Artículo de Revisión

Rev. Fitotec. Mex. Vol. 42 (1): 3 - 11, 2019

SOME USES OF STABLE ISOTOPE TECHNIQUES IN FOREST SCIENCE

ALGUNOS USOS DE TÉCNICAS DE ISÓTOPOS ESTABLES EN CIENCIA FORESTAL

Leandris Argentel-Martínez[1,2], Jaime Garatuza-Payán[2]*, Enrico A. Yépez González[2]
Marco Garrido-Salina[3] y Edmundo Acevedo-Hinojosa[3]

[1]Tecnológico Nacional de México/Instituto Tecnológico del Valle del Yaqui, Bácum, Sonora, México. [2]Instituto Tecnológico de Sonora, Dirección de Recursos Naturales, Cd. Obregón, Sonora, México. [3]Universidad de Chile, Facultad de Ciencias Agronómicas, La Pintana. Santiago, Chile.

**Autor para correspondencia* (jaime.garatuza@itson.edu.mx)

SUMMARY

It is known that for many latitudes worldwide, significant changes in climate variables are predicted for many latitudes worldwide, which mainly include variations in precipitation patterns, temperature increases and CO_2 concentrations; such events, commonly known as climate change, affect the metabolism of forest species, which due to their importance on earth have received special attention from the scientific community, especially in regard to the description of physiological and biochemical mechanisms involved in plants response and adaptation to abiotic stress. Several studies have been developed on this topic, some of them have used stable isotope techniques, which have allowed a better understanding of processes such as water use efficiency and gas exchange in plants, which are related to the carbon and water balance of ecosystems. The present review aims to compile some of the main advances in the use of stable isotopes in forestry science. Although the advances on this subject are significant in such aspects as the description of the origin of plants water uptake, its photosynthetic pathway, changes in the transpiration rate of forest species, the effects of climatic factors on the differential growth of their tissues, and the nitrogen origin during mineral nutrition, in the future such studies will allow a calculation and modeling of photosynthesis, a better understanding of variations in transpiration and nitrogen nutrition efficiency over time.

Index words: Composition, discrimination, oxygen, carbon, nitrogen, water use efficiency.

RESUMEN

Para muchas latitudes en todo el mundo, se prevén cambios significativos en las variables climáticas, que incluyen principalmente fluctuaciones en los patrones de precipitación, aumentos de temperatura y concentraciones de CO_2; tales eventos, comúnmente conocidos como cambio climático, afectan el metabolismo de las especies forestales, que debido a su importancia en la tierra han recibido especial atención por parte de la comunidad científica, especialmente en lo referente a la descripción de los mecanismos fisiológicos y bioquímicos involucrados en la respuesta de las plantas y la adaptación al estrés abiótico. Varios estudios se han desarrollado sobre este aspecto, algunos de ellos han utilizado técnicas de isótopos estables, lo que ha permitido una mejor comprensión de procesos como la eficiencia en el uso de agua y el intercambio de gases en las plantas, fenómenos relacionados con el balance de carbono y agua de los ecosistemas. La presente revisión tiene como objetivo recopilar algunos de los principales avances en el uso de isótopos estables en la ciencia forestal. Si bien los avances en este tema han sido significativos en aspectos tales como la descripción del origen de la absorción de agua por parte de las plantas, su ruta fotosintética, los cambios en la tasa de transpiración de las especies forestales, los factores climáticos que afectan el crecimiento diferencial de sus tejidos y el origen del nitrógeno durante la nutrición mineral, en el futuro tales estudios permitirán un cálculo y modelado de la fotosíntesis, una mejor comprensión de las variaciones en la transpiración y la eficiencia de la nutrición con nitrógeno a través del tiempo.

Palabras clave: Composición, discriminación, oxígeno, carbono, nitrógeno, eficiencia en el uso de agua.

INTRODUCTION

Climate change, mainly manifested as temperature rise and variation in precipitation patterns, has already induced changes in forest ecosystems (Allen *et al.*, 2015). Tree mortality events are expected to increase in large areas of the planet under the current scenario of climate change, with higher temperature and water stress as a common causal factors (Allen *et al.*, 2010; Berry *et al.*, 2014a). These factors affect plant physiology, metabolism and growth, as well as tree productivity (Giammarchi *et al.*, 2017) since forest productivity is the result of convergent physiological and biochemical aspects acting in a coordinated way. Such climate variations have had a negative effect on the adaptive response of forest ecosystems (Cristiansen, 2016).

Nowadays fast and precise analytical techniques, such as isotopic analyses, have been used in forest science to understand mechanisms of plant adaptation to climate change and productivity. These techniques are mainly based on the evaluation of photosynthetic activity, water relations and nitrogen use efficiency (Di Matteo *et al.*, 2017). The use of stable isotope analysis is an important research line since 1927, showing significant advances in the last 20 years; nevertheless, there are still several limitations for the understanding of its relationship with plant development promotion and the explanation of possible physiological mechanisms of adaptation to conditions commonly present in forest ecosystems.

Recibido: 18 de enero de 2018
Aceptado: 18 de diciembre de 2018

The present review compiles some of the scientific results achieved by various researchers that focus their studies on the forest species response to the environment. The topics to be addressed are on the use of stable isotopes for the measurement of photosynthetic activity, water absorption, transpiration, and water and nitrogen use efficiency. In addition, some medium-term perspectives of the isotopic composition and discrimination of ^{13}C, ^{18}O, and ^{15}N involved in forest trees metabolism and productivity will be considered.

HISTORY OF STABLE ISOTOPES AND THEORETICAL ASPECTS

Carbon- ^{13}C

Around 1927 and 1932, light bioelements such as carbon, nitrogen, hydrogen and oxygen isotopes were discovered (Lehmann, 2017). The first studies of the carbon isotope composition in plants showed that it had lower ^{13}C value than carbonates. Later, studies of variations in abundance of ^{13}C in marine and terrestrial plants lead to propose a schematic representation of isotopic fractionation during the photosynthetic cycle for terrestrial plants (Craig and Gordon, 1965). This fractionation on plants is based on the existence of two stable carbon isotopes (^{12}C and ^{13}C) forming part of the atmospheric carbon dioxide (CO_2), which are in approximate proportions of 99 % and 1%, respectively. Plant tissues have a different distribution of $^{13}C/^{12}C$ isotopes in comparison to the atmosphere, mainly associated to the biologic carbon incorporation into the plants, which includes processes of intracellular uptake and diffusion, and photosynthetic fixation of atmospheric CO_2 (O'Leary, 1981). Farquhar *et al.* (1982) developed the theory of isotopic composition in plants, where isotopic discrimination was modeled for diffusion, carboxylation, photorespiration and respiration effects.

The composition of ^{13}C in a plant tissue is determined through mass spectrometry, as an isotopic ratio (R) of the sample of interest in relation to a standard ratio (O'Leary, 1981) as described in Equation 1.

$$\delta^{13}\,C(^{o}/_{oo}) = \left(\frac{\text{R sample}}{\underset{\text{(Pee Dee Belemnite)}}{\text{R standard}}} - 1\right) \times 1000 \qquad \text{[Eq. 1]}$$

Where $\delta^{13}C$ is an isotopic signature, a measure of the ratio of stable isotopes ^{13}C:^{12}C, reported in parts per thousand; R is the molar abundance ratio $^{13}CO_2/^{12}CO_2$ for the sample of interest and the standard, where the standard relation is 0.01124.

It is recommended to use the term isotopic discrimination of ^{13}C, as this provides directly an integrated value of the biologic processes that interacted throughout the plants biological cycle (Dawson *et al.*, 2002; Farquhar *et al.*, 1989), and also provides an independent value of the standard isotopic relation and the source isotopic ratio (atmosphere) (Farquhar *et al.*, 1989). Then, the isotopic discrimination value is expressed as a difference between the source and sample isotopic compositions, where the atmosphere is the source (O'Leary, 1981) (Equation 2).

$$\Delta = \frac{\delta^{13}C\ (source) - \delta^{13}C\ (product)}{1 + [\delta^{13}C\ (source) + 1000]} \qquad \text{[Eq. 2]}$$

For plants with C_3 metabolism, a comprehensive model of photosynthetic discrimination was developed (Equation 3), where the discrimination is a consequence of Rubisco fractionation, stomatal conductance, mesophyll conductance, respiration, and photorespiration, as well as ternary effects (Farquhar and Cernusak, 2012; Ubierna and Farquhar, 2014). Nevertheless, because the equation has several parameters that are to estimate or to measure directly, a simplified discrimination model becomes relevant (Equation 4) (Ubierna and Farquhar, 2014). In this expression, the discrimination values are obtained for the variation of photosynthetic capacity or stomatal aperture (conductance).

$$\begin{aligned}\Delta_{com} = {} & \frac{1}{1-t}\left[a_b\frac{c_a-c_s}{c_a} + a_s\frac{c_s-c_i}{c_a}\right] \qquad \text{[Eq. 3]}\\ & + \frac{1+t}{1-t}\left[a_m\frac{c_i-c_c}{c_a} + b\,\frac{c_c}{c_a} - \frac{ea_bR_d(C_c-\Gamma*)}{a_ec_a(A+R_c)} - \frac{fa_b\Gamma*}{a_fc_a}\right]\end{aligned}$$

$$\Delta^{13}C_{sim} = a + (b - a)\,\frac{c_i}{c_a} \qquad \text{[Eq. 4]}$$

Where a is the discrimination caused by CO_2 diffusion through the boundary layer and the stomatal pore, which has 4.4 % and b is the discrimination realized by the carboxylation enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco, C_3 metabolism) which has a 27 % value; C_i and C_a are the CO_2 partial pressures inside the leaf and in the atmosphere, respectively (Farquhar *et al.*, 1989).

Oxigen- ^{18}O

Gonfiantini *et al.* (1965) demonstrated that ^{18}O be used for studies on water-plant relationships, as isotopic water composition on the leaf is enriched in heavy isotopes when transpiration occurs. ^{16}O and ^{18}O, used in plant tissues studies have a proportion of 99.7 % and 0.21 %, respectively (Barbour, 2007).

Analogous to the carbon 13 ($\delta^{13}C$) composition, oxygen composition on vegetal tissue is determined as the isotopic ratio of the sample related to a standard ratio which, for oxygen, is commonly Vienna-Standard Mean Oceanic Water (VSMOW), the mean concentration of present-day ocean water with a molar abundance ratio value $^{18}O/^{16}O$ of 2.0052×10^{-3} (Equation 5) (Barbour, 2007; Dansgaard, 1964).

$$\delta^{18}O\ (\%) = \left(\frac{R\ sample}{R\ standard}\right) \times 1000 \qquad [\text{Eq.5}]$$

(Vienna - Standard Mean Ocean Water)

Nevertheless, it is recommended to use the term isotopic enrichment of oxygen 18, which eliminates the variability factor in isotopic composition terms of water source (Barbour, 2007), it also provides an independent value of the isotopic standard relation. Therefore, the isotopic enrichment value is expressed as a difference between the source and the sample isotopic compositions, where soil water is the source (Equation 6).

$$\Delta = \frac{\delta^{18}O\ (source) - \delta^{18}O\ (sample)}{1 + \left[\delta^{18}O\ (source) / 1000\right]} \qquad [\text{Eq.6}]$$

Oxygen stable isotopes in plants can be used for identification of water source (Ehleringer *et al.*,1991), a record of precipitation patterns in growth rings in woody plants and evaporative enrichment of leaf water as a consequence of physical and physiological factors (Barbour and Farquhar, 2000; Roden and Ehleringer, 1999). Specifically, evaporative enrichment of leaf water above source water, can be represented by applying the Craig and Gordon (1965) model for evaporative enrichment for the free water surface, adding some modifications (Equation 7) (Farquhar and Lloyd,1993).

$$\Delta^{18}O_{es} = \varepsilon^{+} + \varepsilon_{k} + (\Delta^{18}O_{v} - \varepsilon_{k})\, e_{a}/e_{i} \qquad [\text{Eq. 7}]$$

Where, e_a and e_i are vapor pressures for atmosphere and intercellular spaces, ΔO_v is the isotopic relationship of water vapor relative to a water source, ε_k is the diffusive fractionation trough boundary layer and stomata and ε^{+} is a proportional depression of equilibrium vapor pressure of $H_2{}^{18}O$ respect to $H_2{}^{16}O$.

Nitrogen- ^{15}N

Two stable isotopes conform to atmospheric nitrogen (^{14}N and ^{15}N), the light isotope is 99.6337 %, while the heavy one is 0.3663 % approximately (Handley and Raven, 1992). In atmosphere $^{15}N/^{14}N$ ratio is very constant; therefore, atmospheric N_2 is used as a standard to report values of ^{15}N isotopic composition (Shearer and Kohl, 1993; Stewart, 2013).

Analogous to isotopic discrimination of ^{13}C, for ^{15}N will exist fractionation during biochemical, biogeochemical and physiological processes. Isotopic composition of N can provide information about inputs of N fertilizer, sources of N available for plant growth, N inputs through N_2 fixation by free-living and symbiotic organisms, and estimates of plant fixation, because non-fixing plants have higher $\delta^{15}N$ than fixing plants (Boddey *et al.*, 2000; Dawson *et al.*, 2002); however, ^{15}N measurements have been less used than other isotopes (Robinson *et al.*, 2000), but these measurements are most useful when there is a multiple isotope approximation (Griffiths, 1991).

Stable isotopes studies in the measurement of photosynthesis

Plants may react to rising levels of atmospheric CO_2 and temperature by increasing the water-use efficiency (WUE) through either an increased photosynthetic rate or by a reduced water transpiration at a higher vapor pressure deficit (VPD) (Farquhar *et al.*, 1989). These two physiological processes result in carbon and oxygen isotopic fractionation. The response of tree growth to increased CO_2 is far from being straightforward and seems to be strongly dependent on site conditions, as it can interact with other drivers, such as warming-induced drought and physiological acclimation to high carbon dioxide levels, potentially reducing the ability of forests to act in the long-term as carbon sinks (Gómez-Guerrero *et al.*, 2013). Tree growth and intrinsic water-use efficiency (iWUE) have been observed not to increase as expected or even decline (Lévesque *et al.*, 2014).

It is well known that stable isotopes can serve as integrators or tracers of many key physical and biological processes. Carbon fixation during the process of photosynthesis discriminates against the heavier stable isotope of carbon (^{13}C) in favor of the lighter isotope (^{12}C), but the intensity of this discrimination ($\Delta^{13}C$) depends on environmental conditions such as vapor pressure deficit, soil-water availability, as well as physiological responses such as stomatal conductance. The carbon isotopic composition of C_3 plant tissues is often expressed as carbon isotope discrimination, a parameter used to track how environmental conditions affect leaf gas exchange (Cornejo-Oviedo *et al.*, 2017).

Stable isotope ratios also provide time-integrated information on plant ecophysiological responses to changing abiotic conditions and can also help to characterize species-specific plant water use strategies. Plant $\delta^{13}C$ provides an integrated record of the ratio of intercellular to atmospheric CO_2 concentrations (Ci/Ca) during the period in which the carbon was fixed (Herrero *et al.*, 2013). Factors affecting net photosynthetic rate and stomatal conductance (g_s) influence Ci, and thus, plant carbon isotope ratio (Martin-Benito *et al.*, 2017).

Carbon isotope discrimination is typically employed as a proxy for a plant intrinsic water-use efficiency, or a ratio of net assimilation to stomatal conductance. Tree-rings are composed of annual increments of xylem tissue, so increment cores can be used to retrospectively estimate past diameter or basal area growth (Cornejo-Oviedo *et al.*, 2017). Furthermore, carbon stable isotopes in the tree ring record a canopy-integrated signal of annual leaf gas-exchange (Martin-Benito *et al.*, 2017).

Measurements of the stable carbon isotope ratio ($\delta^{13}C$) on annual tree rings offer new opportunities to evaluate mechanisms of variations in photosynthesis and stomatal conductance under changing CO_2 and climate conditions, especially in conjunction with process-based biogeochemical model simulations.

A possible perspective for the mid-future should be to estimate photosynthesis along time using stable isotope analysis. Although it has been a recurrent theme for physiologists, biochemists and geneticists there is no agreement and the use of IRGA (Infrared gas analyzer system) for its measurement has been continued.

Stable isotope studies on water uptake and water use efficiency determination (iWUE)

Several forest species worldwide are strongly impacted by different scenarios of climate change (Feng *et al.*, 2016); for example, in arid and semi-arid regions precipitation patterns have changed in relation to species seasonal-phenology. Under such conditions, water availability decreases considerably because precipitation takes place during plant dormancy (Wang *et al.*, 2017). When plants recover cellular dynamism, a significant metabolic readjustment takes place to guarantee water uptake (Hsiao and Acevedo, 1974). The rainfall can be used by plants although a significant volume of water may be lost (Ehleringer and Dawson, 1992; Saiter *et al.*, 2016). Even though there is relatively low precipitation in arid and semi-arid regions, individual rainfall events cause water source isotopic enrichment in the short-term, which plays important roles in shaping plant adaptation in water use strategies (Farquhar *et al.*, 1989).

Stable isotope studies of ^{13}C, ^{18}O and ^{2}H have proved useful in identifying the origin of water consumed, in knowing the photosynthetic pathway and studying possible changes in the transpiration rate in many species; an example of this is in *Prosopis tamarugo* Phil. (Garrido *et al.*, 2016).

The isotopic composition of the water source, plant water, and growth rings cellulose can be used to analyze the water use efficiency patterns in short and long periods. As a medium-term perspective for ecosystems, which have been affected by climate change scenarios, stable isotope studies can provide accurate and consistent information on the plants stress intensity and its relationship with lumber quality.

Foliar water uptake describes the process by which plants absorb water into their leaves, resulting in a net increase in the mass of water in the leaf (Ehleringer and Dawson, 1992; Cernusak and Kahmen, 2013). This occurs when saturated atmospheric water vapor conditions result in a driving gradient for water to enter the leaf that is at a more negative water potential (Vesala *et al.*, 2017). The conditions necessary for this phenomenon to occur are often observed in dew- and fog-affected ecosystems such as coastal Mediterranean ecosystems (Baguskas *et al.*, 2016) and tropical montane cloud forests (Schwerbrock and Leuschner, 2017), where fog, often leading to leaf wetting, serves as an alternative plant water source during the dry season. The effects of precipitation events are similar and foliar water uptake has now been described as affecting plant water and carbon relations in more than 70 species from several different ecosystems (Berry *et al.*, 2014b). The capacity for species to do foliar water uptake has frequently been established by means of water isotope labeling experiments (Cassana *et al.*, 2016).

Stable isotope signatures in trees wooden tissues have been successfully used both in natural and controlled conditions, to detect and understand the physiological causes of iWUE changes (Guerrieri *et al.*, 2015). On this respect, Scheidegger *et al.* (2000) suggested a conceptual physiological model that links changes in C and O isotopic ratios to clarify the role of net assimilation and stomatal conductance in determining iWUE changes, based on the response of the plant to different VPD scenarios. More recently, other authors such as Battipaglia *et al.* (2013) used this conceptual model to better understand the effect of the environmental drivers and climate change on iWUE changes. Tree size and age can also affect iWUE, probably because of a decrease in stomatal conductance with increasing tree height.

The oxygen isotope compositions of plant tissue in foliar organs have been of interest because current understanding suggests that a sequential environmental and plant physiological record may be preserved from oldest tissues to the newest tissue. Such results could be used to evaluate lumber quality; although several advances have been achieved, still some quality lumber elements remain unclear.

The $\delta^{13}C$ measured in the dry matter has been a precise estimator of the intrinsic time-integrated water use efficiency for C_3 plants (Farquhar *et al.*, 1989). Also, $\delta^{13}C$ has been successfully used in the iWUE study of eucalyptus (*Eucalyptus*) forests (Zolfaghar *et al.*, 2017) and *Prosopis tamarugo* (Garrido *et al.*, 2016) submitted to depth gradients of water level, as well as roots depth estimation and use of groundwater in different forest communities (Rumman *et al.*, 2018).

Since the observed variation in $\delta^{13}C$ may be due to the increase of net assimilation (with a constant stomatal conductance), or to the stomatal conductance decrease (with net assimilation constant), Scheidegger *et al.* (2000) proposed an analysis model based on the use of $\delta^{13}C$ and $\delta^{18}O$: the Dual Isotope Model. The use of $\delta^{18}O$ is based on its negative association with stomatal conductance when the latter is the limiting factor for transpiration (Farquhar *et al.*, 2007). This scenario has been observed in forest communities where relative humidity and water source have a similar isotopic composition (Saurer *et al.*, 2001).

Although Scheidegger *et al.* (2000) developed their interpretation model in herbaceous species under natural conditions, it has also been applied in woody species such as *Quercus frainetto* Ten. under natural conditions, as well as in retrospective studies in *Fagus sylvatica* and *Nothofagus* spp. (Tognetti *et al.*, 2014), and in Scots pine (*Pinus sylvestris*) (Voltas *et al.*, 2013).

The efficacy of the model has been evaluated by Roden and Farquhar (2012) by measuring $\delta^{13}C$ and $\delta^{18}O$ in growth rings of *Eucalyptus globulus* Labill. and *Pinus radiata* D. Don. under controlled conditions. The authors evaluated the effect of different abiotic stresses (low irradiance, nitrogen deficit, heat and drought), simulating climate change, on the isotopic composition of wood and cellulose. In that assay, the authors hypothesized that it was possible to predict net assimilation and stomatal conductance behavior from the obtained values. Although the dual isotopic model did not perform well under all the evaluated scenarios, it was particularly efficient under drought and heat stress (under low relative humidity conditions and light restriction), while it had no predictive power under nitrogen deficit stress.

The application of the dual isotope model depends to a great extent on the fulfillment of a series of assumptions, which were summarized in Roden and Siegwolfs (2012). One of the most relevant assumptions is that the variation observed in $\delta^{18}O$ must be explained by stomatal conductance, which implies that the variables that affect the evaporative enrichment of water and organic tissue must be constant (mainly water source, leaf temperature and relative humidity). On the other hand, it is necessary to consider the buffer effect that wood capacitance and the presence of reserves can have on the isotopic composition of $\delta^{18}O$ and $\delta^{13}C$ respectively, which also perform independently.

Although the use of the dual isotopic model implies a careful experimental design and an extensive knowledge of the experimental conditions, its use allows understanding $\delta^{13}C$ and $\delta^{18}O$ variability under climate change scenarios in forest ecosystems.

Stable isotope studies on nitrogen nutrition measurement

There are different definitions and perspectives for nitrogen use efficiency (NUE) measurement. One of the definitions states that the NUE can be calculated by the yield ratio per unit of available nitrogen in the soil, including residual nitrogen present in the soil and nitrogen fertilizer in forest ecosystems where nitrogen fertilizer is applied, or available nitrogen in non-fertilized soil (Tarvainen and Näsholm, 2017); however, not all the available nitrogen in the plant comes from nitrogen fertilizer. The NUE is a function of the edaphic structure, climatic conditions, interactions between soil and bacterial processes, and the nature of organic and inorganic nitrogen sources, which generally is not included during NUE measurement from (plants nitrogen uptake from the field)/(nitrogen applied). The easiest way to calculate the NUE is based on a partial nitrogen balance (Salamanca-Jimenez *et al.*, 2017).

Given the complexity of the NUE measurements and the variability of loss rates among ecosystems, innovative techniques have been generated for their evaluation, such as stable isotope measurements (Cornejo-Oviedo *et al.*, 2017).

Nitrogen pattern valuation is important given their impact on plant biochemistry and physiology, and their consequences for the structuring of plant communities (Lambers *et al.*, 2008), particularly when it is well known that most forest ecosystems are generally affected by low rainfall distribution, affecting nitrogen nutrition (Balzotti *et al.*, 2016).

Plant uptake of dissolved organic nitrogen (DON) has been proposed to explain some inconsistencies of N balance of semi-arid ecosystem (Houlton *et al.*, 2007) but the direct evidence for the importance of the role of DON in plant nutrition in these ecosystems remains elusive under field conditions, particularly when water availability is low (Pardo *et al.*, 2013). Plant N-limitation is a widespread phenomenon in these ecosystems by the fact that the

N supply and the N plants and microbes demand may be discontinuous and temporally asynchronous in arid and semiarid ecosystems (Yahdjian *et al.*, 2011) due to the variability of water availability (Calvo-Rodriguez *et al.*, 2017).

Nitrogen is readily available at higher concentrations during the time that plants and microbes are relatively inactive due to dry soil conditions (Austin *et al.*, 2004), but the large increases in plant N uptake and bursts of microbial activity rapidly exhaust the available N when water stress is alleviated (Evans and Burke, 2013). Nowadays new and efficient methodologies are applied to study the natural abundance of ^{15}N measurements (δ^{15}N) of specific soil N sources, root, shoot and leaf material (Huygens *et al.*, 2016). On the strict condition that the isotopic abundance significantly differs among potential plant N sources, this method provides time-integrated information on preferential N uptake patterns under undisturbed plant rhizosphere conditions and natural resource availabilities. Intrinsically, this approach has the potential to overcome the methodological limitations that call into question the validity of many previous assessments of plant N source partitioning (Jones *et al.*, 2013).

Huygens *et al.* (2016) published the first report of quantitative time-integrated information on plant N source partitioning patterns under *in situ* conditions for a semi-arid ecosystem. It was found that all plant species showed similar N preferences and dominantly relied on NO_3^- for their N nutrition. Dissolved organic N was an insignificant plant N source in this semi-arid model ecosystem. Additionally, the observed δ^{15}N patterns of soil N pools and plant biomass provide further insight into the soil N cycle and competitive interactions among plants and microbes for N sources (Cornejo-Oviedo *et al.*, 2017).

To the extent that growth responses to N fertilization are influenced by net assimilation/stomatal conductance, differences in carbon isotope discrimination should provide insight into the mechanisms of fertilization response; for example, Brooks and Coulombe (2009) measured tree-ring growth and both carbon and oxygen stable isotopes in response to three levels of nitrogen fertilization (157, 314, 417 kg ha^{-1}) in an 85 year-old Douglas-fir plantation; the annual basal area increment of these trees peaked in the third growing season after fertilization, after which the values decreased slowly back to control levels over the next 20 years. In response to nitrogen fertilization, Δ^{13}C was reduced and iWUE was increased in both earlywood and latewood components, but only for the first three years before returning to pretreatment levels. They interpreted this short-term Δ^{13}C response to an increase in leaf nitrogen and photosynthesis, while the longer-term growth response was attributed to an increase in leaf area. Brooks and Mitchell (2011) found a similar response to nitrogen fertilization (448 kg N ha^{-1} as urea) in a 41 year-old Douglas-fir plantation. The direct effect of nitrogen on tree growth lasted six years after application, but Δ^{13}C was only reduced for the first few years, again related to an increase in leaf nitrogen and photosynthesis, prior to a subsequent increase in leaf area, which sustained the longer-term increase in growth; in contrast, Balster *et al.* (2009) did not find a decrease in Δ^{13}C in fertilized Douglas-fir plantations.

Analyses of tissue N concentration and N stable isotopic composition have provided very important information on resource acquisition (Givnish, 2002). A high tissue N concentration could benefit in tropical dry forest plants by enabling high rates of photosynthesis and maximizing carbon gain opportunities during the short-wet season (Santiago *et al.*, 2017). Additionally, a high tissue N concentration has the potential to maximize carbon gain for a given stomatal conductance (Wright *et al.*, 2003) during water deficit. N isotopic composition (δ^{15}N) of plant tissue reflects N sources, and alternative N sources such as biological N-fixation or atmospheric deposition of N might allow contrasting growth forms unique mechanisms to support carbon gain (Craine *et al.*, 2015).

Although studies conducted during the last 10 years have shown significant advances, even in relation to nitrogen discrimination, there are still some hints in carbon and oxygen isotopes that need to be clarified. An important aspect to treat as a future perspective would be to evaluate the nitrogen use efficiency in plants in the decomposed way between the structural and protein nitrogen accumulation.

CONCLUSIONS

This review gathers information on the most recent uses of stable isotope techniques in forest science, mainly based on the variability of response of the physiological variables: gas exchange, water use efficiency and nitrogen nutrition. These analyses have been useful to study the response of trees to climate change in most latitudes, and for the explanation of biochemical mechanisms activated during plant adaptation and productivity. Significant advances in the use of stable isotope techniques in forest science have been reported in scientific literature, we consider that such studies will allow us to calculate and model photosynthetic activity and nitrogen nutrition over time but not specific measurements on those areas are currently being carried out. Such studies will allow a retrospective assessment of possible climatic and biochemical variations that could affect metabolism and adopt forestry alternatives for productivity increase.

BIBLIOGRAPHY

Allen C. D., D. D. Breshears and N. G. McDowell (2015) On underestimation of global vulnerability to tree mortality and forest die-off from hotter drought in the Anthropocene. *Ecosphere* 6:1-55, https://doi.org/10.1890/ES15-00203.1

Allen C. D., A. K. Macalady, H. Chenchouni, D. Bachelet, N. McDowell, M. Vennetier, T. Kitzberger, A. Rigling, D. D. Breshears, E. H. Hogg, P. Gonzalez, R. Fensham, Z. Zhang, J. Castro, N. Demidova, J. H. Lim, G. Allard, S. W. Running, A. Semerci and N. Cobb (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* 259:660-684, https://doi.org/10.1016/j.foreco.2009.09.001

Austin A. T., L. Yahdjian, J. M. Stark, J. Belnap, A. Porporato, U. Norton, D. A. Ravetta and S. M. Schaeffer (2004) Water pulses and biogeochemical cycles in arid and semiarid ecosystems. *Oecologia* 141:221-235. https://doi.org/10.1007/s00442-004-1519-1

Baguskas S. A., C. J. Still, D. T. Fischer, C. M. D'Antonio and J. Y. King (2016) Coastal fog during summer drought improves the water status of sapling trees more than adult trees in a California pine forest. *Oecologia* 181:137-148. https://doi.org/10.1007/s00442-016-3556-y

Balster N. J., J. D. Marshall and M. Clayton (2009) Coupling tree-ring d^{13}C and d^{15}N to test the effect of fertilization on mature Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) stands across the Interior northwest, USA. *Tree Physiology* 29:1491-1501, https://doi.org/10.1093/treephys/tpp090

Balzotti C. S., G. P. Asner, P. G. Taylor, C. C. Cleveland, R. Cole, R. E. Martin, M. Nasto, B. B. Osborne, S. Porder and A. R. Townsend (2016) Environmental controls on canopy foliar nitrogen distributions in a Neotropical lowland forest. *Ecological Applications* 26:2451-2464, https://doi.org/10.5061/dryad.ck585

Barbour M. M. (2007) Stable oxygen isotope composition of plant tissue: a review. *Functional Plant Biology* 34:83-94, https://doi.org/10.1071/FP06228

Barbour M. M. and G. D. Farquhar (2000) Relative humidity- and ABA-induced variation in carbon and oxygen isotope ratios of cotton leaves. *Plant, Cell and Environment* 23:473-485, https://doi.org/10.1046/j.1365-3040.2000.00575.x

Battipaglia G., M. Saurer, P. Cherubini, C. Calfapietra, H. R. McCarthy, R. J. Norby and M. F. Cotrufo (2013) Elevated CO$_2$ increases tree-level intrinsic water use efficiency: insights from carbon and oxygen isotope analyses in tree rings across three forest FACE sites. *The New Phytologist* 197:544-554, https://doi.org/10.1111/nph.12044

Berry Z. C., N. M. Hughes and W. K. Smith (2014a) Cloud immersion: an important water source for spruce and fir saplings in the southern Appalachian Mountains. *Oecologia* 174:319-326, https://doi.org/10.1007/s00442-013-2770-0

Berry Z. C., J. C. White and W. K. Smith (2014b) Foliar uptake, carbon fluxes and water status are affected by the timing of daily fog in saplings from a threatened cloud forest. *Tree Physiology* 34:459-470, https://doi.org/10.1093/treephys/tpu032

Boddey R. M., M. B. Peoples, B. Palmer and P. J. Dart (2000) Use of the ^{15}N natural abundance technique to quantify biological nitrogen fixation by woody perennials. *Nutrient Cycling in Agroecosystems* 57:235-270, https://doi.org/10.1023/A:1009890514844

Brooks J. R. and A. K. Mitchell (2011) Interpreting tree responses to thinning and fertilization using tree-ring stable isotopes. *New Phytologist* 190:770-782, https://doi.org/10.1111/j.1469-8137.2010.03627.x

Brooks J. R. and R. Coulombe (2009) Physiological responses to fertilization recorded in tree rings: isotopic lessons from a long-term fertilization trial. *Ecological Applications* 19:1044-1060, https://doi.org/10.1890/08-0310.1

Calvo-Rodriguez S., A. G. Sanchez-Azofeifa, S. M. Duran and M. M. Espírito-Santo (2017) Assessing ecosystem services in Neotropical dry forests: a systematic review. *Environmental Conservation* 44:34-43, https://doi.org/10.1017/S0376892916000400

Cassana F. F., C. B. Eller, R. S. Oliveira and L. R. Dillenburg (2016) Effects of soil water availability on foliar water uptake of *Araucaria angustifolia. Plant and Soil* 399:147-157, https://doi.org/10.1007/s11104-015-2685-0

Cernusak L. A. and A. Kahmen (2013) The multifaceted relationship between leaf water ^{18}O enrichment and transpiration rate. *Plant Cell and Environment* 36:1239-1241, https://doi.org/10.1111/Pce.12081

Christiansen S. M. (2016) Climate Conflicts - A Case of International Environmental and Humanitarian Law. Springer International Publishing. Cham, Switzerland. 245 p, https://doi.org/10.1007/978-3-319-27945-9

Cornejo-Oviedo E. H., S. L. Voelker, D. B. Mainwaring, D. A. Maguire, F. C. Meinzer and J. R. Brooks (2017) Basal area growth, carbon isotope discrimination, and intrinsic water use efficiency after fertilization of Douglas-fir in the Oregon Coast Range. *Forest Ecology and Management* 389:285-295, https://doi.org/10.1016/j.foreco.2017.01.005

Craig H. and L. I. Gordon (1965) Deuterium and Oxygen-18 Variations in the Ocean and the Marine Atmosphere. Laboratory of Geology and Nuclear Science. Pisa, Italy. 122 p.

Craine J. M., E. N. J. Brookshire, M. D. Cramer, N. J. Hasselquist, K. Koba, E. Marin-Spiotta and L. Wang (2015) Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. *Plant and Soil* 396:1-26, https://doi.org/10.1007/s11104-015-2542-1

Dansgaard W. (1964) Stable isotopes in precipitation. *Tellus* 16:436-468, https://doi.org/10.3402/tellusa.v16i4.8993

Dawson T. E., S. Mambelli, A. H. Plamboeck, P. H. Templer and K. P. Tu (2002) Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics* 33:507-559, https://doi.org/10.1146/annurev.ecolsys.33.020602.095451

Di Matteo G., P. Nardi and G. Fabbio (2017) On the use of stable carbon isotopes to detect the physiological impact of forest management: the case of Mediterranean coppice woodland. *Forest Ecology and Management* 389:158-166, https://doi.org/10.1016/j.foreco.2016.12.030

Ehleringer J. R. and T. E. Dawson (1992) Water uptake by plants: perspectives from stable isotope composition. *Plant, Cell and Environment* 15:1073-1082, https://doi.org/10.1111/j.1365-3040.1992.tb01657.x

Ehleringer J. R., S. L. Phillips, W. S. F. Schuster and D. R. Sandquist (1991) Differential utilization of summer rains by desert plants. *Oecologia* 88:430-434, https://doi.org/10.1007/BF00317589

Evans S. E. and I. C. Burke (2013) Carbon and nitrogen decoupling under an 11-year drought in the shortgrass steppe. *Ecosystems* 16:20-33, https://doi.org/10.1007/s10021-012-9593-4

Farquhar G. D. and J. Lloyd (1993) Carbon and oxygen isotope effects in the exchange of carbon dioxide between terrestrial plants and the atmosphere. *In*: Stable Isotopes and Plant Carbon-Water Relations. J. R. Ehleringer, A. E. Hall and G. D. Farquhar (eds.). Academic Press. New York. pp:47-70, https://doi.org/10.1016/B978-0-08-091801-3.50011-8

Farquhar G. D., J. R. Ehleringer and K. T. Hubick (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 40:503-537, https://doi.org/10.1146/annurev.pp.40.060189.002443

Farquhar G. D., M. H. O'Leary and J. A. Berry (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology* 9:121-137, https://doi.org/10.1071/PP9820121

Farquhar G. D. and L. A. Cernusak (2012) Ternary effects on the gas exchange of isotopologues of carbon dioxide. *Plant, Cell and Environment* 35:1221-1231, https://doi.org/10.1111/j.1365-3040.2012.02484.x

Farquhar G. D., L. A. Cernusak and B. Barnes (2007) Heavy water fractionation during transpiration. *Plant Physiology* 143:11-18, https://doi.org/10.1104/pp.106.093278

Feng W., H. Lindner, N. E. Robbins II and J. R. Dinneny (2016) Growing out of stress: the role of cell- and organ-scale growth control in plant water-stress responses. *The Plant Cell* 28:1769-1782, https://doi.org/10.1105/tpc.16.00182

Garrido M., P. Silva and E. Acevedo (2016) Water relations and foliar isotopic composition of *Prosopis tamarugo* Phil., an endemic tree of the Atacama Desert growing at three levels of water table depth. *Frontiers in Plant Science* 7:375, https://doi.org/10.3389/fpls.2016.00375

Giammarchi F., P. Cherubini, H. Pretzsch and G. Tonon (2017) The increase of atmospheric CO_2 affects growth potential and intrinsic water-use efficiency of Norway spruce forests: insights from a multi-stable isotope analysis in tree rings of two alpine chronosequences. *Trees* 31:503-515, https://doi.org/10.1007/s00468-016-1478-2

Givnish T. J. (2002) Adaptive significance of evergreen vs. deciduous leaves: solving the triple paradox. *Silva Fennica* 36:703-743.

Gómez-Guerrero A., L. C. R. Silva, M. Barrera-Reyes, B. Kishchuk, A. Velázquez-Martínez, T. Martínez-Trinidad, F. O. Plascencia-Escalante and W. R. Horwath (2013) Growth decline and divergent tree ring isotopic composition ($\delta^{13}C$ and $\delta^{18}O$) contradict predictions of CO_2 stimulation in high altitudinal forests. *Global Change Biology* 19:1748-1758, https://doi.org/10.1111/gcb.12170

Gonfiantini R., S. Gratziu and E. Tongiorgi (1965) Oxygen isotopic composition of water in leaves. *In*: Isotopes and Radiation in Soil-Plant Nutrition Studies. Proceedings of a Symposium. Ankara, June 28-July 02, 1965. International Atomic Energy Agency. Vienna, Austria. pp:405-410.

Griffiths H. (1991) Applications of stable isotope technology in physiological ecology. *Functional Ecology* 5:254-269, https://doi.org/10.2307/2389263

Guerrieri R., E. I. Vanguelova, G. Michalski, T. H. E. Heaton and M. Mencuccini (2015) Isotopic evidence for the occurrence of biological nitrification and nitrogen deposition processing in forest canopies. *Global Change Biology* 21:4613-4626, https://doi.org/10.1111/gcb.13018

Handley L. L. and J. A. Raven (1992) The use of natural abundance of nitrogen isotopes in plant physiology and ecology. *Plant Cell and Environment* 15:965-985, https://doi.org/10.1111/j.1365-3040.1992.tb01650.x

Herrero A., J. Castro, R. Zamora, A. Delgado-Huertas and J. I. Querejeta (2013) Growth and stable isotope signals associated with drought-related mortality in saplings of two coexisting pine species. *Oecologia* 173:1613-1624, https://doi.org/10.1007/s00442-013-2707-7

Houlton B. Z., D. M. Sigman, E. A. G. Schuur and L. O. Hedin (2007) A climate-driven switch in plant nitrogen acquisition within tropical forest communities. *Proceedings of the National Academy of Sciences of the United States of America* 104:8902-8906, https://doi.org/10.1073/pnas.0609935104

Hsiao T. C. and E. Acevedo (1974) Plant responses to water deficits, water-use efficiency, and drought resistance. *Agricultural Meteorology* 14:59-84. https://doi.org/10.1016/0002-1571(74)90011-9

Huygens D., S. Díaz, R. C. Urcelay and P. Boeckx (2016) Microbial recycling of dissolved organic matter confines plant nitrogen uptake to inorganic forms in a semi-arid ecosystem. *Soil Biology and Biochemistry* 101:142-151, https://doi.org/10.1016/j.soilbio.2016.07.006

Jones D. L., P. L. Clode, M. R. Kilburn, E. A. Stockdale and D. V. Murphy (2013) Competition between plant and bacterial cells at the microscale regulates the dynamics of nitrogen acquisition in wheat (*Triticum aestivum*). *New Phytologist* 200:796-807, https://doi.org/10.1111/nph.12405

Lambers H., F. S. Chapin III and T. L. Pons (2008) Growth and allocation. *In*: Plant Physiological Ecology. H. Lambers, F. S. Chapin III and T. L. Pons (eds.). 2nd ed. Springer. New York, USA. pp:321-374, https://doi.org/10.1007/978-0-387-78341-3_10

Lehmann W. D. (2017) A timeline of stable isotopes and mass spectrometry in the life sciences. *Mass Spectrometry Reviews* 36:58-85, https://doi.org/10.1002/mas.21497

Lévesque M., R. Siegwolf, M. Saurer, B. Eilmann and A. Rigling (2014) Increased water-use efficiency does not lead to enhanced tree growth under xeric and mesic conditions. *New Phytologist* 203:94-109, https://doi.org/10.1111/nph.12772

Martin-Benito D., K. J. Anchukaitis, M. N. Evans, M. del Río, H. Beeckman and I. Cañellas (2017) Effects of drought on xylem anatomy and water-use efficiency of two co-occurring pine species. *Forests* 8:332, https://doi.org/10.3390/f8090332

O'Leary M. H. (1981) Carbon isotope fractionation in plants. *Phytochemistry* 20:553-567, https://doi.org/10.1016/0031-9422(81)85134-5

Pardo L. H., P. Semaoune, P. G. Schaberg, C. Eagar and M. Sebilo (2013) Patterns in $\delta^{15}N$ in roots, stems, and leaves of sugar maple and American beech seedlings, saplings, and mature trees. *Biogeochemistry* 112:275-291, https://doi.org/10.1007/s10533-012-9724-1

Robinson D., L. L. Handley, C. M. Scrimgeour, D. C. Gordon; B. P. Forster and R. P. Ellis (2000) Using stable isotope natural abundances ($\delta^{15}N$ and $\delta^{13}C$) to integrate the stress responses of wild barley (*Hordeum spontaneum* C. Koch.) genotypes. *Journal of Experimental Botany* 51:41-50, https://doi.org/10.1093/jexbot/51.342.41

Roden J. S. and J. R. Ehleringer (1999) Hydrogen and oxygen isotope ratios of tree-ring cellulose for riparian trees grown long-term under hydroponically controlled environments. *Oecología* 121:467-477, https://doi.org/10.1007/s004420050953

Roden J. S. and G. D. Farquhar (2012) A controlled test of the dual-isotope approach for the interpretation of stable carbon and oxygen isotope ratio variation in tree rings. *Tree Physiology* 32:490-503, https://doi.org/10.1093/treephys/tps019

Roden J. and R. Siegwolf (2012) Is the dual-isotope conceptual model fully operational? *Tree Physiology* 32:1179-1182, https://doi.org/10.1093/treephys/tps099

Rumman R., J. Cleverly, R. H. Nolan, T. Tarin and D. Eamus (2018) Speculations on the application of foliar ^{13}C discrimination to reveal groundwater dependency of vegetation and provide estimates of root depth and rates of groundwater use. *Hydrology and Earth System Sciences* 22:4875-4889, https://doi.org/10.5194/hess-22-4875-2018

Saiter F. Z., P. V. Eisenlohr, M. R. V. Barbosa, W. W. Thomas and A. T. Oliveira-Filho (2016) From evergreen to deciduous tropical forests: how energy-water balance, temperature, and space influence the tree species composition in a high diversity region. *Plant Ecology and Diversity* 9:45-54, https://doi.org/10.1080/17550874.2015.1075623

Salamanca-Jimenez A., T. A. Doane and W. R. Horwath (2017) Nitrogen use efficiency of coffee at the vegetative stage as influenced by fertilizer application method. *Frontiers in Plant Science* 8:223, https://doi.org/10.3389/fpls.2017.00223

Santiago L. S., K. Silvera, J. L. Andrade and T. E. Dawson (2017) Functional strategies of tropical dry forest plants in relation to growth form and isotopic composition. *Environmental Research Letters* 12:115006, https://doi.org/10.1088/1748-9326/aa8959

Saurer M., R. Siegwolf and Y. Scheidegger (2001) Canopy gradients in $\delta^{18}O$ of organic matter as ecophysiological tool. *Isotopes in Environmental and Health Studies* 37:13-24, https://doi.org/10.1080/10256010108033278

Scheidegger Y., M. Saurer, M. Bahn and R. Siegwolf (2000) Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: a conceptual model. *Oecologia* 125:350357, https://doi.org/10.1007/s004420000466

Schwerbrock R. and C. Leuschner (2017) Foliar water uptake, a widespread phenomenon in temperate woodland ferns? *Plant Ecology* 218:555-563, https://doi.org/10.1007/s11258-017-0711-4

Shearer G. and D. H. Kohl (1993) Natural abundance of ^{15}N: fractional contribution of two sources to a common sink and use of isotope discrimination. *In*: Nitrogen Isotope Techniques. R. Knowles and T. H. Blackburn (eds.). Academic Press. New York. pp:89-125, https://doi.org/10.1016/B978-0-08-092407-6.50009-2

Stewart G. R. (2013) What do $\delta^{15}N$ signatures tell us about nitrogen relations in natural ecosystems? *In*: Stable Isotope Techniques in the Study of Biological Processes and Functioning of Ecosystems. M. J. Unkovich, J. S. Pate, A. McNeill and D. J. Gibbs (eds.). Springer. Dordrecht, The Netherlands. pp:91-101, https://doi.org/10.1007/978-94-015-9841-5_5

Tarvainen L. and T. Näsholm (2017) Can adjustments in foliar nitrogen-use efficiency reduce drought stress impacts on boreal trees? *Tree Physiology* 37:415-417, https://doi.org/10.1093/treephys/tpx003

Tognetti R., F. Lombardi, B. Lasserre, P. Cherubini and M. Marchetti (2014) Tree-ring stable isotopes reveal twentieth-century increases in water-use efficiency of *Fagus sylvatica* and *Nothofagus* spp. in Italian and Chilean mountains. PLoS ONE 9:e113136, https://

doi.org/10.1371/journal.pone.0113136

Ubierna N. and G. D. Farquhar (2014) Advances in measurements and models of photosynthetic carbon isotope discrimination in C_3 plants. *Plant, Cell and Environment* 37:1494-1498, https://doi.org/10.1111/pce.12346

Vesala T., S. Sevanto, T. Grönholm, Y. Salmon, E. Nikinmaa, P. Hari and T. Hölttä (2017) Effect of leaf water potential on internal humidity and CO_2 dissolution: reverse transpiration and improved water use efficiency under negative pressure. *Frontiers in Plant Science* 8:54, https://doi.org/10.3389/fpls.2017.00054

Voltas J., J. J. Camarero, D. Carulla, M. Aguilera, A. Ortiz and J. P. Ferrio (2013) A retrospective, dual-isotope approach reveals individual predispositions to winter-drought induced tree dieback in the southernmost distribution limit of Scots pine. *Plant, Cell and Environment* 36:1435-1448, https://doi.org/10.1111/pce.12072

Wang J., B. Fu, N. Lu and L. Zhang (2017) Seasonal variation in water uptake patterns of three plant species based on stable isotopes in the semi-arid Loess Plateau. *Science of the Total Environment* 609:27-37, https://doi.org/10.1016/j.scitotenv.2017.07.133

Wright I. J., P. B. Reich and M. Westoby (2003) Least-cost input mixtures of water and nitrogen for photosynthesis. *The American Naturalist* 161:98-111, https://doi.org/10.1086/344920

Yahdjian L., L. Gherardi and O. E. Sala (2011) Nitrogen limitation in arid-subhumid ecosystems: a meta-analysis of fertilization studies. *Journal of Arid Environments* 75:675-680, https://doi.org/10.1016/j.jaridenv.2011.03.003

Zolfaghar S., R. Villalobos-Vega, M. Zeppel, J. Cleverly, R. Rumman, M. Hingee, N. Boulain, Z. Li and D. Eamus (2017) Transpiration of *Eucalyptus* woodlands across a natural gradient of depth-to-groundwater. *Tree Physiology* 37:961-975, https://doi.org/10.1093/treephys/tpx024