REVIEW

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 (^{13}C), oxygen (^{18}O) and nitrogen (^{15}N).

2. We start by explaining how environmental and physiological effects define the isotopic composition of wood in tropical trees. Abundance of ^{13}C is mainly driven by water, light and nutrient availability. Here ^{18}O values are chiefly determined by those of rainwater and additionally by rooting depth and factors determining leaf water evaporation. The ^{15}N levels are determined by the ^{15}N 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 δ^{13}C and δ^{18}O was correlated with precipitation and El Niño Southern Oscillation variability. Seasonality in δ^{13}C and δ^{18}O was successfully used for delimiting visually non-distinct annual rings. Tropical tree responses to increasing atmospheric [CO_2] were effectively quantified, using δ^{13}C as a measure of intrinsic water use efficiency. And finally, anthropogenic changes in the nitrogen cycle in tropical forests have been inferred from δ^{15}N.

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,

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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.

$$\delta_{\text{sample}} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000(\text{‰}) \qquad \text{eqn 1}$$

[Correction added after online publication on 17 July 2017: Equation 1 was updated to change the final number from 100(‰) to 1000(‰). The authors and editors are satisfied that this does not affect the results or conclusions of this study.]

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 δ^{13}C in plant tissue compared to atmospheric CO_2. The V-PDB standard for δ^{13}C measurement is rather arbitrarily chosen, resulting in δ ^{13}C values that provide little insight. A more insightful way of expressing the isotope effect is discrimination against ^{13}C (Δ^{13}C = $R_{\text{atm}}/R_{\text{plant}}$ − 1). This can be calculated from δ-values as follows:

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

If long time series of $\delta^{13}C_{\text{plant}}$ are analysed, the gradual decrease in $\delta^{13}C_{\text{atm}}$, from a preindustrial value of −6·4‰ to lower than −8‰ at present, must be taken into account

(Treydte *et al.* 2001; McCarroll & Loader 2004). Also, seasonal variation in $\delta^{13}C_{atm}$ can be significant for intra-annual 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):

$$\Delta^{13}C = a + (b - a)C_i/C_a (\permil), \qquad \text{eqn 3}$$

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 ($4.4\permil$) and b the discrimination by Rubisco ($27\permil$). 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 $\delta^{13}C_{atm}$ 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.

$$iWUE = A/g_s = (C_a - C_i)/1.6 (\mu mol\,mol^{-1}) \qquad \text{eqn 4}$$

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
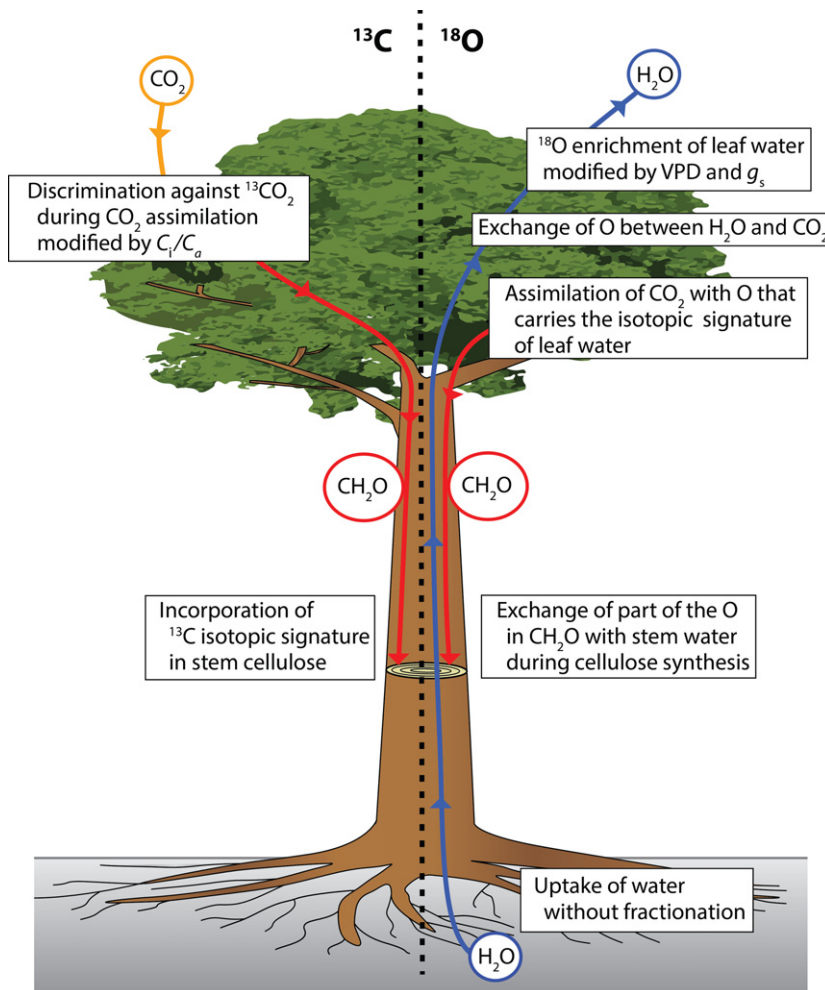
Fig. 1. Schematic representation of physiological processes in a tree that determine $\delta^{13}C$ (left) and $\delta^{18}O$ (right) in tree rings. Discrimination against ^{13}C occurs during diffusion and assimilation of CO_2. Assimilates (CH_2O) are transported to the cambium where they are incorporated into the wood. This carries the ^{13}C signature of the assimilates after some downstream modifications. Evapotranspiration enriches leaf water with ^{18}O. The O in H_2O and CO_2 are exchanged, which transfers the isotopic signature to CO_2. The resulting enriched assimilates (CH_2O) are transported to the cambium where part of the O is exchanged with stem water during cellulose synthesis. Progressive cambial activity builds an archive of temporal changes in the above-mentioned processes during a tree's lifetime. C_i, intercellular CO_2 concentration; C_a, atmospheric CO_2 concentration; g_s, stomatal conductance; VPD, vapour pressure deficit.

Figure labels:
^{13}C | ^{18}O
CO_2 / H_2O
Discrimination against $^{13}CO_2$ during CO_2 assimilation modified by C_i/C_a
^{18}O enrichment of leaf water modified by VPD and g_s
Exchange of O between H_2O and CO_2
Assimilation of CO_2 with O that carries the isotopic signature of leaf water
CH_2O | CH_2O
Incorporation of ^{13}C isotopic signature in stem cellulose
Exchange of part of the O in CH_2O with stem water during cellulose synthesis
Uptake of water without fractionation
H_2O

availability as a result of stomatal closure (Lambers, Chapin & Pons 2008). The above described theory thus predicts that $\Delta^{13}C$ is lower in drought conditions as a result of decreased g_s and consequently C_i. 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: $\delta^{13}C$ of leaves collected in the rainy season were lower than those collected in the dry season (Buchmann *et al.* 1997) and wood $\delta^{13}C$ of trees near a creek were lower than that of trees on the ridge (Pons *et al.* 2005).

Co-occurring tropical forest tree species can exhibit large variation in $\delta^{13}C$ (Guehl *et al.* 1998; Bonal *et al.* 2000b), which is partly associated with their successional status. The hierarchy of species in terms of $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ sequences from different species cannot be readily combined for reconstructions of environmental conditions.

OXYGEN

About 0·20% of global oxygen consists of ^{18}O. Plant tissue has typically a higher $\delta^{18}O$ compared to that of the water taken up [source water (sw)]. This enrichment is also expressed with a Δ-value ($\Delta^{18}O = R_{plant}/R_{sw} - 1$), but source and product are interchanged (Barbour 2007).

$$\Delta^{18}O_{plant} = \frac{(\delta^{18}O_{plant} - \delta^{18}O_{sw})}{(\delta^{18}O_{sw}/1000 + 1)} (‰) \qquad \text{eqn 5}$$

During water uptake by roots no fractionation of oxygen isotopes occurs, thus xylem water has the same $\delta^{18}O$ as absorbed soil water (Dawson & Ehleringer 1991). Transpiration causes enrichment of leaf water due to a lower evaporation rate of $H_2^{18}O$. 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 ($\Delta^{18}O_e$) can be calculated from the temperature-dependent fractionation between water in the liquid and vapour phase (ε^+), the kinetic fractionation with diffusion from leaf to atmosphere (ε_k), the isotopic composition of water vapour ($\Delta^{18}O_v$) and the ratio of vapour pressure in atmosphere and leaf (e_a/e_i).

$$\Delta^{18}O_e = \varepsilon^+ + \varepsilon_k + (\Delta^{18}O_v - \varepsilon_k)e_a/e_i \qquad \text{eqn 6}$$

For calculating the ^{18}O enrichment of bulk leaf water ($\Delta^{18}O_L$) the mixing of xylem water and enriched water from the site of evaporation must be taken into account. This Péclet effect ($\wp$) 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 $H_2^{18}O$ in water (D).

$$\Delta^{18}O_L = \Delta^{18}O_e (1 - e^{-\wp}) / \wp \qquad \text{eqn 7}$$

where $\wp = (L \cdot E)/(C \cdot D)$

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 CO_2 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 $\delta^{18}O$ signature of leaf water instead of the $\delta^{18}O$ signature of the absorbed CO_2 (DeNiro & Epstein 1979). Further fractionation in Calvin cycle reactions and sucrose synthesis results in ^{18}O enrichment (ε_{wc}), which is estimated at 27‰, independent of species and growth conditions. When macromolecules are synthesized from sucrose, exchange in O with local water occurs (p_{ex}). 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 (p_x), which is likely to be close to unity in the cambium. The isotopic composition of cellulose relative to source water ($\Delta^{18}O_{cel}$) can then be calculated as follows:

$$\Delta^{18}O_{cel} = \Delta^{18}O_L(1 - p_x p_{ex}) + \varepsilon_{wc} \qquad \text{eqn 8}$$

Evidence indicated that ε_{wc} and p_{ex} values are not constant. Species or condition-specific variation can cause uncertainty about the extent to which wood cellulose $\delta^{18}O$ is predominantly determined by humidity and stomatal conductance or by source water (Sternberg 2009; Gessler *et al.* 2014). The $\delta^{18}O$ 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 $\delta^{18}O$ on tree ring cellulose is also true for large trees is unknown.

The $\delta^{18}O$ of precipitation water varies seasonally. It is depleted during the rainy season and heavy precipitation events (Villacís, 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 $\delta^{18}O$ 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 $\delta^{18}O$ of source water. The soil depth of water uptake has been estimated by measuring natural abundance of ^{18}O in the soil profile and in xylem water (Jackson *et al.* 1995; Hasselquist, Allen & Santiago 2010; Ellsworth & Sternberg 2015; Schwendenmann *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 $\delta^{18}O$. Yet, such information is usually lacking in isotopic studies on tropical trees.

NITROGEN

The stable isotope of nitrogen, ^{15}N, comprises 0·37% of global N. Its natural abundance in plants and other ecosystems compartments is expressed as $\delta^{15}N$, the isotopic ratio relative to atmospheric N_2 as a standard (eqn 1). The value of plant $\delta^{15}N$ depends for a large part on the $\delta^{15}N$ of the N source used by the plant. Fractionation associated with uptake of the major inorganic N sources for plants, NH_4^+ and NO_3^-, 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 N_2-fixation (Högberg 1997). Plant $\delta^{15}N$ thus largely reflects the isotopic signature of the N source. N_2-fixing plants therefore tend to exhibit a $\delta^{15}N$ value of 0‰, with fluctuations depending on the fraction of N derived from N_2-fixation (Högberg 1997). However, the $\delta^{15}N$ of soil derived N can vary substantially and is regulated in a complex manner.

Fractionation associated with soil microbial processes can generate differences in $\delta^{15}N$ between NH_4^+ and NO_3^- and cause variation over time and with soil depth (Hobbie & Ouimette 2009). Processes influencing plant and ecosystem $\delta^{15}N$ have recently been reviewed (Hobbie & Högberg 2012; Gerhart & McLauchlan 2014) and are shortly summarized here and illustrated in Fig. 2. Discrimination against ^{15}N with nitrification is an important reason for patterns in soil $\delta^{15}N$. Leaching under surplus precipitation causes export of the depleted NO_3^- out of the system, leaving isotopically enriched NH_4^+ for uptake by plants and further nitrification. There is also substantial discrimination against ^{15}N with denitrification, causing an outflow of depleted gaseous N_2 and N_2O and leaving isotopically enriched NO_3^-. The relative importance of these processes

determines the $\delta^{15}N$ of the plant available forms of N, and depending on the fraction taken up as NH_4^+ and NO_3^- determine plant $\delta^{15}N$. The picture can be further complicated when ectomycorrhizal fungi utilize organic N. When ecosystem NO_3^- losses are large, N cycling is predicted to increase soil and plant $\delta^{15}N$. When losses are small compared to total N-cycling, $\delta^{15}N$ tends to be lower.

For tropical lowland forests, higher soil and foliage $\delta^{15}N$ is reported compared to temperate and boreal forests (Martinelli *et al.* 1999; Amundson *et al.* 2003). Low $\delta^{15}N$ 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 N_2-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. N_2-fixing trees are virtually lacking in temperate forest where obligate actinorhizal N_2-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 $\delta^{15}N$ has been investigated experimentally with fertilizer addition in temperate and tropical forests. The experiments in temperate forest showed increases in tree $\delta^{15}N$ under additional N treatments (Elhani *et al.* 2005), which were paralleled by soil N-losses (Högberg & Johannisson 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 $\delta^{15}N$ after N-addition and increased $\delta^{15}N$ of soil NO_3^-, but not NH_4^+. This partly confirms the predicted effects of a more open N-cycle on $\delta^{15}N$ in tropical forests.

$\delta^{15}N$ 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 $\delta^{15}N$ 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 CO_2 concentration and N-deposition. Increased CO_2 increases potential growth rate of trees and thus demand for N. This can be expected to cause an increased N-limitation, less NO_3^- losses and thus a decrease in soil and tree $\delta^{15}N$. On the other hand, N-deposition will increase N-availability and can be expected to cause increased NO_3^- losses resulting in an increase of $\delta^{15}N$ in the system. The balance between these two processes, in conjunction with other processes, will ultimately determine the resulting change in $\delta^{15}N$ of tropical trees.
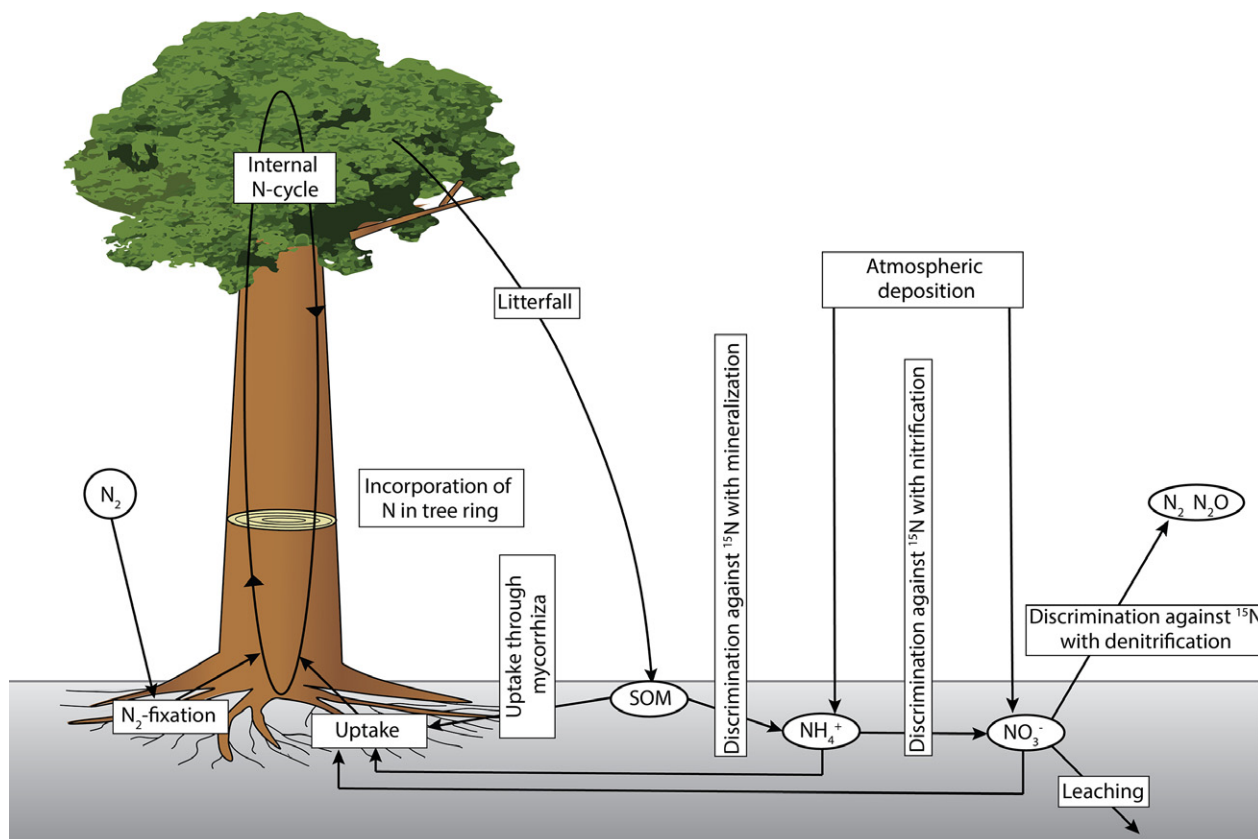
Fig. 2. Schematic representation of the processes in trees and forests that determine $\delta^{15}N$ in tree rings. Relevant components of the nitrogen cycle in the tree and the soil are represented. Discrimination against ^{15}N occurs with mineralization of soil organic matter (SOM), nitrification of the resulting NH_4^+ to NO_3^-, and denitrification of NO_3^- to N_2 and N_2O. ^{15}N depleted NO_3^- can also leave the system by leaching. Gain of N can occur through atmospheric N-deposition and N_2-fixation. N can be taken up by the tree in different forms depending on microbial associations and availability. Once absorbed, it enters the tree's internal N-cycle. Amino acids are incorporated in cell wall proteins in wood, and so forming an archive of the $\delta^{15}N$ of the N taken up by the tree during its lifetime.

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 pattern) 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 ^{13}C-depleted CO_2 (Buchmann *et al.* 1997), thus enforcing the increasing trend of $\delta^{13}C$ with tree size. Sub-canopy trees are exposed to slightly ^{13}C enriched CO_2 due to high photosynthetic activity in the canopy, whereas larger trees presumably take up CO_2 with similar $\delta^{13}C$ compared to atmospheric CO_2 (Buchmann *et al.* 1997). Additionally, a changing contribution of bark photosynthesis to wood formation could also modify its $\delta^{13}C$ (Cernusak *et al.* 2001). Although, changes in $\delta^{13}C$ 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 $\delta^{18}O$ is less straightforward. Individual trees can show trends in $\delta^{18}O$ 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 $\delta^{18}O$, 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 ^{18}O enrichment. Growing hydraulic resistance with size can reduce g_s and thus further add to isotopic enrichment. These factors can be responsible for increasing trends in tree ring $\delta^{18}O$ in individual trees (Poussart & Schrag 2005). However, trends not related to age or size could also be involved (Brienen *et al.* 2012; van der Sleen 2014), such as a gradual change in source water $\delta^{18}O$.

Little is known about changes in $\delta^{15}N$ with tree development. In a study on six tropical tree species, van der Sleen *et al.* (2015b) found evidence for changes in $\delta^{15}N$ with tree diameter, possibly caused by increasing rooting depth or otherwise changes in the $\delta^{15}N$ 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 CO_2 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 ^{14}C. 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 $\delta^{15}N$ 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 ^{13}C. 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 $\delta^{13}C$ and/or $\delta^{18}O$. 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 throughput techniques (e.g. Schulze *et al.* 2004; Schollaen, Heinrich & Helle 2014).

For $\delta^{13}C$, 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 $\delta^{18}O$ 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 $\delta^{15}N$, 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 & McLauchlan 2014).

Stable isotopes sequences in tropical trees

CARBON

Early measurements of $\delta^{13}C$ 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*, $\delta^{13}C$ 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 $\delta^{13}C$ was found compared to the

pattern described above. Intra-annual variation in $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ sampling for identifying non-distinct annual rings. The intra-annual variation in $\delta^{13}C$ of up to 3‰ in combination with similar inter-annual variation in $\delta^{18}O$ 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, $\delta^{18}O$ proved to be more useful compared to $\delta^{13}C$ and that will be discussed in the next section.

Several studies have investigated the inter-annual variation in $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ sequences with measurements of $\delta^{18}O$ (Table 1; Table S3). In some of these, $\delta^{13}C$ showed correlation with other precipitation variables than those found for $\delta^{18}O$ (Cullen & Grierson 2007; Schollaen *et al.* 2013). Nevertheless, $\delta^{18}O$ series generally yielded stronger correlations with precipitation variables than $\delta^{13}C$ 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 $\delta^{13}C$ 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

Table 1. Summary of the main usage and outcomes of studies measuring stable isotope in tropical tree rings

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

Reviewed publications are summarized in Tables S1, S2 and S3 in Supporting Information; those on ^{15}N are reviewed in the text.
ENSO, El Niño Southern Oscillation; iWUE, intrinsic water-use efficiency; g_s, 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 *Peltogyne* cf *heterophylla* saplings to gap formation in a tropical forest. The trees that showed increased growth after gap formation had also a decreased $\Delta^{13}C$, suggesting that increased light and possibly nutrients availability had stimulated growth. The trees without growth stimulation generally had an increased $\Delta^{13}C$, 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 $\delta^{13}C$ 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 $\delta^{13}C_{atm}$ over that period, a rather constant ^{13}C discrimination ($\Delta^{13}C$) generally remains. This leads to the conclusion (based on eqns 2 and 3) that C_i/C_a was basically constant over time and that, as a result of the increasing atmospheric CO_2 concentration, iWUE has increased consistently over time (Silva & Anand 2013). An increasing iWUE was also found in FACE experiments were CO_2 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 $\delta^{13}C$, stable C_i/C_a 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 CO_2 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).

OXYGEN

Studies of stable oxygen isotopes ($\delta^{18}O$) in tropical trees have started more recently. Earlier work with temperate trees had shown correlations of inter-annual variation in tree ring cellulose $\delta^{18}O$ with temperature, humidity and isotopic composition of precipitation (Burk & Stuiver 1981; Saurer *et al.* 1997). Later, models were developed that satisfactorily describe environmental and physiological controls of cellulose $\delta^{18}O$ (Section Stable isotope ecophysiology). Its potential for recording past environmental factors also in tropical trees was recognized and several studies followed (Table 1; Table S2).

The first $\delta^{18}O$ studies on tropical trees quantified radial variation of $\delta^{18}O$ in a tree stem to reconstruct annual ring

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, $\delta^{13}C$ was also measured, but $\delta^{18}O$ was found to be generally superior for this purpose (Table 1; Table S3). The suitability of $\delta^{18}O$ 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 $\delta^{18}O$ of stem wood. High $\delta^{18}O$ 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 $\delta^{18}O$ 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 $\delta^{18}O$ 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 $\delta^{18}O$ in the rainy season, but this pattern was reversed in Southern India as monsoon rains exhibit a different $\delta^{18}O$ signature (Managave *et al.* 2010, 2011a).

Most studies conclude that trees in closed humid tropical forest primarily record variation in $\delta^{18}O$ 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 $\delta^{18}O$ is large. This is the case in the western parts of the Amazon basin, where $\delta^{18}O$ 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 $\delta^{18}O$ 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 $\delta^{18}O$ values, resulting in large seasonal variation in *Ocotea tenera* (up to 9‰) at 1500 m altitude in Cost 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 in $\delta^{18}O$ 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 $\delta^{18}O$ signature of rainwater in stem wood, time series of tree ring $\delta^{18}O$ can be used to quantify past variability in precipitation. Tree ring $\delta^{18}O$ has been correlated with basin-wide precipitation in the Amazon (Ballantyne *et al.* 2011; Brienen *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 $\delta^{18}O$ 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 $\delta^{18}O$ is developing into a powerful tool for reconstructing the variability of precipitation on regional scales.

Several species were found that show good synchronization of $\delta^{18}O$ 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 $\delta^{18}O$ 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 (Boysen, Evans & Baker 2014). However, $\delta^{18}O$ synchronization between individuals may be low for certain species or sites (e.g. Poussart & Schrag 2005; Baker *et al.* 2015). For $\delta^{18}O$ in *Toona ciliata* low $\delta^{18}O$ 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 $\delta^{18}O$ values exploit other water sources than recent precipitation. Deep rooting species likely use ground water, which has a $\delta^{18}O$ signature that may be formed over several years. Shallow-rooting trees are more likely to take up recent rainwater and thus more closely record $\delta^{18}O$ variability in rainwater. This suggests that shallow rooting tree species on well-drained soils have the highest probability to record the $\delta^{18}O$ 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 $\delta^{18}O$ 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 $\delta^{18}O$ in Andean ice cores (Thompson *et al.* 2006) and Andean lake sediments (Bird *et al.* 2011). Thus, the increasing trend in $\delta^{18}O$ in tree rings likely reflects a pan-tropical increase in precipitation $\delta^{18}O$. 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 $\delta^{13}C$ 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 (Roden & 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 *Metrosideros 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 (Roden & 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, Dünisch & Wanek 2010; Hietz *et al.* 2011; van der Sleen, Groenendijk & Zuidema 2015). Hietz, Dünisch & 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 likely 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 $\delta^{15}N$ in tropical tree rings could be enhanced NO_3^- losses associated with increased forest disturbance, as initially proposed by Hietz, Dünisch & Wanek (2010).

Tropical montane forest trees have lower foliage $\delta^{15}N$ compared to lowland forest (Brearley 2013), which is ascribed to slower mineralization at low temperature and, hence, lower NO_3^- losses. Global warming effects in lowland forest could thus include increased mineralization and nitrification, leading to increased NO_3^- losses. Unfortunately, the interpretation of temporal changes in $\delta^{15}N$ in the few available tropical tree ring studies is strongly hampered by a limited understanding of the factors that influence $\delta^{15}N$ 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 extent well-beyond observation records. Thus, pre-industrial conditions, such as CO_2 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 ^{18}O 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 (^{13}C and ^{18}O) approach allowing better assessment whether changes in C_i are a consequence of changes in A, g_s, 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 ^{15}N data; (v) further experimental work on the manipulation of the N-cycle and its effect on $\delta^{15}N$ 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 ^{18}O 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 C_i (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 expended 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.

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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.