Stable isotopes in tropical tree rings: Theory, methods and applications

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Stable isotopes in tropical tree rings: theory, methods and applications

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Summary

1. The notion that many tropical tree species form annual growth rings has triggered research on their growth and its environmental drivers over long periods of time. Even more recently, a large number of studies have also analysed the natural abundance of stable isotopes in tropical tree rings. The rapid developments in this young field call for a review. Here, we focus on stable isotopes of carbon (13C), oxygen (18O) and nitrogen (15N).

2. We start by explaining how environmental and physiological effects define the isotopic composition of wood in tropical trees. Abundance of 13C is mainly driven by water, light and nutrient availability. Here 18O values are chiefly determined by those of rainwater and additionally by rooting depth and factors determining leaf water evaporation. The 15N levels are determined by the 15N signature of nitrogen uptake, which in turn depends in complex ways on various processes in the nitrogen cycle.

3. We then discuss methodological aspects of isotopes studies in tropical tree rings. An important requirement is that rings are reliably dated. Furthermore, a key methodological concern is that temporal changes in isotopic values can be confounded by tree-size driven changes, which can be avoided by sampling from a fixed diameter range or accounted for statistically.

4. Next, 50 studies are reviewed that measured stable isotopes of C, O, and/or N in tree rings of a total of 85 tropical tree species. Temporal variation in both d13C and d18O was correlated with precipitation and El Niño Southern Oscillation variability. Seasonality in d13C and d18O was successfully used for delimiting visually non-distinct annual rings. Tropical tree responses to increasing atmospheric [CO2] were effectively quantified, using d13C as a measure of intrinsic water use efficiency. And finally, anthropogenic changes in the nitrogen cycle in tropical forests have been inferred from d15N.

5. We conclude with methodological and ecophysiological recommendations for isotope studies in tropical tree rings. Future perspectives include the analysis of intramolecular isotopic distributions of isotopes in glucose that can advance our understanding of environmental effects on tropical tree physiology. Finally, we recommend that tropical tree ring isotope data are deposited in open access databases.

Key-words: carbon, climate, global change, nitrogen, oxygen, stable isotopes, tree rings, tropical forest

Introduction

Tropical forests harbour an incredible biodiversity and provide many ecosystem services on which millions of people depend. They are a major component of the global carbon cycle, storing some 25% of the total terrestrial carbon and accounting for a third of net primary production (Bonan 2008). Understanding the functioning of these forests and their responses to global change is therefore of crucial importance. The analysis of annual growth rings in...
the stem of trees is a relatively new tool in tropical forest, and logically follows the well-established methods developed for trees growing at higher latitudes.

The existence of annual rings in tropical trees was not generally recognized until recently. For a long time, the tropical environment was associated with year-round favourable growth conditions that likely inhibited the formation of distinct annual growth rings. However, most tropical environments are seasonal to various degrees and the formation of annual tree rings in deciduous species growing in tropical climates with a pronounced dry season has been known for a long time (Coster 1927). Although tree rings in the humid tropics are generally less distinct than those formed in temperate regions, the formation of clear annual growth rings has been shown for a large number of tropical tree species (e.g. Worbes 2002; Rozendaal & Zuidema 2011; Zuidema, Brienen & Schöngart 2012; Brienen, Schöngart & Zuidema 2016). In addition to drought, other seasonally changing environmental factors, such as flooding and salinity, are known to induce the formation of ring boundaries (Schöngart et al. 2002; Chowdhury et al. 2008).

Given that trees can become hundreds of years old, their rings contain a long-term archive of growth rates, which can be used to assess the responses of trees to environmental variability. Changing environmental conditions, however, do not only influence diameter growth rate of the tree stem (and thus ring width), but can also affect the chemical composition of the wood. The discovery of annual rings in many tropical trees and developments in stable isotope analysis (McCarroll & Loader 2004) has recently triggered studies on the variation in the natural abundance of isotopes in these trees. Developments in stable isotope theory has further enhanced interpretation of these data (Farquhar, Ehleringer & Hubick 1989; Roden, Lin & Ehleringer 2000; Dawson et al. 2002; Barbour 2007; Robertson et al. 2008; Gessler et al. 2014). In several recent studies, the variation in stable isotope composition in tree rings was used for a retrospective analysis of environmental changes at the local, regional and global scales. Another set of studies used the reverse argumentation: for tree species lacking (visually) distinct ring boundaries, the seasonal variation in isotope composition of the wood can be used to delineate tree rings.

The number of publications on the natural abundance of stable isotopes of carbon ($^{13}$C) and oxygen ($^{18}$O) in annual growth rings of tropical trees has increased rapidly in recent years, and even more recently the first studies on nitrogen ($^{15}$N) isotopes appeared. In this review, we address the theory of fractionation of these stable isotopes and discuss its potential for the reconstruction of past environmental conditions. We also cover the methodological aspects of isotopic studies in the tropics. Finally, we review the results of stable isotope studies on tropical trees, including species without distinct annual rings. In the discussion, we address the question to what extent isotope analyses on tropical trees have been able to (i) reconstruct historical environmental variation; (ii) help in the detection of tree ring boundaries in species without visible rings; and (iii) explain temporal fluctuation, as well as, long-term trends in tree growth.

**Stable isotope ecophysiology**

The stable isotopes $^{13}$C, $^{18}$O and $^{15}$N form a small fraction of the total pools of these elements on earth. The majority consists of the lighter forms $^{12}$C, $^{16}$O and $^{14}$N. Chemical and physical processes involving these elements are often accompanied by isotopic fractionation as a result of small difference in the rate of transport and in chemical reactions. The factors influencing fractionation are well understood, and this knowledge forms the basis for deriving past environment and physiological factors and ecosystem processes from isotopic data. In the interpretation of such data, it is important to distinguish the source of the elements and the product in which they are used by plants. In the context of tree ring studies, stem wood or the cellulose therein is the product, and the sources are typically atmospheric CO$_2$ for $^{13}$C and water absorbed by the roots for $^{18}$O. For $^{15}$N, trees use different sources, mainly NH$_4^+$, NO$_3^-$ and atmospheric N$_2$.

Isotopic composition is expressed as the atomic ratio of the heavier over the common lighter isotope relative to an internationally recognized standard.

$$\delta_{sample} = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 100(\%) \quad \text{eqn 1}$$

Standards are Pee Dee Belemnite rock for $^{13}$C (V-PDB), mean ocean water for $^{18}$O (V-SMOW) and atmospheric N$_2$ for $^{15}$N.

**CARBON**

About 1-11% of global carbon is in the form of $^{13}$C. Plant tissue is typically depleted of the heavier isotope relative to atmospheric CO$_2$ as a result of slower diffusion of $^{13}$CO$_2$ and lower reaction constant for $^{13}$CO$_2$ of the carboxylation reaction catalysed by Rubisco compared to $^{12}$CO$_2$. The whole process of CO$_2$ assimilation in C$_3$ plants thus discriminates against $^{13}$C causing a lower $\delta^{13}$C in plant tissue compared to atmospheric CO$_2$. The V-PDB standard for $\delta^{13}$C measurement is rather arbitrarily chosen, resulting in $\delta^{13}$C values that provide little insight. A more insightful way of expressing the isotope effect is discrimination against $^{13}$C ($\Delta^{13}C = \frac{R_{atm}/R_{plant}}{R_{plant}} - 1$). This can be calculated from $\delta$-values as follows:

$$\Delta^{13}C = \frac{\left(\delta^{13}C_{atm} - \delta^{13}C_{plant}\right)}{\left(\delta^{13}C_{plant}/1000 + 1\right)} \times 100(%) \quad \text{eqn 2}$$

If long time series of $\delta^{13}C_{plant}$ are analysed, the gradual decrease in $\delta^{13}C_{atm}$, from a preindustrial value of $-6-4\%_{oo}$ to lower than $-8\%_{oo}$ at present, must be taken into account (Treydte et al. 2001; McCarroll & Loader 2004). Also,
seasonal variation in $\delta^{13}C_{\text{atm}}$ can be significant for intrannual sampling. In dense tropical forests with limited air turbulence, the correct estimation of source $\delta^{13}C$ is further complicated because the CO$_2$ in the air just above the forest floor can be depleted in $^{13}C$ as a result of respiration and enriched in the canopy because of photosynthetic activity (Buchmann et al. 1997).

A mechanistic model developed by Farquhar, O’Leary & Berry (1982); Farquhar, Ehleringer & Hubick (1989) quantifies the two processes involved in the discrimination during CO$_2$ assimilation, and was further specified for tree rings (Francey & Farquhar 1982; Fig. 1):

$$\Delta^{13}C = a + (b - a) C_i/C_a \%,$$

where $C_i$ and $C_a$ are, respectively, the CO$_2$ concentrations in a leaf’s intercellular space and in the atmosphere; $a$ represents the magnitude of the effect of diffusion in air through the stomata (4-4 $\%$) and $b$ the discrimination by Rubisco (27 $\%$). The latter is somewhat lower than the Rubisco effect itself, as it includes effects such as photorespiration and mesophyll conductance (Flexas et al. 2012) that cannot be quantified. During transport of the primary assimilates and synthesis of the macromolecules in the wood further fractionation occurs, causing stem cellulose to have a 1–2 $\%$ higher $\delta^{13}C$ (Fig. 1). Equation (3) is widely used to derive a time integrated measure for $C_i$, which is relevant to photosynthetic rate. However, given the quantitative uncertainties associated with $\delta^{13}C_{\text{atm}}$ in a forest, mesophyll conductance and downstream fractionation (Gessler et al. 2014) the absolute value of a single measurement may have a substantial error. Nonetheless, when assessing temporal trends in a particular species it is reasonable to assume that at least the latter two are constant over time.

$C_i$ is also relevant to water use efficiency of photosynthesis, and $\Delta^{13}C$ allows the calculation of intrinsic water use efficiency (iWUE) through the calculation of $C_i$:

$$i\text{WUE} = A/g_s = (C_a - C_i)/1.6 (\text{mol} \text{mol}$^{-1})$$

In this equation, the value of 1.6 accounts for the lower diffusivity in air of CO$_2$ compared to water vapour. For the calculation of actual water use efficiency (WUE), the net CO$_2$ assimilation rate ($A$) is not divided by the stomatal conductance ($g_s$) but by the actual transpiration rate ($E$). Data on the vapour pressure difference between leaf and atmosphere are necessary for that purpose.

It is known from physiological measurements that $C_i/C_a$ decreases and iWUE increases with decreasing water
availability as a result of stomatal closure (Lambers, Chapin & Pons 2008). The above described theory thus predicts that δ13C is lower in drought conditions as a result of decreased g, and consequently Ci. This was experimentally confirmed, initially for crops (Farquhar & Richards 1984) and more recently also for tropical trees (Cernusak, Winter & Turner 2009). Correlative evidence suggests that such a relationship holds also in high rainfall areas: such a relationship holds also in high rainfall areas (Winter & Turner 2009). Correlative evidence suggests that such a relationship holds also in high rainfall areas.

Co-occurring tropical forest tree species can exhibit large variation in δ13C (Guehl et al. 1998; Bonal et al. 2000b), which is partly associated with their successional status. The hierarchy of species in terms of δ13C was found to be similar in different forests and when the same species were grown in a greenhouse (Bonal et al. 2007). These interspecific differences in δ13C were indeed associated with independently measured WUE (Bonal et al. 2007; Cernusak et al. 2008), but the relationship was not always strong and differed across species in some cases (Cernusak et al. 2007). Such interspecific differences in isotopic composition are likely related to ecophysiological traits, but until now, they are not fully understood. This implies that δ13C sequences from different species cannot be readily combined for reconstructions of environmental conditions.

OXYGEN

About 0-20% of global oxygen consists of 18O. Plant tissue has typically a higher δ18O compared to that of the water taken up [source water (sw)]. This enrichment is also expressed with a Δ-value (Δ18O = Rplant/Rsw − 1), but source and product are interchanged (Barbour 2007).

\[
\Delta^{18}O_{\text{plant}} = \frac{\left(\delta^{18}O_{\text{plant}} - \delta^{18}O_{\text{sw}}\right)}{(\delta^{18}O_{\text{sw}}/1000 + 1)} \quad \text{eqn 5}
\]

During water uptake by roots no fractionation of oxygen isotopes occurs, thus xylem water has the same δ18O as absorbed soil water (Dawson & Ehleringer 1991).

Transpiration causes enrichment of leaf water due to a lower evaporation rate of H218O. This process and its significance for tree ring cellulose has been described in several papers (Farquhar, Ehleringer & Hubick 1989; Roden, Lin & Ehleringer 2000; Barbour 2007), will be shortly summarized here and is schematically represented in Fig. 1.

Enrichment at the site of evaporation in the leaf relative to source water (Δ18Oex) can be calculated from the temperature-dependent fractionation between water in the liquid and vapour phase (εv), the kinetic fractionation with diffusion from leaf to atmosphere (εk), the isotopic composition of water vapour (Δ18Ov) and the ratio of vapour pressure in atmosphere and leaf (εa/εv).

\[
\Delta^{18}O_e = \epsilon^v + \epsilon_k + \left(\Delta^{18}O_v - \epsilon_k\right)\epsilon_a/\epsilon_v \quad \text{eqn 6}
\]

For calculating the 18O enrichment of bulk leaf water (Δ18Oℓ) the mixing of xylem water and enriched water from the site of evaporation must be taken into account. This Péclet effect (ϕ) depends on the ratio of the two, and is calculated from the transpiration rate (E), the effective path length over which the process takes place (L), the molar density of water (C) and the diffusivity of H218O in water (D).

\[
\Delta^{18}O_{\ell} = \Delta^{18}O_e \left(1 - \epsilon^v\right)/\phi \quad \text{eqn 7}
\]

where \(\phi = (L/E)/(C\cdot D)\)

Leaf water enrichment is thus negatively related to atmospheric humidity and transpiration rate, and thus partly to stomatal conductance. Uncertainties are associated with estimation of L and the deviation from steady state for which these calculations are valid.

Before CO2 is used by the carboxylation enzyme Rubisco, it has effectively exchanged its O with water in the mesophyll, a reaction that is catalysed by carbonic anhydrase. As a consequence, the triose-phosphate that is formed in the chloroplast and that forms the basis for sucrose synthesis, is strongly imprinted with the δ18O signature of leaf water instead of the δ18O signature of the absorbed CO2 (DeNiro & Epstein 1979). Further fractionation in Calvin cycle reactions and sucrose synthesis results in 18O enrichment (εwc), which is estimated at 27%/oo, independent of species and growth conditions. When macro-molecules are synthesized from sucrose, exchange in O with local water occurs (εwc). This was estimated at 42% for cellulose, the wood compound on which stable isotopes are often measured in tree ring studies. This exchange of O causes the effect of enriched leaf water to be partly reverted.

The deviation of local cellular water from source water is accounted for by the proportion of source water (ps), which is likely to be close to unity in the cambium. The isotopic composition of cellulose relative to source water (Δ18Ocel) can then be calculated as follows:

\[
\Delta^{18}O_{\text{cel}} = \Delta^{18}O_{\ell} \left(1 - p_s\epsilon_{\text{ex}}\right) + \epsilon_{\text{wc}} \quad \text{eqn 8}
\]

Evidence indicated that εwc and ps values are not constant. Species or condition-specific variation can cause uncertainty about the extent to which wood cellulose δ18O is predominantly determined by humidity and stomatal conductance or by source water (Sternberg 2009; Gessler et al. 2014). The δ18O of stems of potted tropical tree seedling was imprinted by the calculated leaf water enrichment (Cernusak et al. 2008), but whether such a strong effect of leaf water δ18O on tree ring cellulose is also true for large trees is unknown.

The δ18O of precipitation water varies seasonally. It is depleted during the rainy season and heavy precipitation events (Villacés, Vimeux & Taupin 2008; Kurita et al. 2009) and can be enriched by evaporation from exposed
soil surface in the dry season (Jackson et al. 1995). These processes lead to variation of δ^{18}O of water in the soil profile (Bonal et al. 2000a).

It is generally unknown from what depth root systems of tropical trees take up water, which complicates the determination of the δ^{15}N of source water. The soil depth of water uptake has been estimated by measuring natural abundance of δ^{18}O in the soil profile and in xylem water (Jackson et al. 1995; Hasselquist, Allen & Santiago 2010; Ellsworth & Sternberg 2015; Schwendemann et al. 2015) or by labelling soil water (Stahl et al. 2013). These studies showed that deciduous trees tend to take up water from shallower depths than evergreens, and that the depth of water uptake increased not clearly with tree age. Since unmodified rainwater is more likely to be absorbed by shallow-rooting trees, information on depth of water uptake is important for the interpretation of intra- and inter-annual variation in δ^{18}O. Yet, such information is usually lacking in isotopic studies on tropical trees.

NITROGEN

The stable isotope of nitrogen, 15N, comprises 0.37% of global N. Its natural abundance in plants and other ecosystems compartments is expressed as δ^{15}N, the isotopic ratio relative to atmospheric N2 as a standard (eqn 1). The value of plant δ^{15}N depends for a large part on the δ^{15}N of the N source used by the plant. Fractionation associated with uptake of the major inorganic N sources for plants, NH4+ and NO3−, is small or absent (Evans et al. 1996; Högberg et al. 1999), particularly under N-limitation as in that case when most N that becomes available from mineralization is utilized. Similarly, there is little or no fractionation associated with N2-fixation (Högberg 1997). Plant δ^{15}N thus largely reflects the isotopic signature of the N source. N2-fixing plants therefore tend to exhibit a δ^{15}N value of 0%o, with fluctuations depending on the fraction of N derived from N2-fixation (Högberg 1997). However, the δ^{15}N of soil derived N can vary substantially and is regulated in a complex manner.

Fractionation associated with soil microbial processes can generate differences in δ^{15}N between NH4+ and NO3− and cause variation over time and with soil depth (Hobbie & Ouimette 2009). Processes influencing plant and ecosystem δ^{15}N have recently been reviewed (Hobbie & Högberg 2012; Gerhart & McLauchlan 2014) and are shortly summarized here and illustrated in Fig. 2. Discrimination against 15N with nitrification is an important reason for patterns in soil δ^{15}N. Leaching under surplus precipitation causes export of the depleted NO3− out of the system, leaving isotopically enriched NH4+ for uptake by plants and further nitrification. There is also substantial discrimination against 15N with denitrification, causing an outflow of depleted gaseous N2 and N2O and leaving isotopically enriched NO3−. The relative importance of these processes determines the δ^{15}N of the plant available forms of N, and depending on the fraction taken up as NH4+ and NO3− determine plant δ^{15}N. The picture can be further complicated when ectomycorrhizal fungi utilize organic N. When ecosystem NO3− losses are large, N cycling is predicted to increase soil and plant δ^{15}N. When losses are small compared to total N-cycling, δ^{15}N tends to be lower.

For tropical lowland forests, higher soil and foliage δ^{15}N is reported compared to temperate and boreal forests (Martinelli et al. 1999; Amundson et al. 2003). Low δ^{15}N values are also reported for tropical montane forest (Brearley 2013). This pattern is considered as evidence of more N losses and thus a more open N cycle in tropical forests compared to temperate forests. The latter are generally more N-limited, whereas the former tend to be more P-limited (Vitousek & Howarth 1991). Leguminous trees are abundant in tropical forests, although not all can form an effective symbiosis with Rhizobia. Nevertheless, facultative leguminous N2-fixers can still be abundant (Menge & Chazdon 2016) and contribute to N-accumulation also in late successional stages of tropical forests (Roggy et al. 1999; Pons et al. 2007), which can alleviate N-limitation. N2-fixing trees are virtually lacking in temperate forest where obligate actinorhizal N2-fixing trees are mostly limited to early successional stages (Menge, Levin & Hedin 2009).

The degree of N-limitation and its effect on soil and tree δ^{15}N has been investigated experimentally with fertilizer addition in temperate and tropical forests. The experiments in temperate forest showed increases in tree δ^{15}N under additional N treatments (Elhani et al. 2005), which were paralleled by soil N-losses (Högberg & Johansson 1993). Only one long-term fertilizer experiment is known from a tropical forest (Mayor et al. 2014). Two of the four species that were measured in a forest in Panama showed increases in tree δ^{15}N after N-addition and increased δ^{15}N of soil NO3−, but not NH4+. This partly confirms the predicted effects of a more open N-cycle on δ^{15}N in tropical forests.

δ^{15}N can be measured both in sapwood and heartwood. The lower concentration of cell wall proteins conserved in heartwood still leaves sufficient N for measurement of δ^{15}N in small heartwood samples from tree rings. This offers a unique opportunity to investigate historical changes in the N-cycle of forests (Gerhart & McLauchlan 2014) based on the mechanisms described above.

Global change factors include increases in atmospheric CO2 concentration and N-deposition. Increased CO2 increases potential growth rate of trees and thus demand for N. This can be expected to cause an increased N-limitation, less NO3− losses and thus a decrease in soil and tree δ^{15}N. On the other hand, N-deposition will increase N-availability and can be expected to cause increased NO3− losses resulting in an increase of δ^{15}N in the system. The balance between these two processes, in conjunction with other processes, will ultimately determine the resulting change in δ^{15}N of tropical trees.
Stable isotope methodology

Identification of tropical tree rings

To establish time series of isotopic values from tree rings, a proper dating of these rings is crucial. This requires that ring boundaries can be observed anatomically, that the study tree species produces rings annually and that the incidence of false or wedging rings is low.

To fulfil the first requirement – observing ring boundaries in the wood – basic knowledge on wood anatomy, proper preparation of wood samples (cutting or sanding) and microscopes are needed (e.g. Stokes & Smiley 1996). Tree ring boundaries are formed just before periods of cambial dormancy or periods of reduced growth, e.g. during a dry season or annual flooding, and can take many different forms. Worbes (1985, 2002) proposed a simple anatomical classification of ring boundaries, or ‘growth zone delimitation’, for tropical trees characterized by: (i) density variations in the wood; (ii) narrow marginal parenchyma bands; (iii) concurring parenchyma and fibre tissue; and (iv) variation in the size or density of vessels (‘ring-porous’). The tree ring boundaries of each tropical tree species can be described by one or more of these classes, and this classification is species specific. Lists of growth zone classification (Worbes 2002) for tropical tree species and information on wood characteristics in databases (e.g. http://insidewood.lib.ncsu.edu/) can assist in recognizing growth zones.

The second requirement is the annual nature of rings formed in the study species. The recent interest in tropical tree ring studies has resulted in a list of 230 tropical tree species that produce rings annually (Zuidema, Brienen & Schöngart 2012; Brienen, Schöngart & Zuidema 2016). Annual ring formation in these species has been verified, using chronology building, climate-growth correlations and/or radio-carbon dating. As tropical dendrochronology is a rapidly expanding field, the number of tropical tree species with proven annual ring formation will undoubtedly increase further in the near future.

The final requirement is that the incidence of false or missing rings is low. False rings are structures that resemble ring boundaries, but are often anatomically distinct, are not formed along the entire circumference of a tree, and may be induced by environmentally harsh conditions during the growing season. Missing rings may occur if
radial growth is very slow and two or more ring boundaries coincide. This often occurs on only part of the circumference of a tree (Worbes 2002; Groenendijk et al. 2014). Some species possess highly irregular, ‘fluted’ stems, implying that during a given year radial wood growth is only realized on certain portions of the circumference. In these trees, tree ring analysis is virtually impossible. A basic methodological tool to verify ring identification in a sample is to check whether the number of rings (and growth patterns) in each direction is the same. In addition, cross-dating of growth patterns between different individuals is an important tool in tree ring research to check the quality of ring dating and to correct dating errors (e.g. Douglass 1941; Fritts 1976).

ONTOGENETIC EFFECTS

When deriving environmental variables from tree rings (such as climate variability), age or size-related trends in stable isotope ratios must be taken into account (McCarroll & Loader 2004; Gessler et al. 2014; Brienen, Schöngart & Zuidema 2016). Juvenile trees growing under a forest canopy are exposed to reduced irradiance and possibly to 13C-depleted CO2 (Buchmann et al. 1997), thus enforcing the increasing trend of δ13C with tree size. Subcanopy trees are exposed to slightly 13C enriched CO2 due to high photosynthetic activity in the canopy, whereas larger trees presumably take up CO2 with similar δ13C compared to atmospheric CO2 (Buchmann et al. 1997). Additionally, a changing contribution of bark photosynthesis to wood formation could also modify its δ13C (Cernusak et al. 2001). Although, changes in δ13C with tree size can provide valuable information in an ecological context (e.g. on changing light conditions), it is an important confounding factor in dendroclimatological studies.

The effect of tree age or size on tree ring δ18O is less straightforward. Individual trees can show trends in δ18O that may be ontogenetically determined (Xu, Sano & Nakatsuka 2011). A developing root system may take up water from increasingly greater soil depths and thus access water with different δ18O, although that was not unequivocally established (Stahl et al. 2013). Evaporative demand increases when trees grow into the canopy and further when emerging above, causing increasing leaf water 18O enrichment. Growing hydraulic resistance with size can reduce gs and thus further add to isotopic enrichment. These factors can be responsible for increasing trends in tree ring δ18O in individual trees (Poussart & Schrag 2005). However, trends not related to age or size could also be involved (Brienent et al. 2012; van der Sleen 2014), such as a gradual change in source water δ18O.

Little is known about changes in δ15N with tree development. In a study on six tropical tree species, van der Sleen et al. (2015b) found evidence for changes in δ15N with tree diameter, possibly caused by increasing rooting depth or otherwise changes in the δ15N of N sources exploited by the tree.

Recovering trends in isotopic ratios independent of ontogeny requires sampling strategies (e.g. van der Sleen et al. 2015a, b) or statistical analyses (e.g. Hietz et al. 2011; Nock et al. 2011) that allow separating ontogenetic from temporal trends.

SAMPLING RESOLUTION

Sampling for isotope analysis in tree rings can be done with different temporal resolutions (intra-annual to decadal), depending on the objective of the study. When gradual changes over longer periods of time are the principle interest, then bulk (or pooled) samples of several years (rings) can be taken. This was used by Hietz, Wanek & Dünisch (2005) and van der Sleen et al. (2015a) for investigating the gradual increase in iWUE during the period of CO2 increase after the beginning of industrialization. This approach is not limited to trees with distinct annual rings: Loader et al. (2011) sampled wood in the radial direction and dated them using 14C. Although the age of the wood may then be less accurately known compared to the annual resolution of tree rings, it is still suitable for the analysis of long-term trends. As N is to some extent mobile in sapwood (Elhani et al. 2003), sampling for δ15N in tree rings is also often done by pooling several adjacent rings (Hietz, Dünisch & Wanek 2010; Hietz et al. 2011; van der Sleen et al. 2015b), which is sufficient for recovering gradual trends.

Studies that aim to evaluate annual climatic fluctuation and its effect on tree physiology require an annual sampling resolution. This approach relies on identification of annual rings, either anatomically or otherwise. Most of the studies mentioned in Section Stable isotopes sequences in tropical trees follow this approach and are discussed there.

Sampling at higher, intra-annual resolution can serve several purposes. For deciduous trees in temperate climates, intra-annual sampling can reveal carry-over effects from 1 year to the next, particularly in the case of 13C. Reserves stored late in the growth season are used for early wood formation next spring and thus carries the isotopic signatures of that part of the previous year (Helle & Schleser 2004). This is likely to be the case for deciduous tropical trees as well but has not been investigated. Evergreen trees store reserves also, but their mobilization is less predictable. Intra-annual sampling often revealed seasonal patterns of δ13C and/or δ18O. These patterns were used to establish the annual nature of anatomical rings (e.g. Verheyden et al. 2004) or to identify annual growth periods in trees with wood that lack visible rings (see discussion below).

PRE-TREATMENT OF WOOD SAMPLES AND CELLULOSE EXTRACTION

Whole-wood samples were used in early studies on isotopes in tree rings (Craig 1954; Libby & Pandolfi 1974). When it was recognized that different wood components...
have different isotopic compositions, and thus that variation in chemical composition of the wood influenced δ-values, it became a standard procedure to extract the principle component cellulose for isotopic analysis. This has the advantage of more straightforward mechanistic modelling opportunities (McCarroll & Loader 2004). However, cellulose extraction has also disadvantages such as the labour intensive and thus costly laboratory procedure, which may limit the number of samples that can be processed in a research project. Larger samples are also required, which limits the resolution with intra-annual sampling (Verheyden et al. 2004; Pons & Helle 2011) and the use of high through put techniques (e.g. Schulze et al. 2004; Schollaen, Heinrich & Helle 2014).

For δ¹³C, it has been shown more recently that suitable data can be generated by using whole-wood after resin extraction. These data are useful for climate reconstruction using a statistical approach, and may even provide better climate correlations, possibly as a result of variation in the fractions of wood constituents with changing environmental conditions, thereby enhancing the seasonal variation in δ-values (Helle & Schleser 2004; Gori et al. 2013; Schleser et al. 2015).

Comparison of δ¹⁸O between whole-wood and cellulose gives generally also good agreement with around 4‰ higher values for the latter (Borella, Leuenberger & Saurer 1999; Barbour, Andrews & Farquhar 2001; Jaggi et al. 2002), although not invariably so (Ferrio & Voltas 2005; Battipaglia et al. 2008). The choice between whole-wood and cellulose thus depends on the research question, whether whole-wood enhances climate correlations in a particular species, and the available facilities.

To improve annual resolution of δ¹⁵N, which is doubtful due to known mobility of nitrogenous compounds between rings (Hart & Classen 2003), it has become practice to extract soluble compounds before analysis. However, whether this does indeed improve annual resolution has not been unequivocally established (Gerhart & McLaughlan 2014).

Stable isotopes sequences in tropical trees

CARBON

Early measurements of δ¹³C in tropical wood (Leavitt & Long 1991) indicated that there is intra-annual variation, similar to what was found for other climatic regions (Table S1, Supporting Information). In the trees with distinct rings from Puerto Rico, Tectona grandis and Pinus caribea, δ¹³C increased in early wood and decreased to lower values in late wood. A roughly similar pattern was later found in temperate and Mediterranean trees using high resolution sectioning of tree rings (Helle & Schleser 2004; Roden, Johnstone & Dawson 2009). Tectona grandis was also investigated in Central Java, Indonesia (Poussart, Evans & Schrag 2004), but a less clearly defined intra-annual variation in δ¹³C was found compared to the pattern described above. Intra-annual variation in δ¹³C cannot be unequivocally understood from current photosynthesis on the basis of the Farquhar, O’Leary & Berry (1982); Farquhar, Ehleringer & Hubick (1989) model as discussed in Section Carbon. It is likely that other processes interfere with the pattern, such as utilization of stored reserves early in the growth season, fractionation downstream from primary carbon fixation, and a varying fraction of C allocated to other processes than diameter increment in the growth season (Helle & Schleser 2004; Kagawa, Sugimoto & Maximov 2006).

Attempts have also been made to identify annual rings in tropical trees that lack visible increment ring boundaries, using the hypothesized intra-annual pattern of stable isotope ratios. Leavitt & Long (1991) measured the radial variation in δ¹³C in some tropical wood samples without a distinct ring structure. The variation of 1–2‰ was similar to the samples with distinct rings and was interpreted as seasonal cycles of changing water availability. The authors showed the potential of high-resolution δ¹³C sampling for identifying non-distinct annual rings. The intra-annual variation in δ¹³C of up to 3‰ in combination with similar inter-annual variation in δ¹⁸O confirmed that the visible ring structures in the mangrove species Rhizophora mucronata from Kenya were annually formed (Verheyden et al. 2004). For the purpose of identifying none-visible growth rings, δ¹⁸O proved to be more useful compared to δ¹³C and that will be discussed in the next section.

Several studies have investigated the inter-annual variation in δ¹³C and its correlation with precipitation amount (Table 1; Table S1). This is based on the Farquhar, O’Leary & Berry (1982) model that predicts an increase in δ¹³C as a result of higher iWUE at low water availability. Notwithstanding the confounding factors mentioned above, the approach proved to be successful in a study using seven species from various sites in tropical America and Africa that widely differed in annual precipitation (Fichtler, Helle & Worbes 2010). Strong correlation of δ¹³C with precipitation was also found for three Acacia species in semi-arid Ethiopia (Gebrekirstos et al. 2009, 2011). The correlation was best for rainy season precipitation when the species were combined.

Other studies combined δ¹³C sequences with measurements of δ¹⁸O (Table 1; Table S3). In some of these, δ¹³C showed correlation with other precipitation variables than those found for δ¹⁸O (Cullen & Grierson 2007; Schollaen et al. 2013). Nevertheless, δ¹⁸O series generally yielded stronger correlations with precipitation variables than δ¹³C series (Poussart & Schrag 2005; Ballantyne et al. 2011; and see discussion in the next section). Stable carbon isotopes can thus be used to analyse the relationship between water availability and growth of tropical trees but they do not always provide a good proxy for precipitation amount.

Variation in δ¹³C is not only influenced by water availability, but also by fluctuations in other environmental factors such as light and nutrient availability (Ehleringer et al. 1986; Saurer et al. 1997; Cernusak, Winter & Turner
D generally had an increased stimulated growth. The trees without growth stimulation that increased light and possibly nutrients availability had ing atmospheric CO2 concentration, iWUE has increased cally constant over time and that, as a result of the increas-

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ENSO, El Niño Southern Oscillation; iWUE, intrinsic water-use efficiency; gs, stomatal conductance.

*Total number of species and those with non-distinct rings between brackets. Some species were involved in more studies, notably Tectona grandis and Cedrela odorata. Total number of species for the three isotopes combined is 85 (35).

2009). This notion was used by van der Sleen et al. (2014) to identify the causes of variation in growth response of Pellogynae cf heterophylla saplings to gap formation in a tropical forest. The trees that showed increased growth after gap formation had also a decreased Δ13C, suggesting that increased light and possibly nutrients availability had stimulated growth. The trees without growth stimulation generally had an increased Δ13C, which suggested that water stress was not underlying this response, but rather that light and/or nutrient availability had not increased sufficiently. Stable carbon isotopes in tree rings can thus provide a retrospective analysis of the potential factors involved in growth regulation other than water.

Studies using longer sequences have consistently shown a declining δ13C trend over the last one or two centuries in temperate and boreal forest (Leavitt & Lara 1994; McCarroll & Loader 2004; Saurer, Siegwolf & Schweingruber 2004; Treydte et al. 2009). After correcting for the decreasing δ13C atm over that period, a rather constant 13C discrimination (Δ13C) generally remains. This leads to the conclusion (based on eqns 2 and 3) that C4/C3 was basically constant over time and that, as a result of the increasing atmospheric CO2 concentration, iWUE has increased consistently over time (Silva & Anand 2013). An increasing iWUE was also found in FACE experiments were CO2 concentration was experimentally increased above present levels (Battipaglia et al. 2013). The more recent analysis of tropical stable carbon isotope sequences yielded similar decreases in δ13C, stable C4/C3 and increasing iWUE (Hietz, Wanek & Dünisch 2005; Brienen, Wanek & Hietz 2011; Loader et al. 2011; Nock et al. 2011; van der Sleen et al. 2015a). The latter two studies also quantified trends in tree growth derived from tree ring width, but found no indications for growth stimulation over the past century. Stem diameter growth is not necessarily linearly linked to photosynthetic activity, because other aspects of the carbon balance of trees may have changed as well, such as phenology, leaf turnover, respiration and biomass allocation. However, if these factors have not changed, these results suggest that photosynthesis did not increase as a result of rising atmospheric CO2 concentration. The increasing iWUE would then imply a decrease in transpiration, and that surplus water must have increased runoff in catchment areas and river basins (Betts et al. 2007).

### Stable isotopes in tropical tree rings

<table>
<thead>
<tr>
<th>Stable isotope</th>
<th>Main usage</th>
<th>Main outcome</th>
<th>Number of species*</th>
<th>Number of publications</th>
<th>Details in</th>
</tr>
</thead>
<tbody>
<tr>
<td>13C</td>
<td>Proxy for iWUE</td>
<td>iWUE increased generally over time</td>
<td>43 (11)</td>
<td>14</td>
<td>Table S1</td>
</tr>
<tr>
<td></td>
<td>Identification of annual rings in ring-less wood</td>
<td>Moderate potential to identify annual rings through intra-annual sampling</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Unraveling isotope-climate relations</td>
<td>Negative correlation with rainfall</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18O</td>
<td>Reconstruction of past climate</td>
<td>Some prospect for rainfall reconstruction</td>
<td>27 (8)</td>
<td>22</td>
<td>Table S2</td>
</tr>
<tr>
<td></td>
<td>Unraveling isotope-climate relations</td>
<td>Correlations with rainfall (both + and -) and ENSO in shallow rooting trees</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13C &amp; 18O</td>
<td>Identification of annual rings in ring-less wood</td>
<td>Good potential to identify annual rings through intra-annual sampling</td>
<td>26 (17)</td>
<td>11</td>
<td>Table S3</td>
</tr>
<tr>
<td></td>
<td>Unraveling isotope-climate relations</td>
<td>Correlations with rainfall and ENSO</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Identification of drivers of changes in iWUE (gs, vs. photosynthesis)</td>
<td>Some evidence that iWUE increased through decreased gs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15N</td>
<td>Effects of increased N deposition on N cycling</td>
<td>Potential to record aspects of nitrogen cycle</td>
<td>9 (0)</td>
<td>3</td>
<td>Text</td>
</tr>
</tbody>
</table>

Reviewed publications are summarized in Tables S1, S2 and S3 in Supporting Information; those on 15N are reviewed in the text. ENSO, El Niño Southern Oscillation; iWUE, intrinsic water-use efficiency; gs, stomatal conductance.

boundaries of tree species without anatomically distinct rings or to confirm the annual nature of ring formation (Evans & Schrag 2004; Poussart, Evans & Schrag 2004; Verheyden et al. 2004; Poussart & Schrag 2005; Evans 2007). In some of these studies, δ18O was also measured, but δ13C was found to be generally superior for this purpose (Table 1; Table S3). The suitability of δ18O for the identification of annual rings is based on its seasonal change in precipitation, with low values in the rainy season, causing annual cycles in δ18O of stem wood. High δ18O values of precipitation during the dry season can be further amplified in tree rings by enrichment of water as a result of evaporation from exposed surface soil and in leaves due to low humidity. This seasonality in δ18O was confirmed in trees with distinct annual rings in Tachigali myrmecophila from a humid tropical forest (Ballantyne et al. 2011) and in T. grandis in Indonesia with a distinct dry season (Poussart, Evans & Schrag 2004). Soil water enrichment during the dry season translated into high δ18O values in early wood of the following growth season (Schollaen et al. 2013). Tectona grandis showed a similar intra-annual pattern in Central India, with lowest tree ring δ18O in the rainy season, but this pattern was reversed in Southern India as monsoon rains exhibit a different δ18O signature (Managave et al. 2010, 2011a).

Most studies conclude that trees in closed humid tropical forest primarily record variation in δ18O of precipitation water (but see below). The identification of annual rings in wood that are not visually distinct can be most successfully done when intra-annual variation in source δ18O is large. This is the case in the western parts of the Amazon basin, where δ18O in precipitation is low due to rain-out of the heavy isotope in the rainy season (Sturm, Hoffmann & Langmann 2007). Evidence for this effect is provided by the lower intra-annual variation in δ18O in evergreen trees from Guyana (1–4‰; Pons & Helle 2011) compared to trees sampled near Manaus, Brazil (3–6‰; Ohashi et al. 2016). A special case are trees in montane forests where, in the rainy season, the uptake of water is from precipitation whereas moisture is predominantly absorbed from clouds in the dry season. These two water sources differ in δ18O values, resulting in large seasonal variation in Ocotea tenera (up to 9‰) at 1500 m altitude in Costa Rica, although less so (1–5‰) in a Pouteria species (Anchukaitis et al. 2008; Anchukaitis & Evans 2010). As the strength of intra-annual variation in δ18O varies across species (Poussart & Schrag 2005; Anchukaitis et al. 2008) and climatic conditions, selection of species and sites is crucial when the goal is to identify annual rings when they are not distinct.

Because some trees incorporate the δ18O signature of rainwater in stem wood, time series of tree ring δ18O can be used to quantify past variability in precipitation. Tree ring δ18O has been correlated with basin-wide precipitation in the Amazon (Ballantyne et al. 2011; Brien et al. 2012; Baker et al. 2015), and regional precipitation in Thailand (Poussart & Schrag 2005). Costa Rica (Anchukaitis & Evans 2010), India (Managave et al. 2011b), Indonesia (Schollaen et al. 2013, 2015), Laos and Vietnam (Xu, Sano & Nakatsuka 2013) and West-Central Africa (van der Sleen, Groenendijk & Zuidema 2015). Particularly El Niño Southern Oscillation variability is often evident in δ18O sequences (Table 1; Table S2) either from positive anomalies (Verheyden et al. 2004; Anchukaitis & Evans 2010; Zhu et al. 2012) or negative ones (Evans & Schrag 2004; Brienen et al. 2012). The analysis of tropical tree ring δ18O is developing into a powerful tool for reconstructing the variability of precipitation on regional scales.

Several species were found that show good synchronization of δ18O among individual trees (e.g. Poussart & Schrag 2005; Managave et al. 2011b; Brienen et al. 2012; van der Sleen, Groenendijk & Zuidema 2015) sometimes over large spatial distances (Baker et al. 2015; Volland, Pucha & Bräuning 2016). Synchronous variability in δ18O can be higher than for ring-width, thus providing an alternative tool for cross dating (Baker et al. 2015; Volland, Pucha & Bräuning 2016) and identification of false and missing rings (Boisen, Evans & Baker 2014). However, δ18O synchronization between individuals may be low for certain species or sites (e.g. Poussart & Schrag 2005; Baker et al. 2015). For δ18O in Toona ciliata low δ18O synchronization occurred (van der Sleen 2014), in spite of ring-width series showing strong synchronization (Vlam et al. 2014). It is likely that trees that lack a common signal in tree ring δ18O values exploit other water sources than recent precipitation. Deep rooting species likely use ground water, which has a δ18O signature that may be formed over several years. Shallow-rooting trees are more likely to take up recent rainwater and thus more closely record δ18O variability in rainwater. This suggests that shallow rooting tree species on well-drained soils have the highest probability to record the δ18O variability of precipitation and thus have the highest potential as tools for climate reconstructions. Ideally, this is confirmed by analysis of xylem water and concurrently formed wood.

A long-term increase of δ18O values has been encountered in several studies conducted on tropical tree species (Poussart & Schrag 2005; Xu, Sano & Nakatsuka 2011; Brienen et al. 2012; van der Sleen 2014; van der Sleen, Groenendijk & Zuidema 2015; Volland, Pucha & Bräuning 2016) Some of these trends could be caused by ontogenetic changes, when sampling design did not correct for this, but a consistent small trend over the past century was also found in studies that did correct for ontogenetic effects (Brienen et al. 2012; van der Sleen 2014; van der Sleen, Groenendijk & Zuidema 2015; Volland, Pucha & Bräuning 2016). For the Amazon region, these results are consistent with similar increases of δ18O in Andean ice cores (Thompson et al. 2006) and Andean lake sediments (Bird et al. 2011). Thus, the increasing trend in δ18O in tree rings likely reflects a pan-tropical increase in precipitation δ18O. The cause of this increase is yet unknown, and it is unclear whether it reflects climate change.
In several studies the two stable isotopes $^{18}$O and $^{13}$C were measured on the same sample (Table 1; Table S3). This was mostly done to investigate to what extent the combination would give superior results for identification of annual rings and/or for climate reconstructions, or to assess which of the two would be superior in this respect. When the two isotopes are combined using a mechanistic interpretation, the so-called dual isotope approach, $A/g_s$ obtained from $\Delta^{13}$C and $g_s$ derived from $\Delta^{18}$O gives an estimate of $A$ (Scheidegger et al. 2000). This approach was adopted by Nock et al. (2011), who interpreted an increase of $\Delta^{18}$O over time as an indication of a decreasing $g_s$. The decreasing $\Delta^{13}$C, and thus increasing $A/g_s$, would then be the result of this decreasing $g_s$ and not an increasing $A$. The absence of an increase in diameter growth was consistent with a lack of increasing photosynthesis with the increasing atmospheric CO$_2$ concentration.

There are several uncertainties when using $^{13}$C of tree ring cellulose as a proxy for $A/g_s$, as explained in Section Carbon, but input variables for the model are reasonably straightforward. Uncertainties with $^{18}$O modelling are, however, greater (Rodén & Siegwolf 2012; Gessler et al. 2014), particularly in large tropical trees where most input variables for the mechanistic models are poorly known. Nevertheless, Evans (2007) obtained modest correlation between modelled and observed tree ring $\delta^{18}$O values. Kahmen et al. (2011) measured all necessary variables for modelling $\delta^{18}$O from physiology (see Section Oxygen) for Merosideros polymorpha, a shrub growing along an altitudinal gradient on Hawaii. They conclude that the effects of temperature and humidity cannot be distinguished as they combine into the leaf to air vapour pressure difference (VPDif). As mentioned above, $\delta^{18}$O has been used for inferring $g_s$. Among others, this is based on experiments where $g_s$ was manipulated independently of VPDif, which showed that increasing $g_s$ negatively affects $\delta^{18}$O (Barbour & Farquhar 2000). Yet, $g_s$ is not part of the $^{18}$O enrichment model (Barbour 2007). Its effect on leaf water enrichment operates through transpiration rate, which is controlled by VPDif and $g_s$. In turn, $g_s$ is partly controlled by VPDif, and that in a species- and conditions-specific manner (Rodén & Siegwolf 2012). This makes it difficult to separate the $g_s$ effect from the VPDif effect on transpiration. Hence, deriving $g_s$ from tree ring cellulose $\delta^{18}$O is only straightforward when VPDif is not an interacting factor such as when comparing species under identical conditions. However, retrospectively deriving temporal trends in $g_s$ is complicated as VPDif may have changed.

**NITROGEN.**

So far, only three studies on $\delta^{15}$N in tree rings have been carried out in tropical forests (Hietz, Dinisch & Wanek 2010; Hietz et al. 2011; van der Sleen, Groenendijk & Zuidema 2015). Hietz, Dinisch & Wanek (2010) using two annual ring forming species in a Brazilian forest, found a gradual increase of $\delta^{15}$N over time after statistical correction for tree age. The authors suggested that this result could be caused by an increase in tree turnover and thus gap formation that generates NO$_3^-$ losses and thereby increasing $\delta^{15}$N of the remaining soil N pool. In a next study, Hietz et al. (2011) reported also an increase in $\delta^{15}$N in three species from monsoon forest in Thailand. They also found a similar increase when comparing 40-year-old herbarium leaves with leaves from the same species and sample location in a Panamanian forest (BCI). The two forest are intensively monitored and there are no indications that the level of disturbance has increased over the past century. The authors conclude that increase $\delta^{15}$N is mostly caused by more N-deposition, which causes higher NO$_3^-$ losses.

In the most recent study van der Sleen et al. (2015b) sampled annual rings from six species from three sites at different continents. They corrected for possible tree size effects by comparing wood sampled at a fixed diameter (20 cm) from different sized trees. Ten-year pooled samples were also collected between 1955 and 2005 from single trees, which showed increasing trends of $\delta^{15}$N in Bolivia and Cameroon. However, the trends were absent in the fixed diameter sampling, showing evidence of ontogenetic effects. A striking result was that no significant trend was found for the species from Thailand, the same site and the same two species as used by Hietz et al. (2011). Also when excluding sapwood, as done by Hietz et al. (2011), no significant increase was found. The discrepancy between the results may have been caused by the low statistical power for the Thailand sampling of van der Sleen et al. (2015b). Anthropogenic emissions are relatively high and increasing around the site in Thailand, which would be consistent with increasing N-losses in that forest as a possible reason for an increasing tree ring $\delta^{15}$N.

The constant or increasing values of $\delta^{15}$N reported in the three tropical tree ring studies mentioned above contrasts with results from temperate forests where trends are typically negative at sites that are sufficiently distant from sources of N-pollution (Gerhart & McLauchlan 2014). This is interpreted as evidence for a decrease in N-availability (Galloway et al. 2008). A possible explanation, albeit one without empirical support, is that the N-limited temperate forests trees are responding to increasing atmospheric CO$_2$ concentrations with an increased demand for N. This would result in reduced N-availability relative to demand and reduced NO$_3^-$ losses, resulting in decreasing $\delta^{15}$N (Hobbie & Högberg 2012).

The situation is different in tropical forests where trees are more P-limited rather than N-limited (Vitousek & Howarth 1991). The P-limitation could prevent an increased growth response to increased atmospheric CO$_2$ (Körner 2009) and thus not stimulate N-demand. This would make their N-cycle more responsive to additional N-input, causing NO$_3^-$ losses with the associated rise in $\delta^{15}$N. However, in a fertilizer experiment where N-addition was large compared to atmospheric deposition, only two out of four species showed an increased $\delta^{15}$N (Mayor et al.
2014), suggesting that not all species are good recorders of alterations in the local nitrogen cycle. An alternative reason for the increasing δ15N in tropical tree rings could be enhanced NO3-I losses associated with increased forest disturbance, as initially proposed by Hietz, Dünisch & Wanek (2010).

Tropical montane forest trees have lower foliage δ15N compared to lowland forest (Brearley 2013), which is ascribed to slower mineralization at low temperature and, hence, lower NO3-I losses. Global warming effects in lowland forest could thus include increased mineralization and nitrification, leading to increased NO3-I losses. Unfortunately, the interpretation of temporal changes in δ15N in the few available tropical tree ring studies is strongly hampered by a limited understanding of the factors that influence δ15N values.

Conclusions and Outlook

Understanding how global change will alter the physiology and growth of tropical trees is an urgent need for ecology, climate science and conservation. The study of stable isotopes in tropical tree rings offers opportunities to quantify how trees respond to environmental change. Given that trees can live for centuries, an important asset of this method is that it allows studying these changes over periods that extend well-beyond observation records. Thus, pre-industrial conditions, such as CO2 concentration, precipitation and atmospheric N-deposition, can be obtained as a benchmark and provide context for interpreting values or trends in recent years.

Nonetheless, there are major methodological and interpretation issues to overcome in this field, including: (i) uncertainties with the parameterization of the models used to calculate carbon and oxygen isotope fractionation; (ii) potential confounding effects of ontogenetic changes on isotope ratios; and (iii) sampling and analysis requirements of tree ring research in general. These can cause substantial uncertainties in the interpretation, and thus the validity of conclusions from isotope analyses. However, because isotope analyses are essentially the only tools available to obtain cost-effective, high-resolution, long-term retrospective data on tree physiology and its environmental drivers, it is crucial that these issues are addressed.

We therefore stress the need for studies on fundamental isotope environmental physiology in tropical trees, including relevant isotopic fractionation in a tree’s environment, such as: (i) characterization of the depth of water uptake from the soil profile for trees used for climate derived δ18O correlations and reconstructions supplemented with xylem-water measurements to address the question to what extent source water is isotopically unmodified rainwater; (ii) further development of the dual isotope (13C and 18O) approach allowing better assessment whether changes in Ci are a consequence of changes in A, gs, or both; (iii) characterization of model parameter values that are specific to species or environmental conditions; (iv) investigation of the N sources taken up, and isotopic fractionation in processes of the N-cycle in the soil profile for interpreting 15N data; (v) further experimental work on the manipulation of the N-cycle and its effect on δ15N in tropical trees; (vi) a better understanding of the causes of ontogenetic effects, which is essential to separate them from time trends, an issue that tends to be neglected in tree ring isotope studies (Peters et al. 2015).

Stable isotope studies on tropical trees are currently centred on the three isotopes discussed in this paper (C, O, and to a lesser degree N). A broadening of these analyses is expected in the near future. A recent development is the analysis of the intramolecular distribution of isotopes. For instance the position of 16O in the glucose moiety in cellulose can be used to separate source water from leaf water enrichment effects (Sternberg 2009; Waterhouse et al. 2013), and the position of 2H was related to the oxygenation/carboxylation ratio that depends on Cj (Ehlers et al. 2015). These techniques can be used to infer more details about environmental effects on tropical trees than is possible with bulk isotopic ratios as done so far.

One of the great advances in the field of dendrochronology has come from the collation of tree chronologies in open access databases, such as the International Tree Ring Data Bank (ITRDB; Grissino-Mayer & Fritts 1997), yielding a large number of influential studies. More recently, the ITRDB has expanded its capacity as a repository for tree ring isotope data (Csank 2009). We see great virtue in making stable isotope data available (published or not) and encourage researchers to submit their data to open access data banks like the ITRDB.

Authors’ contributions

All authors contributed to the writing.

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Data accessibility

This manuscript uses only published data.

References


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**Supporting Information**

Details of electronic Supporting Information are provided below.

**Appendix S1.** Studies where $\delta^{13}$C and/or $\delta^{18}$O were measured in tropical tree-ring sequences.

**Table S1.** Studies using $\delta^{13}$C.
**Table S2.** Studies using $\delta^{18}$O.
**Table S3.** Studies where $\delta^{13}$C and $\delta^{18}$O were combined.