

Stable isotopes in arid and semi-arid forest systems

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Abstract

The use of stable isotope techniques in forest ecology research has grown steadily over the last two decades. This trend will continue, as stable isotopes can serve as valuable integrators of how plants have interacted, today and in the past, with their physical environment or with other organisms. This is particularly relevant in dry areas due to the strong limitation of resources. After a brief introduction about the theoretical background of stable isotopes in plant physiology, this review highlights recent advances in forest ecology that have embraced this methodology, at different spatial and temporal scales, and with special emphasis on arid and semi-arid systems.

Key words: ecophysiology, $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, $\delta^2\text{H}$, drought, water-use efficiency, climate.

Resumen

Isótopos estables en sistemas forestales de ambientes áridos y semiáridos

El empleo de isótopos estables en el ámbito de la ecología forestal ha ido creciendo progresivamente en las últimas dos décadas. Cabe esperar que esta tendencia se mantenga en el futuro, ya que éstos aportan una visión integradora de cómo las plantas, hoy y en el pasado, han interactuado con el medio así como con otros organismos. Su implementación es particularmente relevante en climas secos debido a la fuerte limitación de recursos que en ellos acontece. Tras una breve introducción sobre las bases teóricas de los isótopos estables en fisiología vegetal, esta revisión destaca, sobre diferentes escalas espaciales y temporales, los últimos avances en ecología forestal empleando esta metodología y con un énfasis especial en los sistemas áridos y semiáridos.

Palabras clave: ecofisiología, $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, $\delta^2\text{H}$, sequía, eficiencia en el uso del agua, clima.

Introduction

At present, the development of useful tools for a complete comprehension of forest system dynamics in response to varying environmental conditions is a key issue in ecology research. This is particularly relevant in dry areas, where resources are scarce and may become even more limited in the future. The information obtained from most ecophysiological approaches (e.g. gas exchange or pressure-volume measures), however, is often limited by sample size because time or technical resources are limited. In particular, they are difficult to extrapolate over large

temporal and/or spatial scales. In this context, stable isotopes offer integrated estimates of stress impacts and life history in ecological systems. Here, we will present the theoretical basis and different applications of carbon, oxygen and hydrogen isotopes as integrative tools in a variety of fields related to forestry, from ecology to forest management through to genetics and palaeoclimatology.

Stable isotopes in the biosphere

Isotopes are atoms of the same element that have different number of neutrons, and can be divided in radioactive and non-radioactive. The former disintegrate spontaneously over time to form other isotopes, whereas the latter do not appear to decay into

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other isotopes on geologic time scales, and thus are also known as stable isotopes. The most abundant elements in the biosphere are carbon (C), hydrogen (H) and oxygen (O), $^{13}\text{C}/^{12}\text{C}$, $^2\text{H}(\text{D})/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$ being the isotope pairs of greatest interest in ecology, particularly for arid and semi-arid systems owing to their relevance in the study of plant-water relationships. The stable isotope composition of a given sample is determined by mass spectrometry, and is usually expressed in differential (δ) notation:

$$\delta^N X(\text{‰}) = (R_{\text{sample}} / R_{\text{standard}} - 1) \times 1000 \quad \text{Eq. 1}$$

where $\delta^N X$ stands for the isotopic composition, in parts per mil (‰) of the heavier isotope ($^N X$), as referred to a standard (see Table 1). R denotes the ratio of the heavy to light isotope (e.g., $^{13}\text{C}/^{12}\text{C}$), and R_{sample} and R_{standard} are the ratios in the sample and the standard, respectively. A positive δ value means that the isotopic ratio of the sample is higher than that of the standard; conversely, a negative δ value denotes an isotopic ratio of the sample lower than that of the standard. The isotopic composition of a given element varies considerably between the different pools of the biosphere. This phenomenon is called isotopic fractionation, and is determined by isotope effects occurring during the cleavage or formation of atomic bonds, as well as during other processes affected by atomic mass (e.g. diffusion, phase changes). Thus, some substances are enriched in the heavier isotope, while others become depleted.

Table 1. Standards, notation, abundance, typical range in plants and mean analytical error of the stable isotopes most commonly used in plant physiology. Original data from Epstein *et al.* (1977), Ehleringer and Rundel (1988), Farquhar *et al.* (1989) and Barbour *et al.* (2001)

	Isotope pair		
	$^{13}\text{C}/^{12}\text{C}$	$^{18}\text{O}/^{16}\text{O}$	$^2\text{H}(\text{D})/^1\text{H}$
Standard	PDB ^b	SMOW ^c	SMOW ^c
Differential notation	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	δD
Mean abundance (‰) ^a	1.1	0.20	0.015
Observed range δ (‰)	-35 to -5	+15 to +35	-300 to +20
Analytical error ^d (‰)	0.1	0.05-0.2	4-7

^a Abundances of the heavier isotope against the total pool of the element.

^b PDB, Pee-Dee Belemnite (limestone): already used up, replaced by secondary standards.

^c SMOW, Standard Mean Ocean Water.

^d Overall analytical precision (standard error: sample preparation + internal error of mass spectrometer).

Carbon isotopes theory

The most usual values of $\delta^{13}\text{C}$ in the biosphere range between -40‰ and 0‰ (Fig. 1). Atmospheric CO_2 is the carbon source for terrestrial plants, and thus its variability is especially important for plant physiologists. Current average $\delta^{13}\text{C}$ of atmospheric CO_2 is -8‰, although this value is becoming more negative year by year (*ca.* 0.02-0.03‰/year) due to the synergic effect of deforestation and the use of fossil fuels (McCarroll and Loader, 2004). Atmospheric $\delta^{13}\text{C}$ variations should be considered when comparing species differing in their growing cycles, or distant samples, either in time or space.

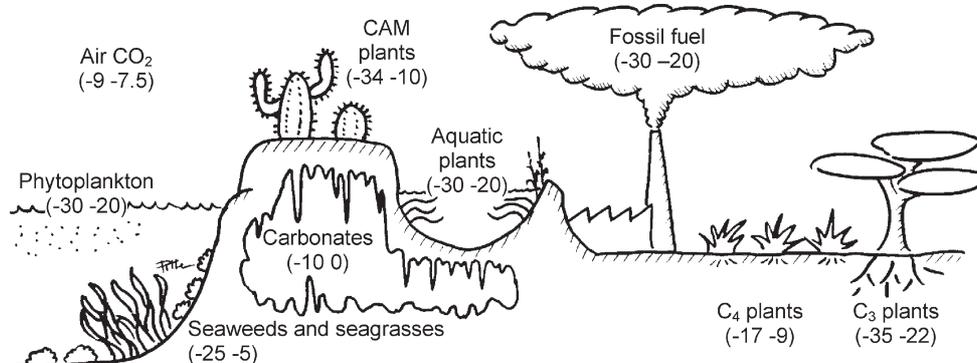


Figure 1. Range of carbon isotopic composition ($\delta^{13}\text{C}$) within the main compartments of environment and biosphere. Redrawn from Mateo *et al.* (2004).

During photosynthesis, C_3 plants take preferentially ^{12}C instead of ^{13}C . The magnitude of this event is affected by several physiological processes, and is expressed in terms of carbon isotope discrimination ($\Delta^{13}C$), as defined by Farquhar *et al.* (1989):

$$\Delta^{13}C_{\text{plant}} = \frac{\delta^{13}C_{\text{air}} - \delta^{13}C_{\text{plant}}}{\left(1 + \frac{\delta^{13}C_{\text{plant}}}{1000}\right)} \quad \text{Eq. 2}$$

where $\delta^{13}C_{\text{air}}$ and $\delta^{13}C_{\text{plant}}$ refer to air and plant composition, respectively. In C_3 species (e.g. most tree species), $\Delta^{13}C$ in plant tissues constitutes an integrated record of the ratio of intercellular to atmospheric concentration of CO_2 (c_i/c_a) during the period in which carbon atoms were fixed, and thus reflects the balance between assimilation rate (A) and stomatal conductance (g_s), that is, intrinsic water-use efficiency (WUE_i). Plants typically react against a decrease in water availability through stomata closure and, although A may also decline, g_s is usually affected to a larger extent, originating a reduction in $\Delta^{13}C$ (Farquhar *et al.*, 1989). The rate of evaporation from the leaf also determines stomatal responses that subsequently affect $\Delta^{13}C$. Indeed, an increase in the leaf-to-air vapour pressure deficit (VPD, the driving force for transpiration) will also cause lower $\Delta^{13}C$ values (Barbour and Farquhar, 2000). This is the basis for the extensively reported relationships between $\Delta^{13}C$ and environmental variables related to water availability or VPD, such as precipitation, relative humidity or potential evapotranspiration (see references in Ferrio *et*

al. (2003b)). Nevertheless, although changes in WUE_i are usually derived from stomatal responses, both genetic and environmental factors may cause variations in A , thus affecting WUE_i and $\Delta^{13}C$.

Oxygen and Hydrogen isotopes theory

During the hydrological cycle, the oxygen and hydrogen isotope compositions ($\delta^{18}O$, δ^2H) of water suffer several fractionations (Fig. 2). On the one hand, light isotopes (^{16}O and 1H) evaporate more rapidly than their heavier counterparts, and thus water vapour is isotopically depleted with respect to source water (e.g. ocean water). On the other, the opposite occurs during precipitation, when the heavier isotopes are selectively precipitated through distillation. The amount of this fractionation varies according to temperature and altitude, resulting in an isotopic composition of precipitation that is positively correlated with temperature, and negatively with altitude. After that, there are several potential fractionations before the water isotopes become fixed in plant tissues (Fig. 2). The first occurs within the soil, as evaporation affects the original isotopic signal, so the residence time and depth of soil water is important (see e.g. Saurer *et al.* (1997)). Although there is no evident fractionation when plant roots absorb soil water, further fractionations occur in the leaves. As a consequence, the $\delta^{18}O$ and δ^2H of plant tissues reflect the variation in (1) isotope composition in source water, (2) evaporative enrichment of leaf water due to

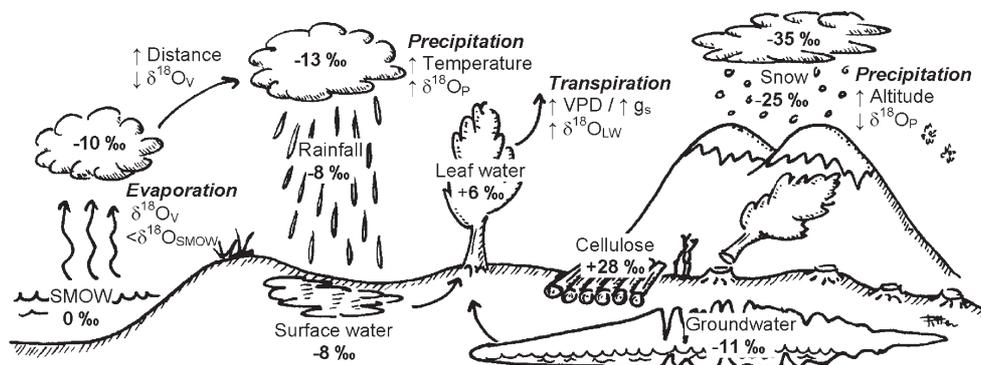


Figure 2. Main fractionation steps and typical values of oxygen isotope composition ($\delta^{18}O$) in a temperate climate. $\delta^{18}O_{\text{SMOW}}$, standard mean $\delta^{18}O$ in ocean water; $\delta^{18}O_v$, $\delta^{18}O_p$, $\delta^{18}O_{\text{LW}}$, $\delta^{18}O$ in water vapour, precipitation (either rainfall or snow) and leaf water, respectively; VPD, vapour pressure deficit; g_s , stomatal conductance. Original data from IAEA/WMO (2001) and Saurer *et al.* (1997). The same fractionation processes are valid for hydrogen isotopes.

transpiration, and (3) biochemical fractionation during the synthesis of organic matter (Yakir, 1992).

The isotopic enrichment in the leaves depends on transpiration rates and is therefore affected by both g_s and VPD (Dongmann *et al.*, 1974, Barbour and Farquhar, 2000). Plants growing at lower VPD are expected to have lower enrichment, while, under identical environmental conditions, plants with higher g_s become less enriched. Leaf water signal is then passed onto the organic molecules formed in the leaf by exchange of oxygen and hydrogen atoms (Yakir, 1992). Besides, in the case of stem cellulose, most of the enrichment signal is further exchangeable with xylem water during the heterotrophic pathways of cellulose biosynthesis (Roden *et al.*, 2000). This is determining for the observed relationship between $\delta^{18}\text{O}/\delta^2\text{H}$ of tree-ring cellulose and precipitation, as it enhances the source-water signal, softening the effect of leaf-level enrichment (Saurer *et al.*, 1997, McCarroll and Loader, 2004).

Applications

Trees in a changing environment: from daily to millennial time scales

The major attraction of stable isotope techniques in ecophysiological studies lies on their ability to integrate plant processes over time. This is particularly interesting if we aim to determine tree responses to environmental changes. Depending on the material of study, the timeframe that can be covered may range from a few days to thousands of years.

Seasonal variability

Carbon stable isotopes, when measured in leaf soluble sugars and/or starch, provide short-term estimations for c_i/c_a and WUE_i in C_3 species (Lauteri *et al.*, 1993, Jäggi *et al.*, 2002). Similar information can be acquired from the analysis of phloem sap sugars collected from incisions in the trunk (Cernusak *et al.*, 2003, Keitel *et al.*, 2003). This last method has the advantage that phloem sap integrates, in a single sample, the photosynthates produced from several branches, thus being far more representative of the overall tree physiological status than a single leaf.

Alternatively, $\delta^{18}\text{O}$ of non-structural carbohydrates may reflect either the seasonal variation of source water or the magnitude of transpirative enrichment (mostly related to g_s and VPD), or both (Jäggi *et al.*, 2003, Cernusak *et al.*, 2003, Keitel *et al.*, 2003). Once the effect of source water is removed, the combined analysis of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in phloem sap has provided evidences that seasonal variation in WUE_i of temperate trees should be attributed to changes in g_s rather than in A (Cernusak *et al.*, 2003, Keitel *et al.*, 2003).

Due to the lower turnover rates of leaf cellulose, when compared to non-structural carbohydrates, the $\delta^{13}\text{C}$ of leaf bulk material is mostly affected by the environmental conditions prevailing during leaf expansion (Damesin *et al.*, 1998). This is particularly relevant in evergreen species, as $\delta^{13}\text{C}$ values in old leaves are nearly stable, showing little response against year-to-year variations (Damesin *et al.*, 1998, Adams and Kolb, 2004). Nevertheless, leaf cellulose analyses have been useful to compare environmental responses for different forest species. For example, Damesin *et al.* (1997) analysed leaf $\delta^{13}\text{C}$ of *Quercus ilex* and *Quercus humilis* growing in mixed stands, varying in water availability. They reported a similar geographic variation in $\delta^{13}\text{C}$ for both species, and concluded that, despite their differences in phenology and habitat preferences, both species were characterised by similar WUE_i . In other cases, however, contrasting performances have been reported for co-occurring species. Adams and Kolb (2004), for example, showed that *Pinus ponderosa* trees growing in their low elevation limit in Northern Arizona exhibited a greater $\delta^{13}\text{C}$ (and WUE) change in response to drought than the co-occurring *Pinus edulis*. In another study, Terwilliger *et al.* (2002) found that, whereas leaf cellulose of *Pinus edulis* showed a negative trend in $\delta^{18}\text{O}$ and $\delta^2\text{H}$ with altitude (as expected from meteoric water variation), the opposite occurred for the accompanying shrub *Yucca glauca*. They attributed such difference to the extensive system of tubers used by the yucca for water storage, which might alter the isotopic signal of the source water used by the leaves.

It is also possible to track the seasonal course of stable isotopes by cutting wood slices along the axis of an increment core. Indeed, several studies on conifers have demonstrated that the isotope composition of consecutive wood layers reflects the environmental conditions of the period in which they are formed (Warren *et al.*, 2001, Barbour *et al.*, 2002, Jäggi *et al.*,

2003). However, Kagawa *et al.* (2005) showed, in a $^{13}\text{CO}_2$ pulse-labelling experiment, that carbon may be incorporated into stem cellulose several weeks after its fixation, which reduces the maximum time resolution of tree rings to about one month. Besides, deciduous species rely strongly on stored carbohydrates for early wood development, which causes unexpected $\delta^{13}\text{C}$ variations in early wood (Helle and Schleser, 2004). Even though this last shortcoming could be ruled out (e.g. in evergreen species), this method is time-consuming, especially for slow-growing species where the slices should be thinner to obtain acceptable resolutions. In this framework, a new technique for high-resolution online determination of $\delta^{13}\text{C}$ in tree rings has been developed by coupling a mass spectrometer to a laser ablation device, which offers a promising future for the study of short-term climate effects on trees (Schulze *et al.*, 2004).

Long-term environmental changes reflected in tree rings

So far, most studies on stable isotopes in tree rings have focused on temperate and boreal environments, where temperature is the main limiting factor for growth. Thus, $\delta^{13}\text{C}$ variations in tree rings have often been related to temperature (Stuiver and Braziunas, 1987, Anderson *et al.*, 1998). However, according to current theory on plant carbon discrimination, little or no direct effect of temperature on $\delta^{13}\text{C}$ is expected. In fact, most of these studies found stronger relationships with other variables, such as relative humidity (Stuiver and Braziunas, 1987) or precipitation (Anderson *et al.*, 1998). Hence, these results are probably derived from indirect relationships between temperature and plant water status. Indeed, $\delta^{13}\text{C}$ in tree rings has shown to be strongly correlated to modelled soil water balance, precipitation and evaporative demand (Dupouey *et al.*, 1993, Korol *et al.*, 1999, Warren *et al.*, 2001, Ferrio *et al.*, 2003a). These relationships are restricted to seasonally dry climates; in other contexts, irradiance, altitude and nutrient availability appear to be the main factors responsible for isotopic variations (Warren *et al.*, 2001). The magnitude of such relationships is species-dependent, varying according to the particular adaptive mechanisms exhibited to cope with drought. For example, species with low reliance on stomatal control (either through tolerance strategies, or

deep-root systems) tend to show little $\delta^{13}\text{C}$ variation in response to changes in aridity (Picon *et al.*, 1996, Ferrio *et al.*, 2003a).

Early studies on $\delta^{18}\text{O}$ and $\delta^2\text{H}$ in tree rings found that both isotopes were strongly correlated with changes in average temperature, associations that were attributed to the isotopic fractionation occurring in precipitation (see references in McCarroll and Loader, (2004)). However, in some cases the leaf transpirative enrichment may be strong enough to hide the original isotopic signal of source water (Cernusak *et al.*, 2003). For example, in their recent work Ferrio and Voltas (2005) found that, over a geographic gradient of aridity and temperature, wood $\delta^{18}\text{O}$ from *Pinus halepensis* was not related to the $\delta^{18}\text{O}$ of precipitation and only poorly related to temperature, but it was closely linked to VPD and, to a lesser extent, to precipitation (Fig. 3). Thus, the environmental factors reflected in $\delta^{18}\text{O}$ were similar to those affecting $\delta^{13}\text{C}$. Since the processes underlying the relationships between both isotopes and climate variables are known to differ, it was concluded that both isotopes provided complementary information on plant reaction to the environment.

Although most of the work cited so far is limited to the last 100 or 200 years, isotope studies of tree rings have been also expanded to some large-scale tree-ring chronologies, providing high resolution climatic reconstructions throughout the Holocene (see references in Ferrio *et al.* (2003b)).

Adaptation, genetics and breeding

The adaptability of forest tree species is related to their potential to respond to changing environmental conditions. In arid and semi-arid climates, where water is the most limiting resource for growth and survival, water availability plays an essential selective role in the physiological mechanisms underlying adaptation. One physiological trait that may be relevant to the capacity of plants to thrive in dry areas is WUE_i (Farquhar and Richards, 1984). Assessments of differences in WUE_i among and within tree species are facilitated by the time-integrative nature of $\Delta^{13}\text{C}$. Provided that plant material collected from different habitats is sampled under uniform garden conditions, then variation in $\Delta^{13}\text{C}$ among geographic origins can be employed to infer genetic diversity in WUE_i . In general, studies on genetic diversity in $\Delta^{13}\text{C}$ for forest tree

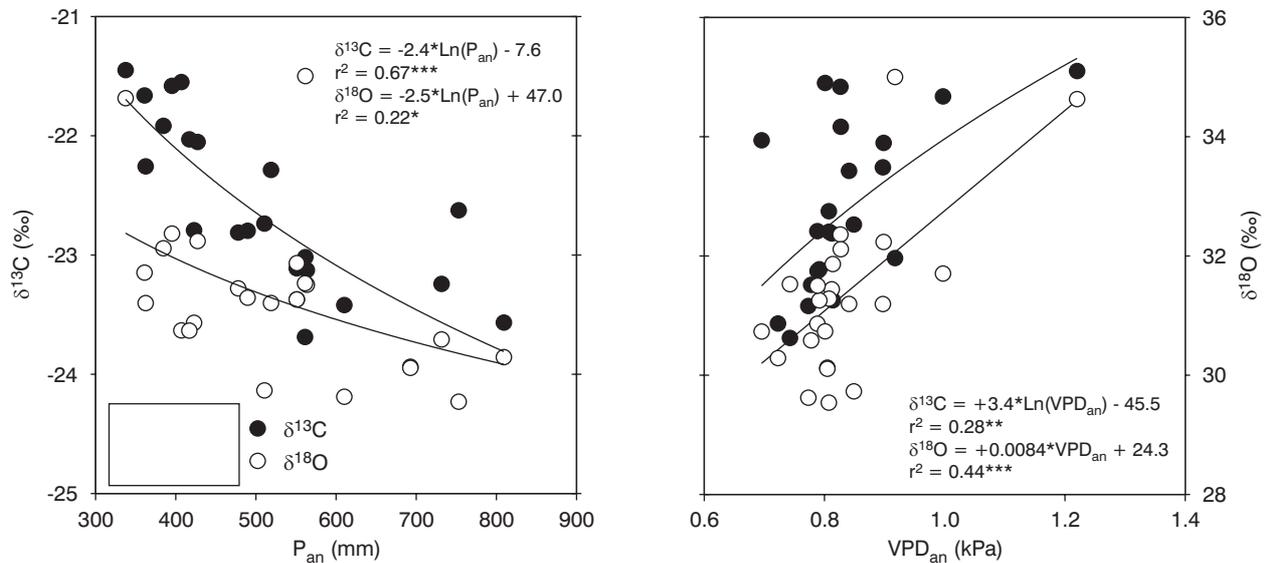


Figure 3. Relationship between annual means of precipitation (P_{an}) and vapour pressure deficit (VPD_{an}) and either carbon ($\delta^{13}C$) or oxygen ($\delta^{18}O$) isotope composition in wood cellulose of *Pinus halepensis* Mill. trees. Original data from Ferrio and Voltas (2005).

species from both mesic and xeric environments suggest the existence of significant variation in WUE_i (e.g. Cregg *et al.* (2000), Li and Wang (2003)). Such variability may be considerable. For example, in a common garden trial with *Pinus halepensis* comprising 25 European populations, the range of variation in tree-ring $\Delta^{13}C$ was about 2.5‰ (Voltas, Chambel and Prada, unpublished data), which suggests a maximum difference in WUE_i among populations of over 25% (Farquhar and Richards, 1984). Understanding genetic variation in WUE_i (through $\Delta^{13}C$) in relation to geographic and/or climatic gradients is essential in evaluating adaptation patterns in drought-prone areas. In this context, several studies have shown that an environmental feature such as VPD can produce, among other factors related to plant water availability, contrasting genetic variation in $\delta^{13}C$ among populations. Comstock and Ehleringer (1992) found that populations of the desert perennial shrub *Hymenoclea salsola* from high-VPD sites showed low $\Delta^{13}C$, but positive and non-significant relationships between VPD and $\Delta^{13}C$ have also been detected, respectively, for the more mesic species *Pseudotsuga menziesii* and *Pinus ponderosa* (Zhang and Marshall, 1995) (Fig. 4). These contrasting results suggest that genetic patterns of variation in $\Delta^{13}C$ within species may be the result of different selection pressures resulting

from the interaction of several climatic variables and physiological strategies.

Since $\Delta^{13}C$ allows for ranking WUE_i among genotypes, the relationship between $\Delta^{13}C$ and growth in arid and semi-arid regions has been investigated in numerous studies. In principle, trees with high WUE_i (i.e. low $\Delta^{13}C$) should maintain higher growth rates under water shortage than trees with lower WUE_i . However, both positive and negative relationships between tree growth and $\Delta^{13}C$ have been reported for different tree species. A feasible explanation of such controversial results originates from the dependence of $\Delta^{13}C$ on changes in either A or g_s (see e.g. Prasolova *et al.* (2001)). If higher WUE_i is related to low g_s , then genotypes with a rapid water use (i.e. high g_s) would develop deeper roots to enhance water supply, hence allowing for a faster growth. Thus, positive relationships between $\Delta^{13}C$ and growth have been described for several *Eucalyptus* species (Pita *et al.*, 2001, Li and Wang, 2003), although opposite associations have also been documented among *Pinus ponderosa* populations as a consequence of limited water loss through reduced g_s in the low $\Delta^{13}C$ trees (Cregg *et al.*, 2000). On the other hand, genetic variation in $\Delta^{13}C$ for *Araucaria cunninghamii* has been linked to changes in A , thus resulting in negative associations between $\Delta^{13}C$ and stem diameter

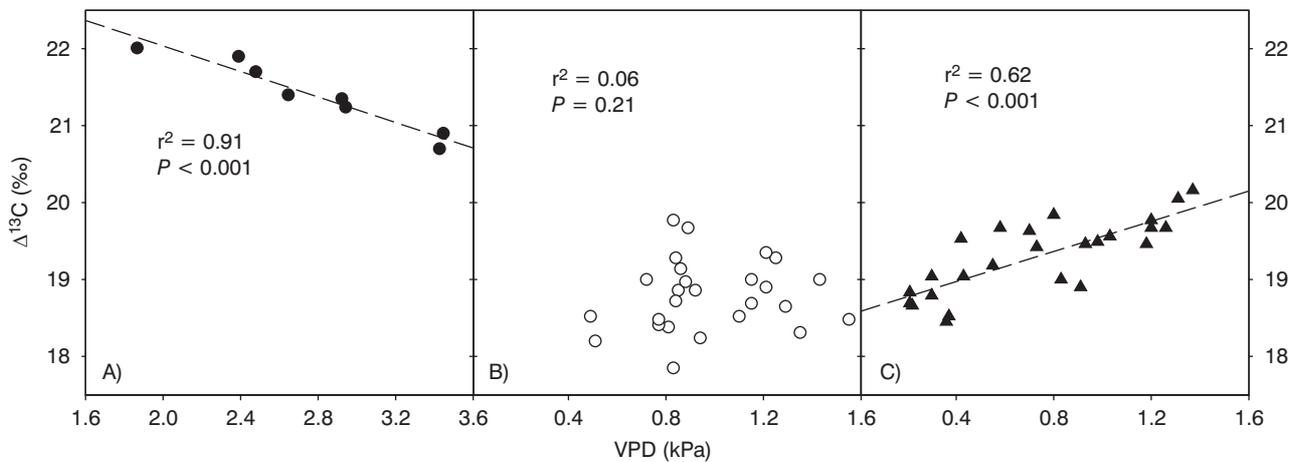


Figure 4. Relationship between growing season vapour pressure deficit (VPD) of seed source and carbon isotope discrimination ($\Delta^{13}\text{C}$) measured on leaf bulk tissue of common garden plants in *Hymenoclea salsola* (A), *Pinus ponderosa* (B), and *Pseudotsuga menziesii* (C). Adapted from Comstock and Ehleringer (1992) and Zhang and Marshall (1995).

(Prasolova *et al.*, 2001). Since factors affecting g_s can also affect the $\delta^{18}\text{O}$ of leaf organic matter, then measuring both $\Delta^{13}\text{C}$ and $\delta^{18}\text{O}$ may help determine the source of variation in $\Delta^{13}\text{C}$ (Yakir, 1992, Barbour and Farquhar, 2000). Whatever the underlying cause of genetic variation in $\Delta^{13}\text{C}$, it appears as a promising tool for genotype selection in dry areas. The relatively high genetic control for $\Delta^{13}\text{C}$ (as indicated by high heritability estimates) has recently launched the interest in dissecting the Mendelian factors controlling this adaptive trait in species such as *Castanea sativa* (Casasoli *et al.*, 2004) or *Pinus pinaster* (Brendel *et al.*, 2002) through QTL analysis. This approach opens new prospects for the characterisation of the genetic architecture of WUE_i and its role in determining tree responses to drought.

Forest management

Cultural practices such as thinning and other disturbances in forest systems are usually associated with a reduction in competition and a higher availability of soil moisture to the remaining trees. Such an increase in water availability is expected to increase g_s , hence causing a reduction in WUE_i , and this effect can be confirmed using carbon isotopes. For example, McDowell *et al.* (2003) analysed $\Delta^{13}\text{C}$ in tree rings before and after thinning in a stand of old, dominant *Pinus ponderosa* trees. In accordance with

the assumption of increasing g_s , the authors observed a progressive increase in $\Delta^{13}\text{C}$ of the remaining trees during the first six years after thinning, after which $\Delta^{13}\text{C}$ became stable. However, stomatal response to greater water availability can be counterbalanced if stand perturbations also increase nutrient concentration and/or enhance the interception of radiation, both having the potential of increasing photosynthetic capacity. Hence, an increase in WUE_i (due to greater A), associated to a decrease in $\Delta^{13}\text{C}$, has been found for *Pinus radiata* and *Pinus pinaster* in heavily thinned stands (Warren *et al.*, 2001) and for *Quercus ilex* resprouts after either fire or tree felling, compared to undisturbed vegetation (Fleck *et al.*, 1996). On the other hand, the sensitivity of $\Delta^{13}\text{C}$ to changes in water and nutrient availability has shown to be useful in tracking the physiological response of trees in watering and fertilising experiments, even when no significant changes in growth and other physiological variables could be detected (Korol *et al.*, 1999, Voltas *et al.*, 2005).

Ecosystem Dynamics

Response to Pulses and Resource Use

Ecosystem dynamics in arid and semiarid regions are controlled by intermittent pulses of precipitation that vary considerably in space and time (Noy-Meir,

1973). The magnitude and timing of precipitation pulses and the translation of these inputs into available soil water drives plant and microbial activity and thus determines gas exchange, biogeochemical processes and productivity of ecosystems. However, plants do not respond equally to inputs of growing season moisture. Intensive root exploiters, such as herbaceous dicots and graminoids, tend to be more responsive to small rainfall pulses than do extensive root exploiters, such as woody perennials (Ehleringer *et al.*, 1991), but large rainfall events that moisten deep soil layers are most efficiently used by extensive root exploiters (Fig. 5) (Golluscio *et al.*, 1998). Soil properties, particularly soil texture, can modify infiltration and percolation and alter the sensitivity of plant species to precipitation inputs (Fravolini *et al.*, 2005). Taken together, interactions among precipitation events, plant functional type and soil hydraulic properties establish a complex mosaic of potential ecosystem responses to growing season precipitation in arid and semiarid landscapes.

Utilisation of individual precipitation pulses and ecosystem-level gas exchange responses can be evaluated with stable isotope measurements. A simple two-component mixing equation can be used to calculate the fractional utilisation of a precipitation pulse event by plants using stable isotope

measurements of water. For deuterium (^2H) the equation takes the form:

$$\delta^2H_p = f(\delta^2H_{\text{prec}}) + (1-f)(\delta^2H_{\text{soil}}) \quad \text{Eq. 3}$$

where δ^2H_p is the hydrogen isotopic composition of non-evaporated plant xylem water after the precipitation event, δ^2H_{prec} is the isotopic composition of the precipitation event and δ^2H_{soil} is that of the antecedent soil moisture used by the plant prior to the precipitation event (Williams and Ehleringer, 2000). The variable f represents the fractional contribution of the precipitation pulse to the plant's transpiration. Such measurements, taken through time, reveal the dynamics of plant water sources with respect to individual precipitation pulses and provide insight into controls on ecosystem gas exchange in water-limited environments. Using this approach with experimentally applied large (35mm) and small (10mm) irrigation events, Fravolini *et al.* (2005) showed that the deep-rooted woody plant, velvet mesquite (*Prosopis velutina*), on clay soils in the Sonoran Desert utilised the large pulse event, but did not respond to the small pulse (Fig. 6). On nearby coarse-textured soils, this species responded to both large and small irrigation pulses. The $\delta^{13}\text{C}$ value of soluble leaf carbohydrates in this species decreased by 2‰ within two days after the large and small irrigation events on the coarse textured soil, but decreases were observed only for plants receiving the large pulse on the clay soil. Such rapid decreases in leaf soluble carbohydrates reflect increases in g_s and potentially enhanced A following the pulse. These results show the power of stable isotope measurements for revealing integrated physiological responses to rainfall events in dryland ecosystems.

Plants have fairly high thresholds for response to rainfall events compared to soil microbial communities. Cable and Huxman (2004) analysed the $\delta^{13}\text{C}$ of respiratory CO_2 from desert soils and partitioned the relative contribution of soil microbial crusts and soil microbes/roots to total respiration of desert surfaces following pulses of precipitation (Fig. 5). The contribution of these ecosystem components to total respiration depended on precipitation pulse size. After a small pulse event (2mm), the contribution of crusts to night-time soil CO_2 efflux increased to over 80%. This was large compared to responses following a large pulse event (25 mm),

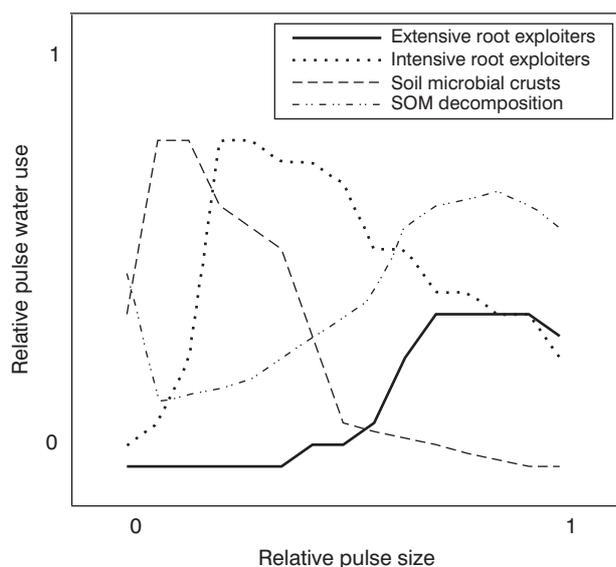


Figure 5. Conceptual figure of the use of pulse water as a function of pulse size by different ecosystem components. Based on data from Ehleringer *et al.* (1991) and Cable and Huxman (2004).

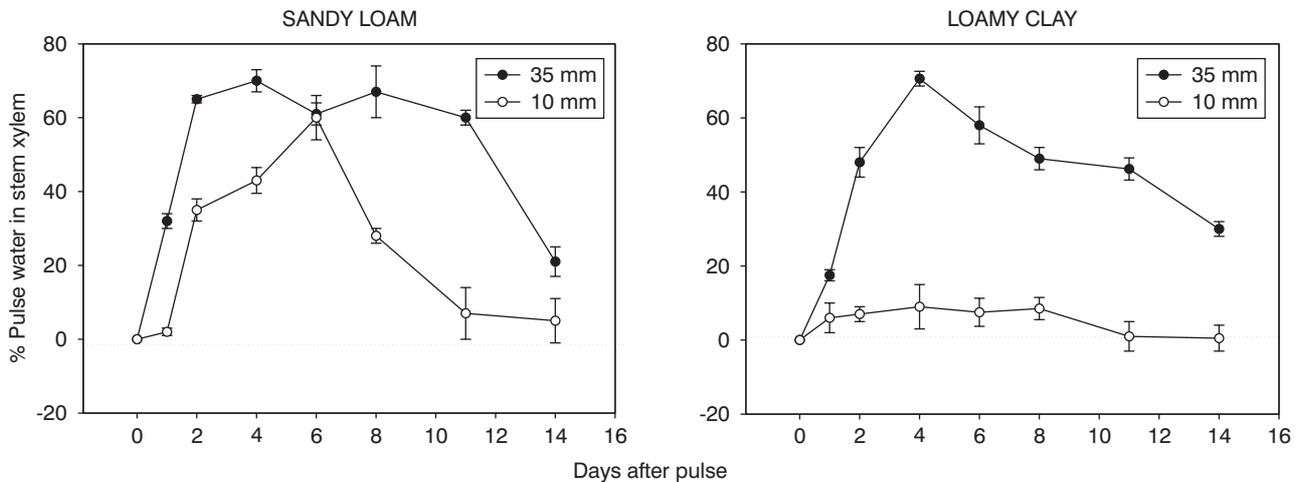


Figure 6. Percentage of pulse water used by *Prosopis velutina* during the two weeks following irrigation across soil textures. Day 0 is 21 June 2002. Error bars indicate ± 1 SE. From Fravolini *et al.* (2005).

where the contribution from soil organic matter decomposition and root respiration dominated the respiration flux (Fig. 5). The vast majority of rainfall events in the three warm deserts of North America (Mojave, Sonoran and Chihuahuan) are smaller than 2 mm. Therefore, in these warm deserts, crusts are potentially the sole contributors to ecosystem carbon cycling following the majority of rain events.

Species Interactions and Water Use Efficiency

Interactions among species, both positive and negative, are strong determinants of community composition (Roughgarden and Diamond, 1986). Their relative importance varies with the age of vegetation. Different plant functional types show specific patterns of water uptake. But these differences are minimised at seed germination and seedling establishment, which are key stages in individual and population success (Grubb, 1977). The amount of water available at this stage, as mediated by community composition, highly influences which species will regenerate. As individuals mature, a spatial partitioning of resources occur (Walter, 1971), where intensive root exploiters take advantage of the water in shallow soil layers and extensive root exploiters explore large volumes of soil. Details of these interactions can be elucidated with stable isotope measurements.

$\Delta^{13}\text{C}$ of leaf bulk tissue may be related to the intensity of competition. Fravolini and Williams (in

prep.) observed lower $\Delta^{13}\text{C}$ values in velvet mesquite seedlings growing with an intensive root exploiter (*Eragrostis lehmanniana*) than in those seedlings growing on bare ground. This higher water stress created lower establishment rates in plots with *E. lehmanniana* (between 0.5 and 2%) than in those on bare ground (between 4 and 9%).

Facilitation in semiarid environments may occur via hydraulic redistribution, the passive movement of water across the soil profile via plant roots (Caldwell and Richards, 1989). Peñuelas and Filella (2003) used labelled water, highly enriched in deuterium, to study the effect of hydraulic redistribution by *Pinus nigra* on the ecophysiological performance of conspecific and heterospecific neighbouring trees. They concluded that neighbouring trees, regardless of species, obtained water hydraulically lifted by *Pinus nigra*.

Challenges and prospects

To date, the utility of stable isotopes for scaling physiological processes across temporal and spatial scales has been firmly established through an increasingly large number of studies in forest ecology. In the following years, research on past, present and future global change will undoubtedly benefit from stable isotope analysis in a variety of materials including tree rings, leaves and soils as indicators of the environment in contemporary and ancient contexts. However, achieving a better integration of the

information provided by the three isotope pairs of greatest biological interest, is still a major challenge that needs to be faced at various levels of plant physiological ecology. This would certainly allow for a more efficient exploitation of these valuable proxy tools, especially in dryland ecosystems.

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