Linking carbon and water cycles using stable isotopes across scales: progress and challenges

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Linking carbon and water cycles using stable isotopes across scales: progress and challenges

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Abstract

Stable isotope analysis is a powerful tool for tracing biogeochemical processes in the carbon and water cycles. One particularly powerful approach is to employ multiple isotopes where the simultaneous assessment of the D/H,$^{18}O/^{16}O$ and/or $^{13}C/^{12}C$ in different compounds provide a unique means to investigate the coupling of water and carbon fluxes at various temporal and spatial scales. Here, we present a research update on recent advances in our process-based understanding of the utilization of carbon, oxygen and hydrogen isotopes to lend insight into carbon and water cycling. We highlight recent technological developments and approaches, their strengths and methodological precautions with examples covering scales from minutes to centuries and from the leaf to the globe.

1 Introduction

Global climate change will alter water availability in many ecosystems worldwide with marked impact on biogeochemical cycles, as water represents one of the key factors constraining ecosystem productivity. Thus, a mechanistic understanding of the linkage between carbon and water cycles within the soil-plant-atmosphere continuum is needed to identify past and future climate and land-use change effects on ecosystem functioning (Heimann and Reichstein, 2008).

Stable isotopes (expressed in δ-notation as the ratio of the heavy to the light isotope of an element relative to the ratio of an international standard, in ‰) are a powerful tool for tracing biogeochemical processes (Yakir and Sternberg, 2000).

The terrestrial carbon and water cycles are coupled at the leaf scale via the stomata by the bidirectional CO_2 and H_2O diffusive exchange during photosynthesis and transpiration and through the soil via soil pores during soil respiration and soil evaporation. During photosynthesis, transpiration, heterotrophic and autotrophic soil respiration and evaporation, isotopic fractionation between $^{13}C/^{12}C$, $^{18}O/^{16}O$ and $^{2}H/^{1}H$,

leave an isotopic imprint on the soil, plant and atmospheric carbon and water pools and associated fluxes which can be traced through the plant-soil-atmosphere continuum. The multiple-isotope approach, i.e. the simultaneous measurements of stable isotope composition of different elements (δ^2H, δ^{18}O and/or δ^{13}C), provides a unique concept to investigate the coupling of the water and carbon fluxes, which has markedly advanced our understanding on the underlying processes at various temporal and spatial scales. Furthermore, the use of biological archives enables extrapolation of this information to longer time scales, such as the recent era of the Anthropocene. Moreover, recent methodological advances allow isotopologue and compound-specific analyses at higher temporal resolutions than ever before, providing new insight into isotope fractionation processes in metabolic pathways and opening new frontiers in ecological and interdisciplinary research across all scales of earth system science.

Here, we present an update on recent research on how the use of carbon and oxygen isotope information is extending our knowledge about water and carbon cycling in diverse ways. We highlight recent insights in δ^{13}C and δ^{18}O isotope fractionation at the leaf level (2.1: leaf gas-exchange (2.1.1), post-photosynthetic processes (2.1.2), and bulk leaf isotope ratios (2.1.3)). We discuss strength and limitations of the use of isotope information of phloem sap to trace plant integrated processes (2.2.1), followed by a brief outlook on plant-soil interaction (2.2.2). The utilization of isotope tracers at the community scales is highlighted in Sect. 2.3 (i.e. for identifying functional plant groups (2.3.1) and water source partitioning (2.3.2)), followed by a critical survey on its application for ecosystem carbon and water fluxes (Sect. 2.4). We survey time-integrated information in different isotope archives (with examples for tree rings, grasslands, animal tissue of grazers and non-vascular plants, 2.5), and at the continental scale utilizing the example of isotope information in precipitation pattern (2.6). Finally a short critical outlook on recent use of isotopes for global-scale modeling is given (2.7). A final section (3) highlights recent technological developments, along with their strengths and methodological precautions, closing with a concluding outlook.

2 Isotope effects across temporal and spatial scales

2.1 Leaf-level processes

2.1.1 Recent advances on fractionation during leaf CO_2 and H_2O exchange

Leaf CO_2 and H_2O exchange is associated with isotopic fractionation, which has been widely used to investigate environmental effects on the physiology of photosynthesis and stomatal conductance (see Farquhar et al., 1989; Brugnoli and Farquhar 2000). Photosynthetic carbon isotope fractionation ($\Delta^{13}C$) is primarily linked to variations in the ratio of leaf intercellular and ambient CO_2 concentration (c_i/c_a) and the principle fractionations during diffusion and carboxylation (Farquhar et al., 1982). However, recent studies measuring diurnal variations in $\Delta^{13}C$ on-line at high resolution have found that fractionations during photorespiration, day respiration and internal transfer of CO_2 cause the linear relationship between $\Delta^{13}C$ and c_i/c_a to diverge at both high and low values of c_i/c_a (e.g. Wingate et al., 2007; Bickford et al., 2009). Much of this discrepancy can be resolved using the more comprehensive description of $\Delta^{13}C$ as proposed by Farquhar et al. (1982) that quantitatively accounts for the isotopic fractionation during photorespiration ($f = 11$, Lanigan et al., 2008), during CO_2 diffusion within the leaf mesophyll (e.g. Wingate et al., 2007; Schäufele et al., 2010) and dark respiration (Ghashghaie et al., 2003; see also 2.1.2). Increasing evidence indicates that the internal mesophyll conductance (g_i) may vary in response to several environmental factors (e.g. light, temperature, drought, salinity and CO_2, see Flexas et al., 2008) that are also detectable at the canopy-scale (Schäufele et al., 2010). This reversible or adaptive component appears to be associated with variations in the conductance of membranes (Schäufele et al., 2010), that may be modified by the expression of cooporins (Hanba et al., 2004; Flexas et al., 2007): aquaporins capable of transporting CO_2 across plasma membranes. Although the dynamic role of g_i at the timescale of minutes to days remains somewhat controversial it is certain that g_i will vary markedly over the season with changes in leaf morphology and amongst and between species (Warren and Adams, 2006).

Furthermore, our understanding on the contribution of day respiration to $\Delta^{13}C$ dynamics is developing. Recent studies both in the field and laboratory, using either high resolution on-line $\Delta^{13}C$ approaches (Wingate et al., 2007, 2010a; Bickford et al., 2009) or substrate labelling (Tcherkez et al., 2010), indicate that the isotopic composition of the day respiration flux is very unlikely the same as of recent photoassimilates (as was assumed in the original model of Farquhar et al., 1982). Instead, older carbon pools partially fuel this flux necessitating the need for improved models of $\Delta^{13}C$ that account for this process (Wingate et al., 2007; Tcherkez et al., 2010) when modeling dynamic isofluxes in the field and importantly when estimating g_i using $\Delta^{13}C$ approaches.

During photosynthetic CO_2 uptake there is also an exchange of oxygen isotopes between CO_2 and leaf water. This results in an oxygen isotope photosynthetic discrimination ($\Delta^{18}O$) that can be extremely dynamic as it integrates variations in the bi-directional fluxes of CO_2 through the stomatal pores and in the $\delta^{18}O$ composition of leaf water (Farquhar et al., 1993). The impact of terrestrial biosphere $\Delta^{18}O$ on the atmosphere is highlighted in the strong Northern hemisphere meridional gradient and seasonal variations of $\delta^{18}O$ in CO_2 observed by the global flask network (Farquhar et al., 1993; Cuntz et al., 2003a, b; Wingate et al., 2009; Francey and Tans, 1987).

Current leaf water isotopic enrichment models are still based on the work of Craig and Gordon (1965) and Dongmann et al. (1974) and describe how $H_2^{18}O$ enrichment in leaf water develops as a function of the $\delta^{18}O$ in source water (water extracted from the soil by plants) and water vapor, and the ratio of vapor pressure between the atmosphere and the leaf intercellular spaces (i.e. relative humidity expressed relative to leaf temperature). Over the course of a sunny day, leaf water becomes enriched as the air gets dryer and leaf temperature increases leading to higher vapor pressure deficits between the leaf and the air, the driving force of water flux from the soil to the atmosphere. For leaves that have a high leaf water content such as conifer needles, a non-steady-state formulation is required because the turnover time of leaf water is too long to let the water pool readjust to the rapidly varying atmospheric conditions (Cernusak et al., 2002; Seibt et al., 2006, 2007; Cuntz et al., 2007; Wingate et al., 2010b).

Also, most studies seem to indicate that the mixing of enriched water from the sites of evaporation in the leaf mesophyll is incomplete, and that some un-enriched (or only partially-enriched) water pools remain in the mesophyll (Yakir et al., 1998; Farquhar and Lloyd, 1993). However the way this incomplete mixing operates is still a matter of debate (Barbour et al., 2004; Ogée et al., 2007; Ferrio et al., 2009; Kahmen et al., 2009) and difficult to model as it requires knowledge on where precisely in the mesophyll water evaporates or condenses and how water molecules move and self-diffuse (Farquhar et al., 2003; Cuntz et al., 2007; Ogée et al., 2007). For this reason, current models of leaf water enrichment have not markedly evolved since the work of Dongmann et al. (1974) or Farris and Strain (1978). Similarly, and also because of a lack of understanding in the extent of isotopic exchange between CO_2 and water in the leaf especially in C_4 plants, models of oxygen isotope discrimination during CO_2 uptake by leaves are still based on the earlier theory of Farquhar and Lloyd (1993).

However, with new laser spectroscopic techniques it is now possible to capture leaf water enrichment and CO_2-H_2O exchange dynamically by measuring on-line either the $\delta^{18}O$ of the transpiration flux (Welp et al., 2009) or the net CO_2 exchange from leaves (Barbour et al., 2007; Wingate et al., 2010b). By inverting either of these flux signals it is possible to reconstruct leaf water dynamics non-invasively, and to obtain new insights regarding the extent of equilibration between CO_2 and water for different species or environmental conditions. Therefore, the $\delta^{18}O$ of the CO_2 flux is an extremely powerful tracer as $\delta^{18}O$ of leaf water can be reconstructed both during the day and night. This approach has provided compelling evidence for the opening of stomata in the dark (Cernusak et al., 2004; Seibt et al., 2007; Wingate et al., 2010b) and may also indicate subtle variations in the extent of CO_2 hydration by the enzyme carbonic anhydrase between light and dark periods (Cernusak et al., 2004; Barbour et al., 2007; Kahmen et al., 2008). Thus, $\delta^{18}O$ of CO_2 will prove a valuable tracer as we try to understand why stomata open in the dark and how rates of nocturnal stomatal conductance vary over the night and from day-to-day.

2.1.2 Current view on post-carboxylation and respiratory fractionation

The isotope effects during leaf CO_2 and H_2O exchange leave an imprint on the $\delta^{13}C$ and $\delta^{18}O$ signatures of newly produced assimilates, tissues and respired CO_2 (Farquhar et al., 1982). However, several post-carboxylation fractionation processes can alter the isotope signatures of the assimilated carbon compounds in down-stream metabolic processes.

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 enzyme catalyzed reactions (aldolisation and transketolisation) with position specific discrimination during C-C bond making. As a result, the C-3 and C-4 positions within glucose are enriched in ^{13}C and thus a non-uniform intra-molecular distribution of ^{13}C within carbohydrates is established (Rossmann et al., 1991; Tcherkez et al., 2004; Gilbert et al., 2009). Subsequently, photorespiration and starch-sucrose partitioning modify the diurnal changes in photosynthetic discrimination (see Brüggemann et al., 2011). Analyses of sugar $\delta^{13}C$ and its diurnal variations have the potential to trace changes in these metabolic activities.

The non-statistical intra-molecular distribution of ^{13}C in carbohydrates leaves its imprints on metabolites synthesized from these ("fragmentation fractionation" Tcherkez et al., 2004). If one of these products is decarboxylated then respired CO_2 will carry an isotopic signature different from the average sugar signature (see Brüggemann et al., 2011). Moreover, switches between substrates (Tcherkez et al., 2003), in particular during light-dark transition of leaves (i.e. light enhanced dark respiration, Barbour et al., 2007) and the oxidative pentosephosphate pathway can markedly change the $\delta^{13}C$of respired CO_2 (Bathellier et al., 2008, 2009; see Brüggemann et al., 2011). So far a full quantitative understanding of apparent respiratory fractionation has not yet been achieved (Tcherkez 2010; Werner 2010). However, measurements with a high temporal resolution indicated remarkable diel dynamics in respiratory $\delta^{13}CO_2$, which differed between functional plant groups (Priault et al., 2009; Werner et al., 2009; reviewed in Werner and Gessler, 2011). Feeding experiments with positionally labeled 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 to identify differences between functional groups (Priault et al., 2009; Wegener et al., 2010). For studies of changes in respiratory substrates measurements of respired CO_2 and its isotopic composition can be combined with measurements of oxygen exchange (Tcherkez et al., 2003).

The main post-photosynthetic changes in $\delta^{18}O$ of carbohydrates are due to exchange with water pools in other plant compartments (e.g. xylem, phloem, sink tissues) or in the course of time. Thus, the isotopic signature of local water pools will be imprinted on the carbohydrate signature (Farquhar et al., 1998). The equilibration with these pools depends on the residence times within these compartments as well as on the stability of the chemical bonds of the oxygen atoms in individual positions from exchange by e.g. phosphorylation. Recent advances have further led to the improvement of process-based models that predict variability in the oxygen isotope composition of plant organic material (Barbour, 2007). These models are now employed to quantitatively relate stable carbon and oxygen isotopes to plant photosynthetic performance, e.g. leaf physiological responses to environmental changes (cf. Gessler et al., 2009a; Kahmen et al., 2008, 2009, 2011).

Further carbon and oxygen isotope fractionation steps occur during secondary metabolism (see Schmidt and Gleixner, 1998; Schmidt 2003; Schmidt et al., 2001). Fragmentation fractionation and diel variation in anabolic pathways and substrates used for between-organ transport are at the origin of inter-organ differences in isotopic composition (see Hobbie and Werner, 2004; Badeck et al., 2005; Cernusak et al., 2009 for reviews). Future progress in quantifying these effects will open new avenues for analyses of inter-organs differences as indicators of metabolic activities and their variation with plant phenology.

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

Bulk leaf carbon isotope discrimination ($\Delta^{13}C$, i.e. the difference of the leaf organic matter $\delta^{13}C$ relative to the atmospheric source $\delta^{13}C_a$; divided by $\delta^{13}C_a$) provides an integrative record of supply and demand for CO_2 and can be used to determine the relationship between carbon gain versus water loss. As such, variation in $\Delta^{13}C$ may be driven by changes in stomatal conductance (i.e. supply of CO_2), in photosynthetic carboxylation rate (i.e. demand for CO_2), or both. In spite of the above mentioned multiple fractionation processes a remarkably linear relationship between bulk leaf $\Delta^{13}C$ and the intrinsic water use efficiency (WUE_i), i.e. the ratio of net assimilation (P_N) to stomatal conductance (g_s) has been observed under controlled conditions (Farquhar et al., 1989). The use of $\Delta^{13}C$ is therefore a convenient tool to study the variation in carbon water relations retrospectively, e.g. in different biological archives (see 2.5), and has been vastly used as an ecological tracer of water use efficiency at the leaf, plant and ecosystem scale (e.g. Bonal et al., 2000; Evans 2001; Lauteri et al., 2004; Ponton et al., 2006).

However, the carbon isotope ratio alone will not provide information, whether a change in $\Delta^{13}C$ was caused by either a change in g_s or P_N. This can be answered by combining the carbon isotope ratio with information from the oxygen isotope ratio in plant organic matter (Scheidegger et al., 2000; see also Grams et al., 2007; Sullivan and Welker, 2007). The negative relationship between $\Delta^{18}O$ and g_s was used to estimate stomatal aperture for interpreting physiological changes in $\delta^{13}C$ of leaf material. $\delta^{18}O$ of bulk organic matter has further been used to determine whether a change in WUE_i is the result of an increase in the atmospheric CO_2 (Saurer and Siegwolf, 2007), or a change in VPD as a result of an increasing temperature at constant or decreasing precipitation, as it can be observed in the Mediterranean (Sarris et al., 2011) or along a Siberian North – South gradient (Siegwolf et al., unpubl.). These results suggest that the dual isotope approach allows the distinction between the contributions of g_s and/or photosynthetic capacity to changes in $\delta^{13}C$ at the site level.

However, under naturally varying environmental conditions interpretation of Δ^{13}C in terms of WUE$_i$ is complex, particularly when only carbon isotope information is available (e.g. Welker et al., 2003). Under natural conditions, Δ^{13}C variations will not deliver straight-forward information on the "efficient" use of carbon and/or water resources but solely reflects changes in the P_N/g_s ratio. Thus, ecological interpretation must be cautioned if the actual rates of these processes (carbon gain and water loss, the latter requiring knowledge on VPD) are not known. Moreover, under natural conditions the trade-off between efficient carbon gain and reduction of water loss is particularly relevant when species are competing for limited water sources. Thus, ecological evaluation of an efficient resource use strategy requires knowledge on resource availability and plant interaction at the community level.

Moreover, different phenological phases and leaf structure (through its effect in mesophyll conductance, see 2.1.1) can constrain a direct comparison of bulk leaf Δ^{13}C between different species and functional groups (Hanba et al., 2003; Warren and Adams, 2006; Werner and Máguas, 2010). The influence of mesophyll conductance and other post-photosynthetic fractionation factors may also be relevant when comparing the isotopic composition of different plant compartments (e.g., leaves, carbohydrates, phloem, roots and root exudates, Bathellier et al., 2009; Cernusak et al., 2009; Wegener et al., 2010).

For the Mediterranean evergreen sclerophyllic species deviations from the expected linear relationship between leaf Δ^{13}C and c$_i$/c$_a$ ratio are observed (see regression line Fig. 2). The expected reduction in the c$_i$/c$_a$ ratio in response to increasing VPD, drought and temperatures was not manifested in leaf Δ^{13}C (Fig. 2), due to different phenological phases, a cease of growth (in evergreens) and concomitant reduction in g_s and P_N (as identified by δ^{18}O, Werner et al., 2001), structural regulation of plant water loss by leaf abscission (semi-malacophyllous species) and low carboxylation rates in the herbaceous species. Hence, environmental conditions during leaf formation as well as length of the growing period, leaf longevity, structure, and life form do modulate Δ^{13}C. Furthermore, the remobilization of non-structural carbohydrates from older tissues and

fractionation processes during the formation of new leaves might mask the Δ^{13}C signal induced by environmental conditions under which the leaf was formed (Terwilliger et al., 2001).

Thus, while the dual isotope approach has proven a valuable concept for ecological applications, an interpretation of WUE$_i$ based exclusively on Δ^{13}Cg in bulk leaf material requires a careful consideration of the type of plant material and the potential environmental conditions. The complexity of this parameter may constrain its use in ecological field studies. Carbon pools with shorter turn-over times such as leaf soluble sugars or phloem allocated carbon (e.g. Gessler et al., 2004; Scartazza et al., 2004) are therefore better indicators for recent changes in c$_i$/c$_a$ and environmental factors as outlined below (2.2).

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

The transport of newly assimilated carbon within the plant and from the plant to the rhizosphere can be characterized by applying stable isotope techniques. For plant and canopy integrating information as well as for plant internal transport the assessment of the isotopic composition of phloem sugars is central.

2.2.1 Carbon and oxygen isotopic composition of phloem organic matter

For almost 15 years δ^{13}C of phloem organic matter has been used to derive information on C-allocation and canopy integrated water use efficiency in plants as affected by environmental conditions (Yoneyama et al., 1997; Pate and Arthur, 1998; Keitel et al., 2003; Gessler et al., 2004; Scartazza et al., 2004; Barbour et al., 2005; Rascher et al., 2010). A dual isotope approach (δ^{13}C and δ^{18}O see 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; Gessler et al., 2007b, 2008). However, it is now apparent that – even though the isotope composition of phloem organic matter can, in principle, integrate leaf physiology over the whole canopy – several points of uncertainty must be taken into account when interpreting phloem isotopic information.

1. Temporal integration: Short-term variations in the isotopic composition of leaf sugars – induced by either an environmental signal or internal plant processes – might or might not be reflected in the 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 ^{2}H enrichment or carbon isotope fractionation. In the trunks of adult trees, however, the mixing of sugars of different metabolic origins dampens the short-term variations and the isotope signatures provide time-integrated information on canopy processes instead (Keitel et al., 2006; Rascher et al., 2010; Wingate et al., 2010a).

2. Change of the original signal: A further constraint in interpreting phloem isotope signatures is that the original isotope signal imprinted on sugars in the leaf may be altered during basipetal transport in the phloem of trees. 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 lead to the observed isotope patterns (Barnard et al., 2007; Gessler et al., 2009b). The change in δ^{13}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 δ^{13}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 assimilate partitioning in trees.

3. Chemical composition: It is often assumed that only one major sugar, namely sucrose, is present in the phloem. However, besides sucrose, there are other transport carbohydrates such as myo-inositol and raffinose family sugars (Karner et al., 2004) and also hexoses present (van Bel and Hess, 2008). Phloem sugar composition varies with environmental conditions which could be one important factor for changes in phloem δ^{13}C (Merchant et al., 2010), independent of the original leaf level isotope signal, since δ^{13}C differs between different carbohydrate molecules (Schmidt, 2003; Devaux et al., 2009). Compound specific analysis, provided by state of the art LC- and GC-IRMS techniques, might help to differentiate between changes in phloem δ^{13}C that result from changes in the chemical composition or changes in leaf level fractionation. In addition, we should apply comparable methods to characterize the compound specific oxygen isotope composition of phloem organic matter.

Thus, the isotopic composition of phloem organic matter is a useful tool for gaining physiological information on whole tree or canopy processes (Cernusak et al., 2009; Gessler et al., 2009b). There is, however, a strong need to develop more insights into the underlying mechanisms behind the spatio-temporal variation of δ^{13}C and δ^{18}O independent of leaf level processes. This is a necessary prerequisite not only to better interpret the physiological meaning of the phloem isotope signal but also to extend our understanding of whole plant carbon transport and the metabolic background behind variations in ecosystem carbon allocation.

2.2.2 Plant-Soil carbon coupling

Stable isotope approaches did enhance our awareness of tight plant-soil coupling of carbon and water flow in recent years (see accompanying paper by Brüggemann et al., 2011). Besides the application of δ^{13}C (or even radioactive δ^{14}C) enriched CO_2 in pulse labelling experiments (e.g. Högberg et al., 2008; Carbone et al., 2007), natural abundance δ^{13}C approaches have been applied to characterize the coupling between carbon assimilation between vegetation and soil processes (e.g. Tu and Dawson, 2005; Kuzyakov and Gavrichkova, 2010). In brief, these approaches calculated the time lags between changes in environmental conditions and/or δ^{13}C in recent assimilates and δ^{13}C in soil and/or ecosystem respired CO_2 (e.g. Knohl et al., 2005; Werner et al., 2006; Kodama et al., 2008). Only recently the application of near infrared laser spectroscopic techniques have allowed more detailed assessments of the coupling between assimilation and soil and ecosystem respiration with a high temporal resolution over a whole growing season (Wingate et al., 2010b). This study under field conditions supported previous work carried out with $^{13}CO_2$ enriched label under controlled conditions (Ruehr et al., 2009), showing that the speed of link and the degree of coupling (Kayler et al., 2010a) is strongly variable and affected by environmental conditions, especially water supply (for further detail see Brüggemann et al., 2011). Laser spectroscopic techniques will most likely advance our knowledge on the influence of recently assimilated carbon on the soil carbon balance in the near future. They provide the necessary temporal resolution to trace the fate of carbon – from photosynthetic CO_2 assimilation to respiratory CO_2 release by either using artificially ^{13}C labeled CO_2 or with its natural ^{13}C abundance – via isotope measurements in the respired CO_2 through all ecosystem compartments with high temporal resolutions (Bowling et al., 2002).

2.3 Community-scale processes

2.3.1 Tracing functional group response to environmental changes: where do we stand?

Complexity and diversity of plant responses at the community level clearly challenges the use of stable isotope tracers at this level under varying environmental and biotic stresses, as well as the examination of interactions between plants. The definition of functional groups, based on a similar response to an environmental factor (functional response groups) or a common effect on ecosystems function (functional effect groups) (Lavorel and Garnier, 2002), may facilitate the interpretation of isotope effects at the community scale.

As shown in Sects. 2.1.1 and 2.1.2 numerous factors form the isotope signals modified by plant functions and driven by environmental factors, resulting in distinct oxygen and carbon isotopic signatures of bulk tissues. It has been shown that Δ^{13}C is a reliable integral for differences in structural, phenological and physiological leaf traits (Brooks et al., 1997; Werner and Máguas 2010; see Sect. 2.1.3). It allows the distinction of broad plant functional types within one ecosystem based on simple measurement of mean bulk leaf Δ^{13}C from different leaf age classes (Fig. 3). Another example is the distinction of functional traits of exotic invasive species in their bulk leaf isotope signatures, such as, high carbon gain, and high efficiency of water and nutrient uptake (e.g. Hellmann et al., 2011). Moreover, invasive species can affect community functioning by competing directly for resources and by altering nutrient, carbon and hydrological cycles (Richardson and Pyšek, 2006), which can be traced through stable isotopes at the community scale (Rascher et al., 2011). Thus, the integrative nature of bulk leaf isotopic composition, which can constrain its use as indicator of leaf gas exchange under natural conditions (see 2.1.3), may provide an efficient integrative tracer of physiological, phenological and structural attributes (e.g. Bowling et al., 2008), enabling the distinction of broad plant functional types at the community level (Werner and Máguas, 2010).

2.3.2 Detecting water source partitioning in plant communities

The sources of water taken up by different plants within a community can be studied by stable hydrogen or oxygen isotopic measurements in stem water and compared with that of potential water sources (e.g., soil water at multiple depths, ground water, precipitation and stream water) (e.g. Welker, 2000; Dawson et al., 2002; Mora and Jahren, 2003; Dawson and Siegwolf, 2007). Differences in water sources 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).

Several models have been developed to determine the contribution of each water source to plant and ecosystem evapotranspiration: Linear mixing models can be applied if the differences of δ^{18}O among the water sources and xylem plant water are large enough; δD-δ^{18}O plots can be used if the difference between water sources and xylem water is small (Ogle et al., 2004). Hu et al. (2009) showed in a recent review that 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.

The patterns of use and redistribution of soil water by plant species have a considerable impact on hydrological cycles in terrestrial ecosystems (Caldwell and Richards, 1989; Caldwell et al., 1989; Jackson et al., 2000; Lee et al., 2005; Kurz-Besson et al., 2006; Domec et al., 2009). For example, species from a single community may differ substantially in rooting depth and water consumption so that shifts in species composition can significantly alter patterns of ecosystem scale water-use (Jackson et al., 2000). 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). Thus, new insights regarding inter-plant competition for above- and belowground resources and to plant functional traits have been achieved by combining $\delta^{13}CO_2$ and $\delta^{18}H_2O$ in studies at the community level (Ramirez et al., 2009; Grams and Matyssek, 2010).

2.4 Carbon and water cycles of terrestrial ecosystems

At the ecosystem scale, stable isotopes can potentially provide insights into the complex interaction between vegetation, soil and atmosphere exchange in carbon and water fluxes and their responses and feed-backs to environmental drivers (Dawson et al., 2002; Yakir, 2003). However, this requires 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. Almost ten years

ago, canopy photosynthetic discrimination was identified as one of the greatest uncertainties for studying the ecosystem carbon cycle (Baldocchi et al., 2001; Dawson et al., 2002). Nowadays, this picture seems almost naive as the importance of several other physical and biological processes have been identified, which significantly complicates the application of stable isotopes as tracers of carbon and water fluxes at the ecosystem scale.

First of all, it has been shown that the carbon isotope composition of ecosystem respiration ($\delta^{13}C_R$) is complicated by several post-photosynthetic fractionation processes that differ between autotrophic and heterotrophic organs (see 2.1.2 and references therein) and varies with changes in photosynthetic discrimination and respiratory activity, not only seasonally (e.g. Griffis et al., 2004; Ponton et al., 2006; Werner et al., 2006; Alstad et al., 2007; Schaeffer et al., 2008; Wingate et al., 2010a), but also at much higher temporal resolution from days to even a few hours (Bowling et al., 2003; Werner et al., 2006; Kodama et al., 2008; Unger et al., 2010a; Wingate et al., 2010a; Barbour et al., 2011). These observed short-term variations in $\delta^{13}C_R$ are most likely driven by changes in the magnitude of single ecosystem component fluxes and/or their isotope compositions (Unger et al., 2010a; Wingate et al., 2010a; Barbour et al., 2011). For example all ecosystem components exhibited large variations in both flux rates and associated isotopic compositions ($\delta^{13}C_{\text{res}}$) in a Mediterranean oak woodland, with marked changes in natural abundance $\delta^{13}C_{\text{res}}$ both at the diel time-scale and in response to drought (Fig. 4, Unger et al., 2010a). Furthermore, due to changes in the supply and turnover of metabolites, the dominance of certain metabolic pathways during cambial growth are most likely different from those outside the growing season (Högberg et al., 2010; Wingate et al., 2010a; Tu and Dawson, in review). Additional mechanisms, such as different plant regulation in response to drought have also been proposed to contribute to such variations (Werner et al., 2006; Ruehr et al., 2009).

Second, other abiotic processes may be involved. Indeed, isotopic mass balance at the entire ecosystem scale has only rarely been achieved (Tu and Dawson, in review), implying that the mixing-model assumptions used for estimation of $\delta^{13}C_R$ from atmospheric CO_2 isotope measurements are rarely met (Pataki et al., 2003; Kayler et al., 2010a), or that isotopic steady-state is not attained (Nickerson and Risk, 2009b; Kayler et al., 2010b). Recently, an increasing number of studies have shown that factors such as diffusivity of soil CO_2, dissolution of CO_2 from respiration and bicarbonates and advection of soil gas may be responsible for strong isotopic disequilibria between the CO_2 efflux at the soil surface and concurrent soil respiration (Kayler et al., 2008, 2010a; Nickerson and Risk, 2009a; Ohlsson, 2009; Gamnitzer et al., 2011). To characterize subsurface gas transport and subsequent fractionation dynamics, soil CO_2 production and transport models are used (Hashimoto, 2002; Davidson et al., 2006; Nickerson and Risk, 2009c; Ohlsson, 2009; Moyes et al., 2010; Wingate et al., 2010b), but many of the above discussed processes are not yet included (e.g. Brüggemann et al., 2011). Similarly, the oxygen isotope composition of soil respired CO_2 ($\delta^{18}O_S$) not only carries the isotopic signature of the soil water it interacted with, but has been shown to be influenced by the carbonic anhydrase present in soil microorganisms that enhances isotopic equilibration between CO_2 and soil water (Wingate et al., 2009; 2010b). Yet, such processes are rarely included in isotope-enabled ecosystem models.

Obviously, the main lesson learned from these recent studies is that the application of the stable isotope approach at the ecosystem scale to constrain carbon and water fluxes from different pools is more complex than previously thought. These studies also allowed us to improve our understanding of the physical and biological drivers of the isotopic signals for each of the component fluxes. This knowledge can now be imbedded into stable isotope-enabled models and must be combined with data assimilation techniques. The recent development of continuous, rapid in situ stable isotope measurement techniques (see Sect. 3) opens new possibilities to study the carbon and water budgets of terrestrial ecosystems. The assimilation of such high time resolution datasets into isotope-enabled ecosystem models can be used to improve models by constraining component fluxes when non-isotopic methods become limiting.

For example, the eddy covariance (EC) technique is widely used for the development and validation of terrestrial ecosystem models. However, the EC method only provides a net flux while process-based models predict component fluxes. To overcome this long-standing incompatibility problem, it is crucial that the CO_2 or H_2O fluxes can be separated into their component fluxes. Conventional partitioning methods make a priori assumptions about ecosystem functions and typically require several days or weeks of data to cover key phenological periods in order to obtain robust regression parameters, obscuring ecosystem functions at shorter time scales. For example, the response of soil respiration to strong rain events is pulse-like, occurring at time scales of minutes to hours (e.g. Xu et al., 2004; Unger et al., 2010b). Other sudden changes or "switches" of ecosystem states have also been documented (Baldocchi et al., 2006; Lee et al., 2007). It is imperative that the partitioning scheme resolves episodic responses of this kind, because it is the transient, non-equilibrium responses that provide a more rigorous test of model processes and their validity. Assimilating continuous measurements of CO_2 and H_2O fluxes and their isotopic composition (e.g. $\delta^{13}C$, $\delta^{18}O$, δD) into process-based models should provide a better constrained solution. Similarly, assimilating chamber-based flux measurements of these isotopic fluxes should help to understand and constrain our model predictions during metabolic switches, especially when photosynthetic products become limiting such as during droughts (Unger et al., 2010a; Fig. 4), rainy periods (Wingate et al., 2010a) or dawn (Barbour et al., 2011). Ideally, we advocate the use of multiple isotopic tracers ($^{13}CO_2$, $CO^{18}O$, $H_2^{18}O$, HDO) as they provide more distinct information on the governing processes and the resulting component fluxes.

2.5 Isotopic archives and relevant aspects of spatio-temporal integration

2.5.1 Isotopic archives in trees and herbaceous vegetation

Trees rings contain formidable isotopic archives, which enable retrospective analyses of intra- and interannual variation of carbon and oxygen isotope composition and the related ecophysiological drivers over many centuries (see Fig. 5a; Sidorova et al., 2009; Andreu-Hayles et al., 2011; Nock et al., 2011; Knorre et al., 2010). These archives are well suited for detailed reconstructions of Anthropocene climate change impacts from carbon and oxygen isotope discrimination, with comparatively high temporal resolution over long time scales (Fig. 5a). However, single tree ring chronologies provide limited spatial and community integration because they are retrieved from individuals and therefore only report local signals (integrating isotopic information from an area of 10^{-1} to $10^2\,m^2$ depending on the size of the individual). Also, the tree ring signal is, or can be, species-specific. Thus, construction of community- or ecosystem-scale chronologies depends upon combining several tree ring records from the different species composing the community. This requires additional information, such as knowledge of past species dynamics for estimation of species-weighted community isotopic signals.

Life span of herbaceous vegetation is shorter than that of trees, meaning that isotopic records are not as long either; whereby long-lived perennial plants that exhibit annual growth increments can be valuable recorders of ecophysiological changes in plant performance and thus recorders of climate variation. For example, $\delta^{18}O$ and $\delta^{13}C$ values of growth increments of *Cassiopoe tetragon* from the High Arctic depict fluctuations in the AO/NAO (Arctic and North Atlantic Oscillations) which control the amount of snow melt ($\delta^{18}O$) water available to plants in summer and associated changes in leaf gas exchange ($\delta^{13}C$).

However, the isotopic reconstructions of Anthropocene climate change in herbaceous vegetation (crops and grassland) are possible, if plants were sampled and preserved during the epoch. Such archives are relatively rare (Köhler et al., 2010), and are mainly represented by herbaria. In general herbarium specimens have been sampled at different locations, so that long-term isotopic records from these involve a spatially disperse representation (that exhibits inter-local isotopic noise) of a species changing isotopic composition.

Clearly, the elaboration of isotopic records presents very different methodological challenges for the different biomes. But, for grassland, a nice analogy to tree rings

is given by the yearly rings (annuli) of horns (or hoofs) of obligate grazers (Barbosa et al., 2009). These can also yield isotopic records over many years, which reflect to some extent that of grassland vegetation (Barbosa et al., 2010; see Fig. 5b). The spatial integrations provided by tree and horn ring isotope composition are quite contrasting: local and stationary for the tree, and vast and cyclic for grasses, reflecting visits of the different parts of the year-round grazing ground of the grazer. Because of the ephemeral nature of grassland biomass, animal tissues (such as hair, bones or feces) have become an important isotopic proxy of grassland vegetation. Still, the use of grazer tissue for reconstructions of grassland isotopic chronologies rests on a number of assumptions which need to be verified for the different systems (e.g. selective grazing or non-constant relationships between isotopic composition of grazer tissues and diet due to differential digestibility of diet components) (Wittmer et al., 2010).

The carbon isotope composition of grazer tissues such as horn can provide an accurate reflection of that of the grazed vegetation, and this is – at least partially – due to its homogenous chemical composition (keratin), which reduces variation associated with metabolic isotope fractionation which can be a problem in chemically heterogeneous materials. Conversely, the oxygen isotope composition of horn does not directly reflect that of diet, because of modification by the isotope composition of drinking water, atmospheric oxygen and fractionation effects in animal metabolism.

2.5.2 Non-vascular plants as integrators of micro-environmental and long-term changes

A particular case of micro-scale environmental record can be derived from $\delta^{13}C$ and $\delta^{18}O$ of non-vascular plants (NVP), as they respond highly sensitive to micro-environmental changes. NPVs such as cyanobacteria, algae, lichens and bryophytes are often used in environmental assessment and are increasingly recognized for the important roles in biogeochemical cycling (DeLucia et al., 2003; Elbert et al., 2009) and pedosphere-biosphere-atmosphere exchanges (Shimoyama et al., 2004).

Due to the desiccation tolerance and ability to reactivate the metabolism by equilibration with water vapor, δ^{13}C and δ^{18}O of NVP integrate changes of carbon and water on a micro-environmental scale and over a long period (Máguas and Brugnoli, 1996), due to their sensitivity to small-scale environmental conditions, low growth rates and long lifespan. δ^{13}C and δ^{18}O of NVP record isotopically distinct micro-resources which are not commonly traced by higher plants. This includes for example micro-scaled humidity gradients, soil-water-CO_2 exchange by carboanhydrase, soil respiration and evaporation recycled by vegetation cover, or diel courses of water vapor signature (e.g. Lakatos et al., 2007; Hartard et al., 2008). Moreover, organic δ^{13}C of NVP can be used as tracer for carbon acquisition (Meyer et al., 2008), environmental change of respired CO_2 (Flanagan et al., 1999; Lakatos et al., 2007), and global change (Máguas and Brugnoli, 1996). Even fossil bryophytes record ancient CO_2 levels (Fletcher et al., 2005, 2006).

δ^{18}O of absorbed water and respired CO_2 operates as a tracer for partitioning water sources (Flanagan et al., 1999; Hartard et al., 2008) and in full equilibration as vapor trap (Helliker and Griffiths, 2007; Hartard et al., 2009). Because epiphytic lichens are often in equilibrium with water vapor, it is inferred that organic δ^{18}O might serve as an environmental integrator and recorder for atmospheric vapor on a regional and global level (Helliker and Griffiths, 2007; Lakatos et al., 2007; Hartard et al., 2009). Further, several studies have demonstrated the potential of stable isotopes (δ^{18}O, δ^{13}C) in peat mosses as proxies for retrospective studies of environmental change, such as temperature, humidity and/or precipitation shifts (Loader et al., 2007; Lamentowicz et al., 2008; Moschen et al., 2009; Loisel et al., 2010).

Thus, stable isotopes of NVP may be used as ecological indicators for environmental change, especially for variations in CO_2-concentrations and water vapor as well as long term indicators of water accessibility change (Niemi and McDonald, 2004).

2.6 Continental isotope variation in precipitation at the landscape-scale

Continental-scale studies of the water isotope cycle are becoming increasingly central to our understanding of synoptic climates, ecosystem processes and the role of abiotic processes (i.e. temperature of condensation), moisture sources, and storm tracks on the ecohydrology of entire landscapes and continents (e.g. Welker 2000; Vachon et al., 2007; Birks and Edwards 2009; Liu et al., 2010). The ratio of heavy to light isotopes within precipitation for instance, records the history of the moisture, from conditions at the moisture source, moisture recycling during transport, to conditions during condensation (Vachon et al., 2010a, b). If the factors that contribute to stable isotopes in precipitation (SIP) for a given climate system are well understood, SIP represent a valuable means of quantifying changes within a given hydrologic system.

A process-based understanding is important to delineating today's climate trajectories (Welker, 2010) and evapotranspiration fluxes, such as those that have been quantified as air masses progress across the Amazon Basin (Noone and Simmonds, 2002; Henderson-Sellers et. al., 2004) and into the Gulf Coast of North America (Liu et al., 2010). Concurrently, SIP have been paramount in determining which moisture sources provide vapor to regions of the eastern US, Tibetan Plateau and Canada (Tien et al., 2007; Birks and Edwards, 2009; Sjostrom and Welker, 2009).

Interannual climate differences in weather conditions can often be linked to major modes of climate variability, such as the Pacific Decadal Oscillation (PDO), El Niño Southern Oscillation (ENSO) or North Atlantic Oscillation (NAO). These conditions and indices are identified by changes in ocean temperatures, high or low pressure systems, or areas of deep convection. The phase of these modes of climate variability have been shown to affect ecosystem processes and a cascading set of consequences (Holmgren et al., 2001) along with the climate (Ault and St. George, 2010). Commensurate with the phaseology are modifications to air mass trajectories, moisture source regions and regional meteorological parameters (e.g. surface temperatures, relative humidity and precipitation amount) in regions of the globe, far from the source of the perturbations (e.g. Kumar et al., 2006). Such teleconnections make for highly variable weather, depending upon the locale which may have direct effects on ecosystem C balance (Holmgren et al., 2001). An example is given in Fig. 5c, showing that the Pacific Decadal Oscillation (PDO) is reflected in the SIP in the western US as part of an initial temporal trend analysis using USNIP (Cohn et al., 2009). There is a strong correspondence between the PDO index and δ^{18}O in eastern Washington between the years 1989–2002.

Our understanding of the SIP and its potential impact on continental C cycling have benefited from recent discoveries, such as (i) quantifying the impact of seasonality in precipitation, paramount to driving C cycling, on the isotopic composition of annual precipitation (Vachon et al., 2007), (ii) defining how time series of monthly SIP values relate to simultaneously measured monthly temperatures (Vachon et. al., 2010a) and (iii) quantifying the primary environmental causes for the average monthly isoscapes (i.e. spatial distribution maps of isotope records, Vachon et al., 2010b) (Fig. 6). The isoscapes shown in Fig. 6 related to average monthly temperatures in such a manner that the SIP-temperature slopes followed the predicted pattern of steep slopes during the colder months and more shallow slopes during warmer months. However, the observed slopes are constantly ~0.2 δ^{18}O/°C less than is predicted by a theoretical Rayleigh Model. Future studies should explore the storm track and moisture source conditions and examine how annual changes in the strength of such decadal oscillations as the PDO, ENSO and others affect SIP and their relationships with mean annual temperature. This would bring important insight for interpreting climate proxies.

Back trajectory analysis of weather and thus precipitation (HYSPLIT – Draxler and Hess, 2004; Sjostrom and Welker, 2009) is a modeling tool that has been used extensively by the atmospheric chemistry community to quantify long-distance transport of pollutants, and more recently for studies of isotopic characteristics of precipitation (Burnett et al., 2004). More specifically, this methodology has also been very useful in determining the origin of air masses and precipitating moisture, which has been linked to excursions in SIP trends (Sjostrom and Welker, 2009). Indeed, moisture source

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conditions are significant contributors to resultant SIP values. Each moisture source experiences its own interannual and interseasonal temperature fluctuations – key components in the most simplistic deviations of SIP. Developing this understanding will be important to fully account for changes in the C cycle.

2.7 GLOBAL-scale modeling

Globally, the terrestrial biosphere fractionates stable carbon isotopes during CO_2 uptake by about 15‰, which includes contributions from both C_3 plants (with ca. 18‰) and C_4 plants (with ca. 4‰) (Lloyd and Farquhar, 1994). In contrast, the dissolution of CO_2 in the oceans fractionates by only 2‰ (Tans et al., 1993). Large variations in the δ^{13}C of atmospheric CO_2 are therefore strong indicators of terrestrial biosphere activity and their observation provided one of the first corroborations of a large terrestrial carbon sink in the Northern hemisphere and have been used to quantify CO_2 uptake by land masses versus uptake by the oceans on continental scale using atmospheric transport inversion techniques (Ciais et al., 1995; Francey et al., 1995). However, because even small errors in the assumed regional mean fractionations can lead to large uncertainties in derived CO_2 flux partitions, atmospheric δ^{13}C is mostly used in current atmospheric inversion studies as an additional constraint concurrently with other tracers (e.g. Rayner et al., 2008). Over continents, recent studies have also used this tracer to estimate the relative contributions of C_3 and C_4 plants in the continental net CO_2 flux (Ballantyne et al., 2010). Even if δ^{13}C is not providing conclusive evidence on the global scale, it can be used, for example, to decide between different formulations of stomatal conductance in global models (Ballantyne et al., 2010).

However, even when δ^{13}C is not providing conclusive evidence on the global scale, it can be used, for example, to decide between different formulations of stomatal conductance in global models (Ballantyne et al., 2010). As stated above, δ^{13}C is nowadays rather used as a further constrain on other atmospheric inversion studies (e.g. Rayner et al., 2008).

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$\delta^{13}C$ tags the net fluxes of terrestrial and oceanic CO_2 fluxes. However, it is not possible to disentangle the large opposing gross fluxes such as assimilation and respiration with $\delta^{13}C$. $\delta^{18}O$ in CO_2, on the other side, marks photosynthesis and respiration differently and has therefore the potential to quantify sinks and sources of carbon separately. It turns out that the global cycle of $\delta^{18}O$ in atmospheric CO_2 is rather difficult to understand (Francey et al., 1987). The spatial distribution of $\delta^{18}O$-sinks and sources seems to be well understood, while the temporal variation was not yet reproduced successfully (Cuntz et al., 2003b). So either our global carbon cycle models are not yet well adapted for $\delta^{18}O$ in atmospheric CO_2 (Cuntz et al., 2003a) or we are missing processes in the description of the $\delta^{18}O$. One of these missing processes could be the equilibration of oxygen from gaseous CO_2 with water by the enzyme carbonic anhydrase in soils (Wingate et al., 2008). Including accelerated soil hydration in a global model of $\delta^{18}O$ in atmospheric CO_2 eliminated discrepancies on the spatial structure between model and observations (Wingate et al., 2009). However, the temporal change in the model was still out of phase with observations. This awaits an explanation at present and might bear a few more surprises.

3 New technical and methodological developments in stable isotope research

The past decade has seen tremendous progress in the development of new techniques that compliment or rival traditional Isotope Ratio Mass Spectrometry (IRMS) for the determination of stable isotope abundances. Most important for carbon and water cycle research was the development of instruments using alternative means for determining stable isotope abundances, as well as the introduction of innovative compound-specific sample extraction methods.

3.1 Laser spectroscopy

The development of absorption spectroscopy instrumentation provided new dimensions of measurement speed and number of quantifiable isotopologues based on analysis of absorption of light in selected wavelengths in the near and mid-infrared, or cavity ring down techniques, which can be used to determine the abundance of individual isotopologues. The capacity for CO_2, liquid and water vapor analysis is providing opportunities which have never been possible with a field-deployable instrument. 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.

3.2 Compound Specific Isotope Analysis (CSIA)

In parallel with the development of new optical methods, IRMS itself has experienced technological and methodological development allowing for a new dimension of our understanding in plant metabolic processes through Compound Specific Isotope Analysis (CSIA) (e.g. different structural and labile carbohydrates extracted from plant organs, leaf wax alkanes, phloem sap, soil fractions) by coupled gas chromatography (GC-IRMS) or liquid chromatography-combustion (LC-C-IRMS) isotope ratio mass spectrometry. These innovative techniques increased the repeatability, precision and sample turnover considerably, which makes them particularly useful for isotopic studies at natural abundance or in tracer experiments (see Brüggemann et al., 2011). This opens numerous possibilities for a deeper understanding of carbon metabolism, transport and allocation under different environmental conditions. For example, carbon can be traced down the stem into the soil and back into the atmosphere, using natural abundance, i.e. ‘labelling’ by changing environmental conditions, or ^{13}C labelling techniques (pulse labelling for labile, non-structural carbon and long-term labelling for structural carbon) by measuring $\delta^{13}C$ of labile and structural carbon in the phloem, the wood, the soil (or $\delta^{13}C$ of single compounds, e.g. sucrose and cellulose), and respired CO_2. Such analyses can be performed successfully at organ, plant and mesocosm scales in controlled and natural environment systems.

3.3 Rapid grab sampling for CO_2

An alternative method to analyze at high temporal resolution dynamics of δ^{13}C and δ^{18}O of respired CO_2 from different ecosystem compounds can be employed by incubating detached leaves, roots or soil in small vials (Tu et al., 2001). If flushed with CO_2-free air the isotopic composition of the respired CO_2 can be determined directly within 3 min on a gas bench-IRMS (in-tube incubation technique, Werner et al., 2007), or transferred into evacuated/CO_2-free vials, which allows field application and longer storage if the septum caps are pre-treated at 105 °C (Midwood et al., 2006). Heat treatment of the septum caps is required to delay ^{18}O fractionation, as in vials with untreated septa the $\delta^{13}C^{18}O^{16}O$ signals only remain stable for ~20 h (Werner et al., 2007).

3.4 Nuclear Magnetic Resonance (NMR)

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

3.5 Nano-scale secondary ion mass spectrometers (NanoSIMS)

Significant methodological progress has also been made through linking isotopic analysis with high resolution microscopy, providing spatially resolved information on the molecular and isotopic compositions of (biological) materials. The latest models of

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 liberated 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 organization, metabolic pathways and resource fluxes within cells, plants and at the root-fungus-soil interface, in particular in labelling studies.

3.6 Methodological challenges

The pool of various new and improved techniques currently available for application of stable isotopes in environmental, physiological and mechanistic approaches in ecological research is large. However, from the user's perspective three general issues should be resolved:

The first one is related to instrument accuracy and calibration. Calibration of water vapor analyzers is done, for example using a liquid water injector ("dripper") into dry air supplied by compressed air cylinders, by a dry air generator or by purging with drying agents (Lee et al., 2005; Wen et al., 2008; Baker and Griffis, 2010; Griffis et al., 2010; Sturm and Knohl, 2010). However, calibration biases can result from impurity of the dry air, evaporation efficiency of the injected water, and instrument nonlinearity. Alternatively, a heated vaporization system is used wherein the liquid standard is completely vaporized without fractionation. Nevertheless, any concentration dependence inherent in the analyzer itself can bias the overall calibration of the instrument, especially when measuring ambient water vapor of widely varying mixing ratios (Lee et al., 2005; Wen et al., 2008; Schmidt et al., 2010; Sturm and Knohl, 2010). CO_2 calibration is generally accomplished by measurement against two or more gas cylinders of CO_2 balanced by dry air. The mixing ratios and isotope ratios of these working standards are tied to primary international standards. While new instruments for vapor measurements have been introduced, large uncertainties appeared in liquid sample analysis extracted from biological sources due to spectral interference with organic contaminants (West et al., 2010).

The second challenge that researchers face is increased speed of detection at high instrument precision. Free of sample preparation and processing, new optical techniques achieve much faster detection than IRMS. In-situ measurements of CO_2 and H_2O 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 land carbon sink and the tracking of water transport in the atmosphere. However, to measure the source/sink signature properly, one should interface the isotopic analyzer with plant (Barbour et al., 2007; Barthel et al., 2010; Wingate et al., 2010a, b) and soil chambers (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, highly suitable interfaces between the analyzer and the periphery are mandatory together with fast instrument response. The whole system must be robust; designed and tuned for minimal interference, memory effects or signal drifts. This is particularly critical for eddy covariance applications, which require an instrument response to be faster than 10 Hz. Fast detections are also desirable in chamber measurements in the case of episodic events such as short soil CO_2-flux pulses after rain (Santos et al., 2010; Unger et al., 2010b). These requirements should be a high priority in future instrument development efforts and open a new dimension of information on the short-term temporal variation in natural systems (e.g. Unger et al., 2010a).

The third challenge that researchers face is instrument and infrastructure cost. High instrument and maintenance costs limit the broad adoption of new technologies in field research. It would be highly desirable that the costs be brought down to a level

comparable to that of a broadband infrared gas analyzer, which are now an indispensible 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 CO_2 and H_2O to help diagnose changes in biospheric processes and functions. This can become a realistic goal at lower instrument costs.

4 Conclusions

We are in the midst of rapid growth in our process-based understanding of the application of stable isotopes. Moreover, new technological developments accelerated the scientific progress opening new frontiers in ecological and interdisciplinary research across scales.

Significant advances have been made recently in linking the carbon and water cycles via dual isotope approaches ($\delta^{13}C$ and $\delta^{18}O$) at the leaf, plant, and ecosystem scale. This approach largely forwarded the understanding of the coherence between carbon and water relations, in particular by uncovering plant responses to environmental changes, interactions and feed-backs within plant communities and their implications for ecosystem processes.

At the same time, we have achieved a new awareness on multiple isotope fractionation processes during assimilation and transpiration (e.g. fractionation during photorespiration, day respiration, leaf internal CO_2 conductance, or exchange between leaf water pools) and during dark respiration, carbon transport and within metabolic processes. This topic is currently in the focus of numerous investigations with the attempt to answer a large number of open questions regarding metabolic processes and within cell and plant carbohydrate formations and fluxes (see also Werner and Gessler, 2011).

However, a new mechanistic understanding of these processes is essential for studying ecosystem processes, where so far only moderate progress in applying stable isotopes in partitioning studies has been achieved. The recognition that the isotope fractionation during photosynthesis and respiration is subject to a dynamic variability as a function of environmental impacts is a promising step. It will lead to the development of new functional concepts for both carbon and water flux partitioning at the ecosystem level.

The scientific progress is highly stimulated by new analytical developments, such as laser spectroscopy and compound specific isotope analysis, which opens a whole array of new possibilities for process-oriented studies regarding the physiologically linked fractionation of stable isotopes.

Thus, on the one hand we are increasingly recognizing the complexity of δ^{13}C and δ^{18}O fractionation processes and their spatial and temporal variation. On the other hand new technologies enable the record of short- and long-term variability in isotope signatures, which will deliver information-rich data sets and deepen our understanding on the complex interplay of soil, plant, ecosystem and atmosphere processes in the carbon and water cycles.

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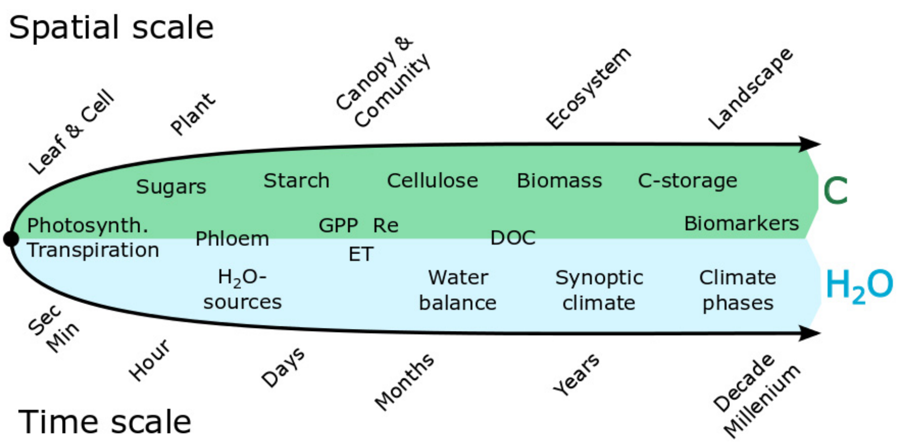

Fig. 1. Use of carbon and water stable isotopes in different compounds/biomarkers to reveal information on carbon and water cycles at different spatial and temporal scales. GPP – Gross primary productivity, Re – respiration, ET – evapotranspiration, DOC – dissolved organic carbon.

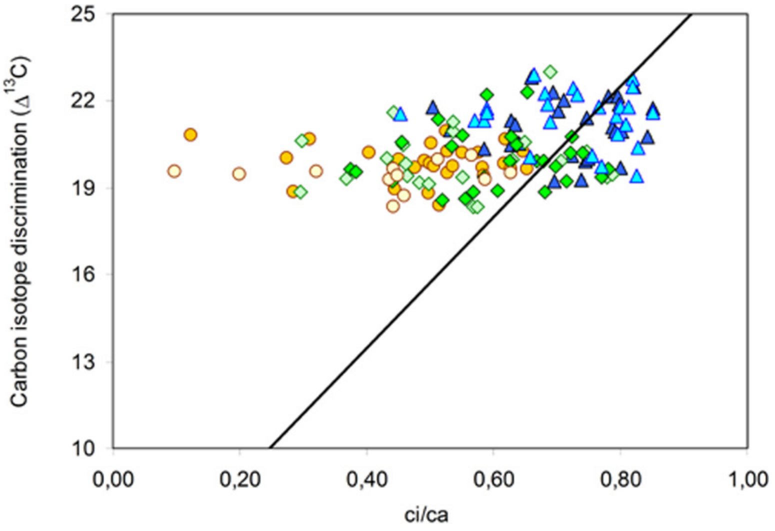

Fig. 2. Relationship between carbon isotope discrimination (Δ^{13}C) of leaf organic matter and the ratio of leaf internal to external CO_2 concentration (c_i/c_a) determined from gas exchange measurements for three Mediterranean species: the evergreen sclerophyllous *Quercus coccifera* L. (circles), the drought semi-deciduous *Cistus albidus* L. (triangles) and the herbaceous *Vinca difformis* Pourret (diamonds). Species were subjected to increasing drought treatment (light symbols) for 12 weeks and compared to well-watered controls (dark symbols) under semi-natural conditions in late spring at the University of Lisbon. Pooled data were collected after week one, six and 11 of the experiments. The line indicates the theoretical relationship from Farquhar et al. (1989). From Unger unpublished.

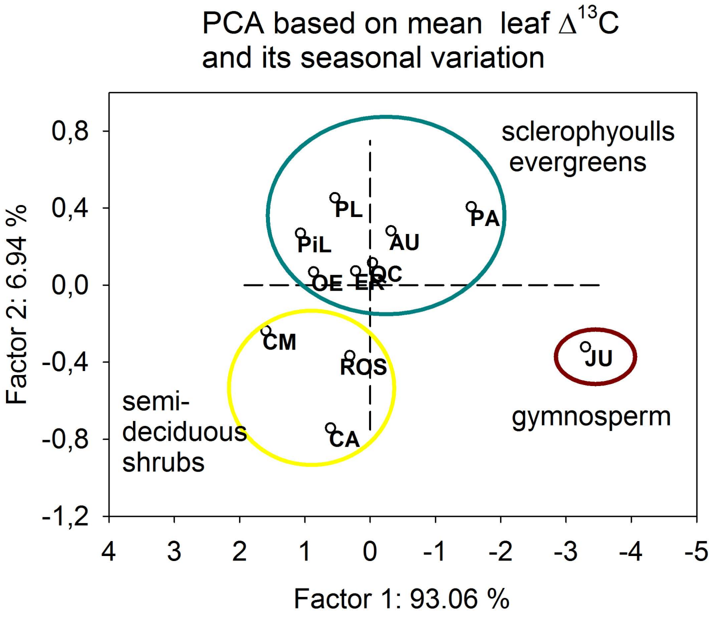

Fig. 3. Principal component analysis (PCA) of eleven co-occurring Mediterranean macchia species based on mean leaf bulk Δ^{13}C of current and of previous year grown leaves. The first axis separated species by annual mean Δ^{13}C, while the second axis was loaded with seasonal changes in Δ^{13}C. Analyzed macchia species from Serra da Arrabida, Southwest Portugal, were drought semi-deciduous shrubs *Cistus albidus* (CA)*, Cistus monspeliensis* (CM) and *Rosmarinus officinalis* (RO), the sclerophyllous evergreens *Quercus coccifera* (QC)*, Arbutus unedo* (AU), *Olea europaea* (OE), *Phillyrea latifolia* (PL), *P. angustifolia* (PA), *Pistacia lentiscus* (PiL), *Erica arborea* (EA) and the gymnosperm *Juniperus phoenicea* (JP).Adapted from Werner and Máguas (2010).

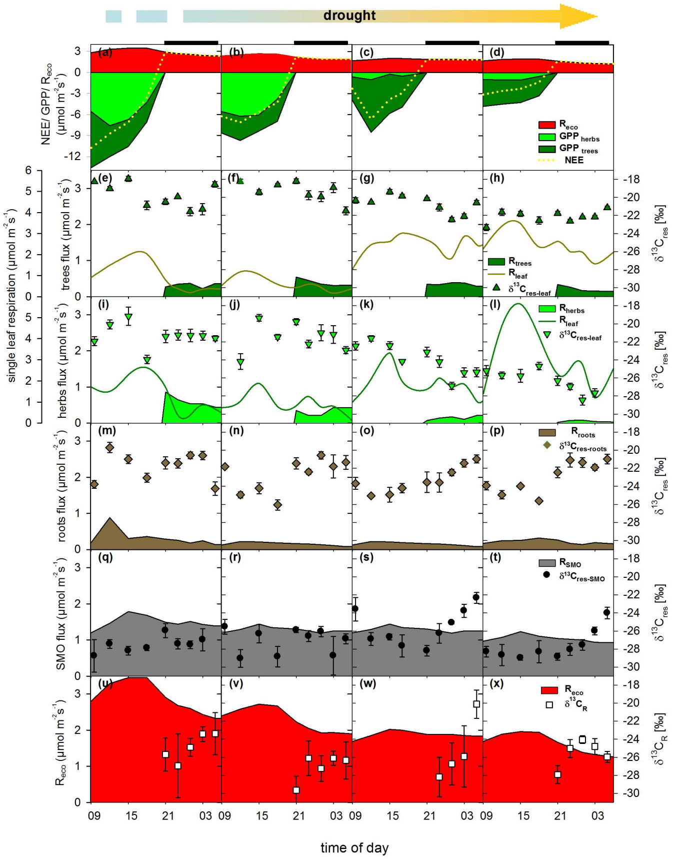

Fig. 4.

Fig. 4 Diurnal dynamics of ecosystem and component fluxes and their isotopic compositions in a Mediterranean oak woodland during the first two weeks of summer drought from 20 May to 3 June 2006. Carbon fluxes were measured by eddy covariance on a 28 m-high tower, a below-canopy eddy covariance system and chamber techniques. Isotopic compositions of carbon fluxes were measured by Intube incubation (see Werner et al., 2007) or estimated by the Keeling plot approach (Keeling, 1958): (**a–d**) Net ecosystem carbon exchange (dotted line), Gross primary production of trees (dark green area) and herbal understory (light green area), ecosystem respiration (red area); (**e–h**) Single leaf respiration of *Q. ilex* (line) and its isotopic composition (triangles); (**i–l**) Single leaf respiration of *Tuberaria guttata* (main understory species, line) and its isotopic composition (upside-down triangles); (**m–p**) Roots respiration (estimated as the difference from chamber measurements on control and root-free trenched plots, brown area) and isotopic composition of *T. guttata* root respiration (diamonds); (**q–t**) Soil microbial respiration (as measured with chambers on root-free trenched plots, grey area) and its isotopic composition (as estimated from chamber Keeling plots, circles); (**u–x**) Ecosystem respiration and its isotopic composition (as estimated from Keeling plots, squares). Black bars indicate night-time (adapted from Unger et al., 2009; 2010a).

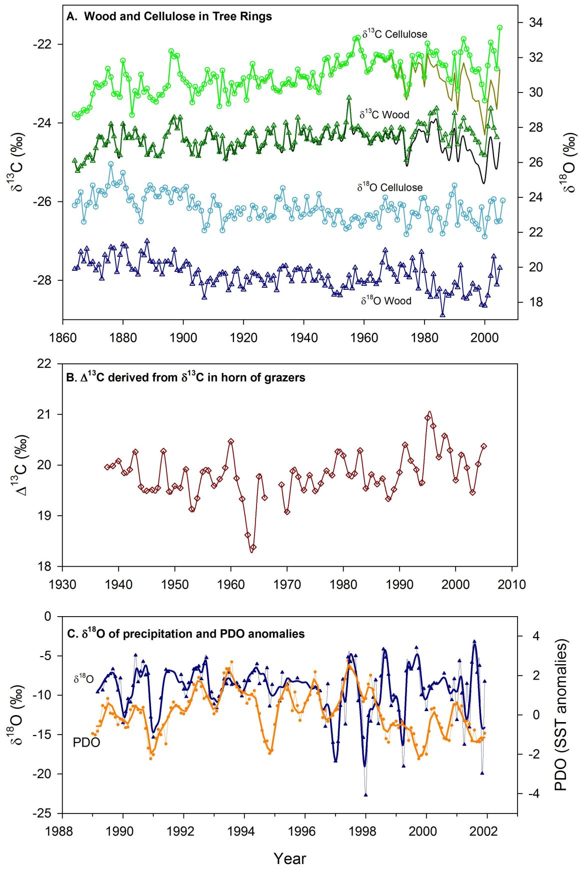

Fig. 5.

Fig. 5 Temporal analysis δ^{13}C and δ^{18}O variations: examples of different samples and archives. **(A)** Tree ring δ^{13}C and δ^{18}O analysis of bulk wood (triangles) and extracted cellulose (circle), data from Sidorova et al. (2009), with two different corrections for the ^{13}C values in tree rings one according to Francey et al. (1999), corrected for the Süess-Effect (lines), the other according to Loader et al. (2010) (lined symbols), which also takes the CO$_2$ response of photosynthesis into account. **(B)** Carbon isotope discrimination (Δ^{13}C) derived from horns of Alpine Ibex (*Capra ibex*) from Augstmatthorn – Brienzer Rothorn between 1938 and 2006, data from Barbosa et al. (2010). **(C)** The monthly ^{18}O precipitation values of a site in eastern Washington in comparison to the Pacific Decadal Oscillation (PDO) index: monthly means values two-days running averages. Note periods of close and fractured correspondence; data from Cohn et al. (2009). Please note different scales on x- and y-axes.

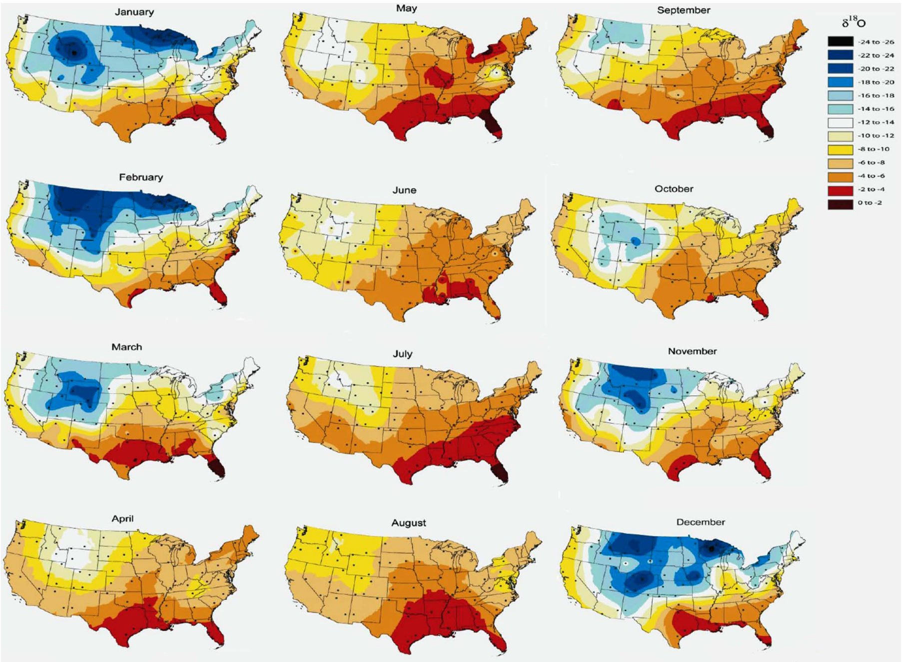

Fig. 6. Changes in the monthly spatial distribution of δ^{18}O of precipitation (isoscapes) across the US. Note the strong depletion of precipitation in the northern Rocky Mountain region. Data from Vachon et al. (2010b).