Pierre, M., Bariac, T., and Loustau, D.: A single-substrate model to interpret intra-annual stable isotope signals in tree-ring cellulose, *Plant Cell Environ.*, 32, 1071–1090, 2009.
- Ogle, K. and Reynolds, J. F.: Plant responses to precipitation in desert ecosystems: integrating functional types, pulses, thresholds, and delays, *Oecologia*, 141, 282–294, 2004.
- Ogle, K., Wolpers, R., and Reynolds, J. F.: Reconstructing plant root area and water uptake profiles, *Ecology*, 85, 1967–1978, 2004.
- Ohlsson, K. E. A.: Reduction of bias in static closed chamber measurement of $\delta^{13}\text{C}$ in soil CO_2 efflux, *Rapid Commun. Mass Sp.*, 24, 180–184, 2009.
- Park, R. and Epstein, S.: Metabolic fractionation of ^{13}C and ^{12}C in plants, *Plant Physiol.*, 36, 133–138, 1961.
- Parnell, A. C., Inger, R., Bearhop, S., and Jackson, A. L.: Source partitioning using stable isotopes: coping with too much variation, *PLoS One*, 5, e9672, doi:10.1371/journal.pone.0009672, 2010.
- Pate, J. and Arthur, D.: $\delta^{13}\text{C}$ analysis of phloem sap carbon: novel means of evaluating seasonal water stress and interpreting carbon isotope signatures of foliage and trunk wood of *Eucalyptus globules*, *Oecologia*, 117, 301–311, 1998.
- Peñuelas, J. and Azcón-Bieto, J.: Changes in leaf $\Delta^{13}\text{C}$ of herbarium plant species during the last 3 centuries of CO_2 increase, *Plant Cell Environ.*, 15, 485–489, 1992.
- Peñuelas, J., Canadell, J. G., and Ogaya, R.: Increased water-use efficiency during the 20th century did not translate into enhanced tree growth, *Global Ecol. Biogeogr.*, 20, 597–608, 2011.
- Phillips, D. L.: Mixing models in analyses of diet using multiple stable isotopes: a critique, *Oecologia*, 127, 166–170, 2001.
- Phillips, D. L. and Gregg, J. W.: Uncertainty in source partitioning using stable isotopes, *Oecologia*, 127, 171–179, 2001.
- Phillips, D. L. and Gregg, J. W.: Source partitioning using stable isotopes: coping with too many sources, *Oecologia*, 136, 261–269, 2003.
- Ponton, S., Flanagan, L. B., Alstad, K. P., Johnson, B. G., Morgenstern, K., Kljun, N., Black, T. A., and Barr, A. G.: Comparison of ecosystem water-use efficiency among Douglas-fir forest, aspen forest and grassland using eddy covariance and carbon isotope techniques, *Glob. Change Biol.*, 12, 294–310, 2006.
- Powers, E. M. and Marshall, J. D.: Pulse labeling of dissolved ^{13}C -carbonate into tree xylem: developing a new method to determine the fate of recently fixed photosynthate, *Rapid Commun. Mass Sp.*, 25, 33–40, 2011.
- Priault, P., Wegener, F., and Werner, C.: Pronounced differences in diurnal variation of carbon isotope composition of leaf respired CO_2 among functional groups, *New Phytol.*, 181, 400–412, doi:10.1111/j.1469-8137.2008.02665.x, 2009.
- Prieto, I., Armas, C., and Pugnaire, F.: Water release through plant roots: new insights into its consequences at the plant and ecosystem level, *New Phytol.*, 193, 830–841, 2012.
- Ramírez, D. A., Querejeta, J. I., and Bellot, J.: Bulk leaf $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ reflect the intensity of intraspecific competition for water in a semi-arid tussock grassland, *Plant Cell Environ.*, 32, 1346–1356, 2009.
- Ramirez, D. A., Parra, A., de Dios, V. R., and Moreno, J. M.: Differences in morpho-physiological leaf traits reflect the response of growth to drought in a seeder but not in a resprouter Mediterranean species, *Funct. Plant Biol.*, 39, 332–341, 2012.
- Rascher, K. G., Máguas, C., and Werner, C.: On the use of phloem sap $\delta^{13}\text{C}$ as an indicator of canopy carbon discrimination, *Tree Physiol.*, 13, 1499–1514, doi:10.1093/treephys/tpq092, 2010.
- Rascher, K. G., Große-Stoltenberg, A., Máguas, C., and Werner, C.: Understorey invasion by *Acacia longifolia* alters the water balance and carbon gain of a Mediterranean pine forest, *Ecosystems*, 14, 904–919, doi:10.1007/s10021-011-9453-7, 2011.
- Rascher, K. G., Hellmann, C., Máguas, C., and Werner, C.: Community scale ^{15}N isoscapes: tracing the spatial impact of an exotic N_2 -fixing invader, *Ecol. Lett.*, 15, 484–491, 2012.
- Reichstein, M., Falge, E., Baldocchi, D., Papale, D., Aubinet, M., Berbigier, P., Bernhofer, C., Buchmann, N., Gilmanov, T., Granier, A., Grunwald, T., Havrankova, K., Ilvesniemi, H., Janous, D., Knohl, A., Laurila, T., Lohila, A., Loustau, D., Matteucci, G., Meyers, T., Miglietta, F., Ourcival, J. M., Pumpanen, J., Rambal, S., Rotenberg, E., Sanz, M., Tenhunen, J., Seufert, G., Vaccari, F., Vesala, T., Yakir, D., and Valentini, R.: On the separation of net ecosystem exchange into assimilation and ecosystem respiration: review and improved algorithm, *Glob. Change Biol.*, 11, 1424–1439, 2005.
- Richter, A., Wanek, W., Werner, R. A., Ghashghaie, J., Jaggi, M., Gessler, A., Brugnoli, E., Hettmann, E., Gottlicher, S. G., Salmon, Y., Bathellier, C., Kodama, N., Nogues, S., Soe, A., Volders, F., Sorgel, K., Blochl, A., Siegwolf, R. T. W., Buchmann, N., and Gleixner, G.: Preparation of starch and soluble sugars of plant material for the analysis of carbon isotope composition: a comparison of methods, *Rapid Commun. Mass Sp.*, 23, 2476–2488, doi:10.1002/rcm.4088, 2009.
- Roscher, C., Schumacher, J., Baade, J., Wilcke, W., Gleixner, G., Weisser, W. W., Schmid, B., and Schulze, E. D.: The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community, *Basic Appl. Ecol.*, 5, 107–121, 2004.
- Rose, K. L., Graham, R. C., and Parker, D. R.: Water source utilization by *Pinus jeffreyi* and *Actostaphylos patula* on thin soils over bedrock, *Oecologia*, 134, 46–54, 2003.
- Rossmann, A., Butzenlechner, M., and Schmidt, H. L.: Evidence for a nonstatistical carbon isotope distribution in natural glucose, *Plant Physiol.*, 96, 609–614, 1991.
- Rothfuss, Y., Biron, P., Braud, I., Canale, L., Durand, J.-L., Gaudet, J.-P., Richard, P., Vauclin, M., and Bariac, T.: Partitioning evapotranspiration fluxes into soil evaporation and plant transpiration using water stable isotopes under controlled conditions, *Hydrol. Process.*, 24, 3177–3194, 2010.
- Rozanski, K., Araguds-Araguds, L., and Gonfiantini, R.: Isotopic patterns in modern global precipitation, in: *Climate Change in Continental Isotopic Records – Geophysical Monograph 78*, edited by: Swart, P. K., Lohman, K. C., McKenzie, J., and Savin, S., American Geophysical Union, Washington, DC, 1–36, 1993.
- Rubino, M., Dungait, J. A. J., Evershed, R. P., Bertolini, T., De Angelis, P., D’Onofrio, A., Lagomarsino, A., Lubritto, C., Merola,

- A., Terrasi, F., and Cotrufo, M. F.: Carbon input belowground is the major C flux contributing to leaf litter mass loss: Evidences from a ^{13}C labelled-leaf litter experiment, *Soil Biol. Biochem.*, 42, 1009–1016, 2010.
- Ryel, R. J., Caldwell, M. M., Leffler, A. J., and Yoder, C. K.: Rapid soil moisture recharge to depth by roots in a stand of *Artemisia tridentata*, *Ecology*, 84, 757–764, 2003.
- Sachse, D., Billault, I., Bowen, G. J., Chikaraishi, Y., Dawson, T. E., Feakins, S. J., Freeman, K. H., Magill, C. R., McInerney, F. A., van der Meer, M. T. J., Polissar, P., Robins, R. J., Sachs, J. P., Schmidt, H.-L., Sessions, A. L., White, J. W. C., West, J. B., and Kahmen, A.: Molecular paleohydrology: interpreting the hydrogen isotopic composition of lipid biomarkers from photosynthetic organisms, *Annu. Rev. Earth Planet. Sci.*, 40, 221–249, 2012.
- Salmon, Y., Barnard, R., and Buchmann, N.: Ontogeny and leaf gas exchange mediate the carbon isotopic signature of herbaceous plants through large non-photosynthetic discrimination, *Plant Cell Environ.*, 34, 465–479, 2011.
- Sanderman, J. and Amundson, R.: A comparative study of dissolved organic carbon transport and stabilization in California forest and grassland soils, *Biogeochemistry*, 89, 309–327, doi:10.1007/s10533-008-9221-8, 2008.
- Santos, E. A., Wagner-Riddle, C., Lee, X., Warland, J. S., Brown, S. E., Staebler, R. M., Bartlett, P. A., and Kim, K.: ^{13}C and ^{18}O temporal variation near the ground and above a temperate deciduous forest, AGU Fall Meeting, San Francisco, Ca, 13–17 Dec, B12B-03, 2010.
- Saurer, M. and Siegwolf, R. T. W.: Human impacts on tree-ring growth reconstructed from stable isotopes, in: *Stable Isotopes as Indicators of Ecological Change*, Terrestrial Ecology Series, edited by: Dawson, T. E. and Siegwolf, R. T. W., Elsevier, Amsterdam, Boston, 49–62, 2007.
- Saurer, M., Borella, S., Schweingruber, F., and Siegwolf, R.: Stable carbon isotopes in tree rings of beech: climatic versus site-related influences, *Trees-Struct. Funct.*, 11, 291–297, 1997.
- Saurer, M., Siegwolf, R. T. W., and Schweingruber, F.: Carbon isotope discrimination indicates improving water-use efficiency of trees in northern Eurasia over the last 100 years, *Glob. Change Biol.*, 10, 2109–2120, 2004.
- Savard, M. M.: Tree-ring stable isotopes and historical perspectives on pollution – An overview, *Environ. Pollut.*, 158, 2007–2013, 2010.
- Scartazza, A., Mata, C., Matteucci, G., Yakir, D., Moscatello, S., and Brugnoli, E.: Comparisons of $\delta^{13}\text{C}$ of photosynthetic products and ecosystem respiratory CO_2 and their responses to seasonal climate variability, *Oecologia*, 140, 340–351, 2004.
- Schaeffer, S. M., Anderson, D. E., Burns, S. P., Monson, R. K., Sun, J., and Bowling, D. R.: Canopy structure and atmospheric flows in relation to the $\delta^{13}\text{C}$ of respired CO_2 in a subalpine coniferous forest, *Agr. Forest Meteorol.*, 148, 592–605, 2008.
- Schaub, M. and Alewell, C.: Stable carbon isotopes as an indicator for soil degradation in an alpine environment (Urseren Valley, Switzerland), *Rapid Commun. Mass Sp.*, 23, 1499–1507, doi:10.1002/rcm.4030, 2009.
- Schäufele, R., Santrucek, J., and Schnyder, H.: Dynamic changes of canopy-scale mesophyll conductance to CO_2 diffusion of sunflower as affected by CO_2 concentration and abscisic acid, *Plant Cell Environ.*, 34, 127–136, doi:10.1111/j.1365-3040.2010.02230.x, 2011.
- Scheidegger, Y., Saurer, M., Bahn, M., and Siegwolf, R.: Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: A conceptual model, *Oecologia*, 125, 350–357, 2000.
- Schmidt, H. L.: Fundamentals and systematics of the non-statistical distributions of isotopes in natural compounds, *Naturwissenschaften*, 90, 537–552, 2003.
- Schmidt, H. L. and Gleixner, G.: Carbon isotope effects on key reactions in plant metabolism and ^{13}C -patterns in natural compounds, in: *Stable Isotopes: Integration of Biological and Geochemical Processes*, edited by: Griffiths, H., BIOS Scientific Publishers, Oxford, UK, 13–25, 1998.
- Schmidt, M., Maseyk, K., Lett, C., Biron, P., Richard, P., Bariac, T., and Seibt, U.: Concentration effects on laser-based $\delta^{18}\text{O}$ and $\delta^2\text{H}$ measurements and implications for the calibration of vapour measurements with liquid standards, *Rapid Commun. Mass Sp.*, 24, 3553–3561, 2010.
- Schnyder, H., Schäufele, R., Lötscher, M., and Gebbing, T.: Disentangling CO_2 fluxes: direct measurements of mesocosm-scale natural abundance $^{13}\text{CO}_2/^{12}\text{CO}_2$ gas exchange, ^{13}C discrimination, and labelling of CO_2 exchange flux components in controlled environments, *Plant Cell Environ.*, 26, 1863–1874, 2003.
- Schnyder, H., Schäufele, R., and Wenzel, R.: Mobile, outdoor continuous-flow isotope-ratio mass spectrometer system for automated high-frequency ^{13}C - and ^{18}O - CO_2 analysis for Keeling plot applications, *Rapid Commun. Mass Sp.*, 18, 3068–3074, 2004.
- Schnyder, H., Schwertl, M., Auerswald, K., and Schäufele, R.: Hair of grazing cattle provides an integrated measure of the effects of site conditions and interannual weather variability on $\delta^{13}\text{C}$ of temperate humid grassland, *Glob. Change Biol.*, 12, 1315–1329, 2006.
- Schultz, N. M., Griffis, T. J., Lee, X., and Baker, J. M.: Identification and correction of spectral contamination in $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$ measured in leaf, stem, and soil water, *Rapid Commun. Mass Sp.*, 25, 3360–3368, 2011.
- Seibt, U., Rajabi, A., Griffiths, H., and Berry, J.: Carbon isotopes and water use efficiency: sense and sensitivity, *Oecologia*, 155, 441–454, 2008.
- Sidorova, O. V., Siegwolf, R. T. W., Saurer, M., Shashkin, A. V., Knorre, A. A., Prokushkin, A. S., Vaganov, E. A., and Kiryanov, A. V.: Do centennial tree-ring and stable isotope trends of *Larix gmelinii* (Rupr.) Rupr. indicate increasing water shortage in the Siberian north?, *Oecologia*, 161, 825–835, 2009.
- Siegwolf, R., Matyssek, R., Saurer, M., Günthardt-Goerg, M. S., Schmutz, P., and Bucher, J. B.: Stable isotope analysis reveals differential effects of soil nitrogen and nitrogen dioxide on the water use efficiency in hybrid poplar leaves, *New Phytol.*, 149, 233–246, 2001.
- Sjostrom, D. and Welker, J. M.: Storm-track trajectories control the isotope geochemistry of precipitation in the Eastern US, *Exploration Geology-Isobalance*, Special Issue, 102, 103–112, 2009.
- Smedley, M. P., Dawson, T. E., Comstock, J. P., Donovan, L. A., Sherrill, D. E., Cook, C. S., and Ehleringer, J. R.: Seasonal carbon isotope discrimination in a grassland community, *Oecologia*, 85, 314–320, 1991.
- Snyder, K. and Williams, D. G.: Water sources used by riparian trees varies among stream types on the San Pedro river, Arizona, *Agr.*

- Forest Meteorol., 105, 227–240, 2000.
- Steinmann, K. T. W., Siegwolf, R., Saurer, M., and Korner, C.: Carbon fluxes to the soil in a mature temperate forest assessed by ^{13}C isotope tracing, *Oecologia*, 141, 489–501, 2004.
- Sternberg, L. D. and DeNiro, M. J.: Biogeochemical implications of the isotopic equilibrium fractionation factor between the oxygen atoms of acetone and water, *Geochim. Cosmochim. Ac.*, 41, 2271–2274, 1983.
- Sternberg, L. D., Pinzon, M. C., Anderson, W. T., and Jahren, A. H.: Variation in oxygen isotope fractionation during cellulose synthesis: intramolecular and biosynthetic effects, *Plant Cell Environ.*, 29, 1881–1889, 2006.
- Stratton, L. C., Goldstein, G., and Meinzer, F. C.: Temporal and spatial partitioning of water resources among eight woody species in a Hawaiian dry forest, *Oecologia*, 124, 309–317, 2000.
- Sturm, P. and Knohl, A.: Water vapor $\delta^2\text{H}$ and $\delta^{18}\text{O}$ measurements using off-axis integrated cavity output spectroscopy, *Atmos. Meas. Tech.*, 3, 67–77, doi:10.5194/amt-3-67-2010, 2010.
- Sturm, P., Eugster, W., and Knohl, A.: Eddy covariance measurements of CO_2 isotopologues with a quantum cascade laser absorption spectrometer, *Agr. Forest Meteorol.*, 152, 73–82, 2012.
- Sullivan, P. F. and Welker, J. M.: Variation in leaf physiology of *Salix arctica* within and across ecosystems in the High Arctic: test of a dual $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ conceptual model, *Oecologia*, 151, 372–386, 2007.
- Tcherkez, G., Farquhar, G., Badeck, F., and Ghashghaie, J.: Theoretical considerations about carbon isotope distribution in glucose of C_3 -plants, *Funct. Plant Biol.*, 31, 857–877, 2004.
- Tcherkez, G., Mahé, A., and Hodges, M.: $^{12}\text{C}/^{13}\text{C}$ fractionations in plant primary metabolism, *Trends Plant Sci.*, 16, 499–506, doi:10.1016/j.tplants.2011.05.010, 2011.
- Tcherkez, G., Nogues, S., Bleton, J., Cornic, G., Badeck, F., and Ghashghaie, J.: Metabolic origin of carbon isotope composition of leaf dark-respired CO_2 in French bean, *Plant Physiol.*, 131, 237–244, 2003.
- Tcherkez, G., Schäufele, R., Nogués, S., Piel, C., Boom, A., Lanigan, G., Barbaroux, C., Mata, C., Elhani, S., Hemming, D., Máguas, C., Yakir, D., Badeck, F., Griffiths, H., Schnyder, H., and Ghashghaie, J.: On the $^{13}\text{C}/^{12}\text{C}$ isotopic signal of day and night respiration at the mesocosm level, *Plant Cell Environ.*, 33, 900–913, 2010.
- Terwilliger, V.: Changes in the ^{13}C values of trees during a tropical rainy season: some effects in addition to diffusion and carboxylation by Rubisco?, *Am. J. Bot.*, 84, 1693–1700, 1997.
- Terwilliger, V. J., Kitajima, K., Le Roux-Swarthout, D. J., Mulkey, S., and Wright, S. J.: Intrinsic water-use efficiency and heterotrophic investment in tropical leaf growth of two neotropical pioneer tree species as estimated from $\delta^{13}\text{C}$ values, *New Phytol.*, 152, 267–281, 2001.
- Thompson, M. V. and Holbrook, N. M.: Scaling phloem transport: information transmission, *Plant Cell Environ.*, 27, 509–519, 2004.
- Tsialtas, J. T., Handley, L. L., Kassioumi, M. T., Veresoglou, D. S., and Gagianas, A. A.: Interspecific variation in potential water-use efficiency and its relation to plant species abundance in a water-limited grassland, *Funct. Ecol.*, 15, 605–614, 2001.
- Tuzson, B., Mohn, J., Zeeman, M. J., Werner, R. A., Eugster, W., Zahniser, M. S., Nelson, D. D., McManus, J. B., and Emmenegger, L.: High precision and continuous field measurements of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in carbon dioxide with a cryogen-free QCLAS, *Appl. Phys. B.-Lasers O.*, 92, 451–458, 2008.
- Tuzson, B., Henne, S., Brunner, D., Steinbacher, M., Mohn, J., Buchmann, B., and Emmenegger, L.: Continuous isotopic composition measurements of tropospheric CO_2 at Jungfraujoch (3580 m a.s.l.), Switzerland: real-time observation of regional pollution events, *Atmos. Chem. Phys.*, 11, 1685–1696, doi:10.5194/acp-11-1685-2011, 2011.
- Ubierna, N. and Marshall, J. D.: Estimation of canopy average mesophyll conductance using $\delta^{13}\text{C}$ of phloem contents, *Plant Cell Environ.*, 34, 1521–1535, 2011.
- Ubierna, N., Kumar, A. S., Cernusak, L. A., Pangle, R. E., Gag, P. J., and Marshall, J. D.: Storage and transpiration have negligible effects on $\delta^{13}\text{C}$ of stem CO_2 efflux in large conifer trees, *Tree Physiol.*, 29, 1563–1574, doi:10.1093/treephys/tpp089, 2009.
- Unger, S., Máguas, C., Pereira, J. S., Aires, L. M., David, T. S., and Werner, C.: Disentangling drought-induced variation in ecosystem and soil respiration by stable isotope partitioning, *Oecologia*, 163, 1043–1057, doi:10.1007/s00442-010-1576-6, 2010a.
- Unger, S., Máguas, C., Pereira, J. S., David, T. S. and Werner, C.: The influence of precipitation pulses on soil respiration – Assessing the “Birch effect” by stable carbon isotopes, *Soil Biol. Biochem.*, 42, 1800–1810, doi:10.1016/j.soilbio.2010.06.01, 2010b.
- Unger, S., Máguas, C., Pereira, J. S., David, T. S., and Werner, C.: Interpreting post-drought rewetting effects on soil and ecosystem carbon dynamics in a Mediterranean oak savannah, *Agr. Forest Meteorol.*, 154–155, 9–18, doi:10.1016/j.agrformet.2011.10.007, 2012.
- Vachon, R., White, J. W., Welker, J. M., and Gutman, E.: Seasonality of precipitation affects the annual isotopic ($\delta^{18}\text{O}$) values across the United States: a sensitivity study, *Geophys. Res. Lett.*, 34, L21707, doi:10.1029/2007GL030547, 2007.
- Vachon, R. W., Welker, J. M., White, J. W. C., and Vaughn, B. H.: Monthly precipitation isoscapes ($\delta^{18}\text{O}$) of the United States: Connections with surface temperatures, moisture source conditions, and air mass trajectories, *J. Geophys. Res.*, 115, D21126, doi:10.1029/2010JD014105, 2010.
- Van Bel, A. J. E. and Hess, P. H.: Hexoses as phloem transport sugars: the end of a dogma?, *J. Exp. Bot.*, 59, 261–272, 2008.
- Vargas, R., Baldocchi, D. D., Bahn, M., Hanson, P. J., Hosman, K. P., Kulmala, L., Pumpanen, J., and Yang, B.: On the multi-temporal correlation between photosynthesis and soil CO_2 efflux: reconciling lags and observations, *New Phytol.*, 191, 1006–1017, 2011.
- Von Caemmerer, S. and Farquhar, G. D.: Some relationships between the biochemistry of photosynthesis and the gas-exchange of leaves, *Planta*, 153, 376–387, 1981.
- Wang, L. X., Caylor, K. K., Villegas, J. C., Barron-Gafford, G. A., Breshears, D. D., and Huxman, T. E.: Partitioning evapotranspiration across gradients of woody plant cover: Assessment of a stable isotope technique, *Geophys. Res. Lett.*, 37, L09401, doi:10.1029/2010GL043228, 2010.
- Wang, X. and Yakir, D.: Temporal and spatial variations in the oxygen-18 content of leaf water in different plant species, *Plant Cell Environ.*, 18, 1377–1385, 1995.
- Warren, C. R. and Adams, M. A.: Internal conductance does not scale with photosynthetic capacity: implications for carbon isotope discrimination and the economics of water and nitrogen use

- in photosynthesis, *Plant Cell Environ.*, 29, 192–201, 2006.
- Warren, J. M., Iversen, C. M., Garten, C. T., Norby, R. J., Childs, J., Brice, D., Evans, R. M., Gu, L., Thornton, P., and Weston, D. J.: Timing and magnitude of C partitioning through a young loblolly pine (*Pinus taeda* L.) stand using ^{13}C labeling and shade treatments, *Tree Physiol.*, 32, 799–813, 2012.
- Wegener, F., Beyschlag, W., and Werner, C.: The magnitude of diurnal variation in carbon isotopic composition of leaf dark respired CO_2 correlates with the difference between $\delta^{13}\text{C}$ of leaf and root material, *Funct. Plant Biol.*, 37, 849–858, doi:10.1071/FP09224, 2010.
- Welker, J. M.: Isotopic ($\delta^{18}\text{O}$) characteristics of weekly precipitation collected across the United States: An initial analysis with application to water source studies, *Hydrol. Process.*, 14, 1449–1464, 2000.
- Welker, J. M., Rayback, S., and Henry, G.: Arctic and North Atlantic Oscillation phase changes are recorded in the isotopes ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) of *Cassiope tetragona* plants, *Glob. Change Biol.*, 11, 997–1002, 2005.
- Welp, L. R., Lee, X., Kim, K., Griffis, T. J., Billmark, K. A., and Baker, J. M.: $\delta^{18}\text{O}$ of water vapour, evapotranspiration and the sites of leaf water evaporation in a soybean canopy, *Plant Cell Environ.*, 31, 1214–1228, 2008.
- Wen, X. F., Sun, X. M., Zhang, S. C., Yu, G. R., Sargent, S. D., and Lee, X.: Continuous measurement of water vapor D/H and $^{18}\text{O}/^{16}\text{O}$ isotope ratios in the atmosphere, *J. Hydrol.*, 349, 489–500, 2008.
- Werle, P., Mazzinghi, P., D'Amato, F., Rosa, M. D., Maurer, K., and Slemr, F.: Signal processing and calibration procedures for in situ diode-laser absorption spectroscopy, *Spectrochim. Acta A*, 60, 1685–1705, 2004.
- Werner, C. and Gessler, A.: Diel variations in the carbon isotope composition of respired CO_2 and associated carbon sources: a review of dynamics and mechanisms, *Biogeosciences*, 8, 2437–2459, doi:10.5194/bg-8-2437-2011, 2011.
- Werner, C. and Máguas, C.: Carbon isotope discrimination as a tracer of functional traits in a Mediterranean macchia plant community, *Funct. Plant Biol.*, 37, 467–477, doi:10.1071/FP09081, 2010.
- Werner, C., Unger, S., Pereira, J. S., Maia, R., David, T. S., Kurz-Besson, C., David, J. S., and Máguas, C.: Importance of short-term dynamics in carbon isotope ratios of ecosystem respiration ($\delta^{13}\text{C}_R$) in a Mediterranean oak woodland and linkage to environmental factors, *New Phytol.*, 172, 330–346, doi:10.1111/j.1469-8137.2006.01836.x, 2006.
- Werner, C., Wegener, F., Unger, S., Nogués, S., and Priault, P.: Short-term dynamics of isotopic composition of leaf respired CO_2 upon darkening: measurements and implications, *Rapid Commun. Mass Sp.*, 23, 2428–2438, doi:10.1002/rcm.4036, 2009.
- Werner, R. A., Buchmann, N., Siegwolf, R. T. W., Kornel, B. E., and Gessler, A.: Metabolic fluxes, carbon isotope fractionation and respiration – lessons to be learned from plant biochemistry, *New Phytol.*, 191, 10–15, 2011.
- West, A. G., Goldsmith, G. R., Brooks, P. D., and Dawson, T. E.: Discrepancies between isotope ratio infrared spectroscopy and isotope ratio mass spectrometry for the stable isotope analysis of plant and soil waters, *Rapid Commun. Mass Sp.*, 24, 1948–1954, 2010.
- West, J. B., Sobek, A., and Ehleringer, J. R.: A simplified GIS approach to modeling global leaf water isoscapes, *PLoS one*, 3, e2447, doi:10.1371/journal.pone.0002447, 2008.
- Williams, D. G., Cable, W., Hultine, K., Hoedjes, J. C. B., Yepez, E. A., Simonneaux, V., Er-Raki, S., Boulet, G., de Bruin, H. A. R., Chehbouni, A., Hartogensis, O. K., and Timouk, F.: Evapotranspiration components determined by stable isotope, sap flow and eddy covariance techniques, *Agr. Forest Meteorol.*, 125, 241–258, 2004.
- Wingate, L., Seibt, U., Moncrieff, J., Lloyd, J., and Berry, J.: Variations in ^{13}C discrimination during CO_2 exchange in *Picea sitchensis* branches in the field, *Plant Cell Environ.*, 30, 600–616, doi:10.1111/j.1365-3040.2007.01647.x, 2007.
- Wingate, L., Ogee, J., Cuntz, M., Genty, B., Reiter, I., Seibt, U., Yakir, D., Maseyk, K., Pendall, E. G., Barbour, M. M., Mortazavi, B., Burlett, R., Peylin, P., Miller, J., Mencuccini, M., Shim, J. H., Hunt, J., and Grace, J.: The impact of soil microorganisms on the global budget of $\delta^{18}\text{O}$ in atmospheric CO_2 , *P. Natl. Acad. Sci. USA*, 106, 22411–22415, 2009.
- Wingate, L., Ogee, J., Burlett, R. and Bosc, A.: Strong seasonal disequilibrium measured between the oxygen isotope signals of leaf and soil CO_2 exchange, *Glob. Change Biol.*, 16, 3048–3064, doi:10.1111/j.1365-2486.2010.02186.x, 2010a.
- Wingate, L., Ogee, J., Burlett, R., Bosc, A., Devaux, M., Grace, J., Loustau, D., and Gessler, A.: Photosynthetic carbon isotope discrimination and its relationship to the carbon isotope signals of stem, soil and ecosystem respiration, *New Phytol.*, 188, 576–589, 2010b.
- Wittmer, M. H. O. M., Auerswald, K., Schönbach, P., Schäufele, R., Müller, K., Yang, H., Bai, Y. F., Susenbeth, A., Taube, F., and Schnyder, H.: Do grazer hair and faeces reflect the carbon isotope composition of semi-arid C_3/C_4 grassland?, *Basic Appl. Ecol.*, 11, 83–92, 2010.
- Xu, L., Baldocchi, D. D., and Tang, J.: How soil moisture, rain pulses, and growth alter the response of ecosystem respiration to temperature, *Global Biogeochem. Cy.*, 18, GB4002, doi:10.1029/2004GB002281, 2004.
- Yakir, D.: Oxygen-18 of leaf water: a crossroad for plant-associated isotopic signals, in: *Stable Isotopes: Integration of Biological and Geochemical Processes*, edited by: Griffiths, H., BIOS Scientific Publishers, Oxford, 147–168, 1998.
- Yakir, D.: The stable isotopic composition of atmospheric CO_2 , *Treatise on Geochemistry*, 4, 175–212, 2003.
- Yakir, D. and DeNiro, M. J.: Oxygen and hydrogen isotope fractionation during cellulose metabolism in *Lemna gibba* L., *Plant Physiol.*, 93, 325–332, 1990.
- Yakir, D. and Sternberg, L.: The use of stable isotopes to study ecosystem gas exchange, *Oecologia*, 123, 297–311, 2000.
- Yakir, D. and Wang, X.-F.: Fluxes of CO_2 and water between terrestrial vegetation and the atmosphere estimated from isotope measurements, *Nature*, 380, 515–517, 1996.
- Yepez, E. A., Huxman, T. E., Ignace, D. D., English, N. B., Weltzin, J. F., Castellanos, A. E. and Williams, D. G.: Dynamics of transpiration and evaporation following a moisture pulse in semiarid grassland: A chamber-based isotope method for partitioning flux components, *Agr. Forest Meteorol.*, 132, 359–376, 2005.
- Yepez, E. A., Scott, R. L., Cable, W. L., and Williams, D. G.: Intra-seasonal variation in water and carbon dioxide flux components in a semiarid riparian woodland, *Ecosystems*, 10, 1100–

- 1115, 2007.
- Yoneyama, T., Handley, L. L., Scrimgeour, C. M., Fisher, D. B., and Raven, J. A.: Variations of the natural abundances of nitrogen and carbon isotopes in *Triticum aestivum*, with special reference to phloem and xylem exudates, *New Phytol.*, 137, 205–213, 1997.
- Zhao, F. J., Spiro, B., and McGrath, S. P.: Trends in $^{13}\text{C}/^{12}\text{C}$ ratios and C isotope discrimination of wheat since 1845, *Oecologia*, 128, 336–342, 2001.