



## SOME USES OF STABLE ISOTOPE TECHNIQUES IN FOREST SCIENCE

### ALGUNOS USOS DE TÉCNICAS DE ISÓTOPOS ESTABLES EN CIENCIA FORESTAL

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#### 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<sub>2</sub> 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<sub>2</sub>; 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.

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 <sup>13</sup>C, <sup>18</sup>O, and <sup>15</sup>N involved in forest trees metabolism and productivity will be considered.

**HISTORY OF STABLE ISOTOPES AND THEORETICAL ASPECTS**

**Carbon- <sup>13</sup>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 <sup>13</sup>C value than carbonates. Later, studies of variations in abundance of <sup>13</sup>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 (<sup>12</sup>C and <sup>13</sup>C) forming part of the atmospheric carbon dioxide (CO<sub>2</sub>), which are in approximate proportions of 99 % and 1%, respectively. Plant tissues have a different distribution of <sup>13</sup>C/<sup>12</sup>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<sub>2</sub> (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 <sup>13</sup>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.

$$\delta^{13}C (‰) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \quad [\text{Eq. 1}]$$

(Pee Dee Belemnite)

Where δ<sup>13</sup>C is an isotopic signature, a measure of the ratio of stable isotopes <sup>13</sup>C:<sup>12</sup>C, reported in parts per thousand; R is the molar abundance ratio <sup>13</sup>CO<sub>2</sub>/<sup>12</sup>CO<sub>2</sub> 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 <sup>13</sup>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).

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

For plants with C<sub>3</sub> 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).

$$\Delta_{\text{com}} = \frac{1}{1-t} \left[ a_b \frac{C_a - C_s}{C_a} + a_s \frac{C_s - C_i}{C_a} \right] + \frac{1+t}{1-t} \left[ a_m \frac{C_i - C_c}{C_a} + b \frac{C_c}{C_a} - \frac{ea_b R_d (C_c - \Gamma^*)}{a_c C_a (A + R)} - \frac{fa_b \Gamma^*}{a_i C_a} \right] \quad [\text{Eq. 3}]$$

$$\Delta^{13}C_{\text{sim}} = a + (b - a) \frac{C_i}{C_a} \quad [\text{Eq. 4}]$$

Where *a* is the discrimination caused by CO<sub>2</sub> 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<sub>3</sub> metabolism) which has a 27 % value; C<sub>i</sub> and C<sub>a</sub> are the CO<sub>2</sub> partial pressures inside the leaf and in the atmosphere, respectively (Farquhar *et al.*, 1989).

**Oxygen- <sup>18</sup>O**

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

Analogous to the carbon 13 ( $\delta^{13}\text{C}$ ) 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  $^{18}\text{O}/^{16}\text{O}$  of  $2.0052 \times 10^{-3}$  (Equation 5) (Barbour, 2007; Dansgaard, 1964).

$$\delta^{18}\text{O} (\%) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \quad [\text{Eq.5}]$$

(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).

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

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).

$$\Delta^{18}\text{O}_{\text{es}} = \varepsilon^* + \varepsilon_k + (\Delta^{18}\text{O}_v - \varepsilon_k) e_a / e_i \quad [\text{Eq. 7}]$$

Where,  $e_a$  and  $e_i$  are vapor pressures for atmosphere and intercellular spaces,  $\Delta\text{O}_v$  is the isotopic relationship of water vapor relative to a water source,  $\varepsilon_k$  is the diffusive fractionation trough boundary layer and stomata and  $\varepsilon^*$  is a proportional depression of equilibrium vapor pressure of  $\text{H}_2^{18}\text{O}$  respect to  $\text{H}_2^{16}\text{O}$ .

### Nitrogen- $^{15}\text{N}$

Two stable isotopes conform to atmospheric nitrogen ( $^{14}\text{N}$  and  $^{15}\text{N}$ ), the light isotope is 99.6337 %, while the heavy one is 0.3663 % approximately (Handley and Raven, 1992). In atmosphere  $^{15}\text{N}/^{14}\text{N}$  ratio is very constant; therefore, atmospheric  $\text{N}_2$  is used as a standard to report values of  $^{15}\text{N}$  isotopic composition (Shearer and Kohl, 1993; Stewart, 2013).

Analogous to isotopic discrimination of  $^{13}\text{C}$ , for  $^{15}\text{N}$  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  $\text{N}_2$  fixation by free-living and symbiotic organisms, and estimates of plant fixation, because non-fixing plants have higher  $\delta^{15}\text{N}$  than fixing plants (Boddey *et al.*, 2000; Dawson *et al.*, 2002); however,  $^{15}\text{N}$  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  $\text{CO}_2$  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  $\text{CO}_2$  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 ( $^{13}\text{C}$ ) in favor of the lighter isotope ( $^{12}\text{C}$ ), but the intensity of this discrimination ( $\Delta^{13}\text{C}$ ) 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  $\text{C}_3$  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  $\delta^{13}\text{C}$  provides an integrated record of the ratio of intercellular to atmospheric  $\text{CO}_2$  concentrations ( $\text{C}_i/\text{C}_a$ ) during the period in which the carbon was fixed (Herrero *et al.*, 2013). Factors affecting net

photosynthetic rate and stomatal conductance ( $g_s$ ) influence  $C_i$ , 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  $^2H$  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  $\delta^{13}\text{C}$  measured in the dry matter has been a precise estimator of the intrinsic time-integrated water use efficiency for  $\text{C}_3$  plants (Farquhar *et al.*, 1989). Also,  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ : the Dual Isotope Model. The use of  $\delta^{18}\text{O}$  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  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  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  $\delta^{18}\text{O}$  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  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  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 (Tavainen 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-Rodríguez *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  $^{15}\text{N}$  measurements ( $\delta^{15}\text{N}$ ) 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  $\text{NO}_3^-$  for their N nutrition. Dissolved organic N was an insignificant plant N source in this semi-arid model ecosystem. Additionally, the observed  $\delta^{15}\text{N}$  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  $\text{kg ha}^{-1}$ ) 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,  $\Delta^{13}\text{C}$  was reduced and  $w\text{WUE}$  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  $\Delta^{13}\text{C}$  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  $\text{kg N ha}^{-1}$  as urea) in a 41 year-old Douglas-fir plantation. The direct effect of nitrogen on tree growth lasted six years after application, but  $\Delta^{13}\text{C}$  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  $\Delta^{13}\text{C}$  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 ( $\delta^{15}\text{N}$ ) 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.

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