

Research

# Recent trends in the intrinsic water-use efficiency of ringless rainforest trees in Borneo

N. J. Loader<sup>1,\*</sup>, R. P. D. Walsh<sup>1</sup>, I. Robertson<sup>1</sup>, K. Bidin<sup>2</sup>, R. C. Ong<sup>3</sup>,  
G. Reynolds<sup>1,4</sup>, D. McCarroll<sup>1</sup>, M. Gagen<sup>1</sup> and G. H. F. Young<sup>1</sup>

<sup>1</sup>Department of Geography, Swansea University, Swansea SA2 8PP, UK

<sup>2</sup>School of Science and Technology, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia

<sup>3</sup>Forest Research Centre, Sabah Forestry Department, Sandakan, Sabah, Malaysia

<sup>4</sup>Royal Society South East Asia Rainforest Research Programme (SEARRP), Danum Valley Field Centre, Lahad Datu, Sabah, Malaysia

Stable carbon isotope ( $\delta^{13}\text{C}$ ) series were developed from analysis of sequential radial wood increments from AD 1850 to AD 2009 for four mature primary rainforest trees from the Danum and Imbak areas of Sabah, Malaysia. The aseasonal equatorial climate meant that conventional dendrochronology was not possible as the tree species investigated do not exhibit clear annual rings or dateable growth bands. Chronology was established using radiocarbon dating to model age–growth relationships and date the carbon isotopic series from which the intrinsic water-use efficiency (IWUE) was calculated. The two *Eusideroxylon zwageri* trees from Imbak yielded ages of their pith/central wood ( $\pm 1$  sigma) of  $670 \pm 40$  and  $759 \pm 40$  years old; the less dense *Shorea johorensis* and *Shorea superba* trees at Danum yielded ages of  $240 \pm 40$  and  $330 \pm 40$  years, respectively. All trees studied exhibit an increase in the IWUE since AD 1960. This reflects, in part, a response of the forest to increasing atmospheric carbon dioxide concentration. Unlike studies of some northern European trees, no clear plateau in this response was observed. A change in the IWUE implies an associated modification of the local carbon and/or hydrological cycles. To resolve these uncertainties, a shift in emphasis away from high-resolution studies towards long, well-replicated time series is proposed to develop the environmental data essential for model evaluation. Identification of old (greater than 700 years) ringless trees demonstrates their potential in assessing the impacts of climatic and atmospheric change. It also shows the scientific and applied value of a conservation policy that ensures the survival of primary forest containing particularly old trees (as in Imbak Canyon and Danum).

**Keywords:** Borneo; stable isotope; dendrochronology; REDD; water-use efficiency; carbon

## 1. INTRODUCTION

### (a) *Tropical trees as recorders of environmental change*

Decision-making in conservation management is often constrained by unacceptably high levels of uncertainty surrounding estimates of forest response to environmental pressures [1]. An ability to quantify more accurately the age-structure of a forest and the flux of carbon and water through it may help reduce these uncertainties and provide a method with which to evaluate and improve Earth systems models. One area of growing interest is the effect that carbon dioxide concentrations, which have increased during the industrial era from approximately 285 ppm in AD 1850 to greater than 392 ppm in AD 2010 [2–4],

may have on the forest biosphere and its ability to cycle water and nutrients [5].

Plants regulate gas exchange and moisture loss through subtle changes in leaf morphology and metabolic processes that are sensitive to environmental change and which may be investigated through analysis of the stable isotopic composition of the plant [4]. Such changes influence the fluxes of both carbon dioxide and water, thus influencing the climatic system; this process is termed physiological forcing [6,7].

One of the key physiological forcings is changing stomatal conductance. Plant stomata evolved to internalize gas exchange and limit moisture loss, and there is a strong tendency to maintain the balance between the concentration of carbon dioxide outside ( $c_a$ ) and inside ( $c_i$ ) the leaf. As the concentration of carbon dioxide in the atmosphere increases, plants can potentially maintain the ratio  $c_i/c_a$  by reducing stomatal conductance and thus reducing moisture loss per unit of carbon gained (increasing their water-use

\* Author for correspondence (n.j.loader@swansea.ac.uk).

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efficiency) [8]. Climate model-based studies have shown that reductions in plant stomatal conductance can impact both directly and indirectly upon regional climate, soil moisture, stream-runoff and productivity [9–11]. Uncertainty in the magnitude and significance of this physiological forcing arises, because most studies on the response of stomatal conductance to rising CO<sub>2</sub> are extrapolated from short-term, leaf ‘exposure’ studies [11], which cannot take account of acclimation that may alter the stomatal response over longer timescales [6,12] or of potential thresholds to response, which may already have been reached [13,14].

One way to reduce these uncertainties is to investigate naturally occurring archives of plant physiological change, such as the wood of trees. This approach has been applied successfully in high-latitude regions where clear annual rings allow well-replicated, absolutely dated chronologies to be constructed, providing annually resolved archives of information on past environmental change [4]. Across much of the tropics, in areas where seasonality is absent/weakly expressed, trees generally do not produce clear annual rings and so chronologies cannot be constructed by dendrochronology alone. Here that problem is overcome by using radiocarbon dating to estimate tree age and growth rate, providing the first record of changing plant response over time, using ringless trees from the lowland dipterocarp rainforest in Sabah, Malaysia.

### (b) Carbon isotope fractionation and intrinsic water-use efficiency

Farquhar *et al.* [15] proposed a model to describe carbon isotopic fractionation in plants that relates, in its simple form, the carbon isotopic composition of the resulting photosynthates to (i) the isotopic composition and concentration of the source carbon dioxide, (ii) plant regulation of CO<sub>2</sub> entering the leaf (stomatal conductance) and (iii) the rate at which it is fixed by photosynthesis (assimilation rate; equation 1.1). This may be modified to express carbon isotope fractionation in terms of isotopic discrimination ( $\Delta$ ) (equation 1.2).

$$\delta^{13}\text{C}_{\text{plant}} \approx \delta^{13}\text{C}_{\text{atmosphere}} - a - (b - a) \times \frac{c_i}{c_a} \quad (1.1)$$

and

$$\Delta = \frac{(\delta^{13}\text{C}_{\text{atmosphere}} - \delta^{13}\text{C}_{\text{plant}})}{(1 + (\delta^{13}\text{C}_{\text{plant}}/1000))}. \quad (1.2)$$

As the conductance of the leaf to water vapour is 1.6 times that of CO<sub>2</sub>, it is possible to express discrimination in terms of net assimilation ( $A$ ) and conductance to water vapour ( $g_w$ ), which is termed the intrinsic water-use efficiency (IWUE; equation 1.3) [8].

$$\frac{A}{g_w} = \text{IWUE} = \frac{(c_a - c_i)}{1.6}, \quad (1.3)$$

where:

$\delta^{13}\text{C}_{\text{plant}}$ , stable carbon isotopic composition of the photosynthate;

$\delta^{13}\text{C}_{\text{atmosphere}}$ , stable carbon isotopic composition of the atmospheric CO<sub>2</sub> used in photosynthesis;

$a$ , fractionation due to diffusion of CO<sub>2</sub> through the stomata (4.4‰);

$b$ , fractionation by ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) enzyme during carboxylation (approx. 27‰);

$c_i$ , inter-cellular concentration of CO<sub>2</sub> (CO<sub>2</sub> concentration internal to the leaf);

$c_a$ , concentration of atmospheric CO<sub>2</sub> (CO<sub>2</sub> concentration external to the leaf);

$\Delta$ , discrimination;

$A$ , rate of CO<sub>2</sub> assimilation by the plant;

$g_w$ , the conductance of the boundary layer and stomatal pores to the diffusion of water (H<sub>2</sub>O).

Several studies have demonstrated that trees can modify their IWUE through changes in the number, size and density of the stomata as levels of atmospheric carbon dioxide increase [16–19]. Kürschner [20] demonstrated that deciduous European trees responded through a general increase in IWUE, but at CO<sub>2</sub> concentrations of about 340 ppm appeared to reach a threshold beyond which their response levelled off. Other tree-ring-based studies [21–24] have reported broadly similar results. Waterhouse *et al.* [14] concluded that the magnitude and the timing of the diminished response were likely to be both site- and species-specific, and argued that a reassessment of estimated global carbon budgets and the modelling of feedbacks might now be required.

In a study of IWUE in ringed trees from the somewhat seasonal location of Aripuaña, Brazil (10°09' S, 59°26' W), Hietz *et al.* [25] sampled carbon isotope ratios in homogenized blocks of dated tree-rings of *Cedrela odorata* L. and *Swietenia macrophylla* King. Although the pooled samples included several very young trees, their results indicated that the IWUE in that part of Amazonia had increased by 34 per cent for *C. odorata* and 52 per cent for *S. macrophylla* between AD 1850 and AD 2000. As no continuous individual isotope time series were developed, however, it was not possible to determine whether or not the trees had reached a plateau in their response.

The IWUE differs from *instantaneous* water-use efficiency (*instWUE*) as the former does not depend on the vapour pressure gradient between the leaf and the surrounding air. The IWUE has the advantage over *instWUE* in that it allows assessment of the role of biological components in determining water–carbon exchange relationships independently of changing atmospheric conditions [14,26]. The IWUE, however, does not take into account the effects of external climate variability, respiration or mesophyll conductance [27], and without this additional information it is difficult to determine whether changes in the IWUE are driven by assimilation rate, gross conductance or a combination of both factors. Notwithstanding these limitations, tree ring IWUE provides a fundamental insight into plant water-use, both currently and retrospectively.

### (c) Developing chronology from ringless tropical trees

Trees growing in temperate seasonal climates usually display well-defined tree-rings, the widths of which are partly controlled by environmental factors, such

as temperature and moisture availability. This allows cross-dating (synchronization) between trees and between sites, which is the basis for dendrochronology (tree-ring dating). Although some of the earliest tree-ring studies were conducted in the seasonally wet tropics [28,29], it has been generally assumed that regions where climate does not exhibit a strong seasonal control on tree growth are unsuitable for dendrochronology [30]. Although some studies in seasonal tropical environments have demonstrated the presence of coherent growth bands in tropical timbers and thereby provide new opportunities for tree-ring research [29,31,32], over large areas of the tropics trees do not form regular growth bands. Ringless tropical trees may, nevertheless, provide a rich palaeoecological archive because they can be very long-lived [33], and assuming that wood formation proceeds without protracted dormancy, the stable isotopes of carbon, oxygen and non-exchangeable hydrogen fixed in the wood cells could provide a continuous record of changes in environment and in physiological response.

Dendrochronology and dendroclimatology rely upon replication to establish robust chronology and to quantify signal strength. Attempts to produce annually resolved chronologies for ringless trees, using high-resolution analyses [34–39], have resulted in the identification of rhythmic patterns in wood chemistry that may be annual in nature. However, to be useful in dendrochronology, high-resolution isotope series need to be replicated. To analyse five 50 cm long cores at 10  $\mu\text{m}$  resolution would require a minimum of 250 000 isotopic determinations, which constitutes an unrealistic analytical commitment. A shift in emphasis and analytical approach is therefore required, away from the high-frequency analysis of single ringless trees towards larger scale better replicated studies. Radiocarbon dating can provide an estimate of tree age and if dating uncertainty can be addressed and adequately quantified then ringless tropical archives could provide information on lower frequency environmental changes. This approach is essentially no different from that used to investigate peat cores and lake or ocean sediments. By accepting some dating uncertainty, and focusing on changes in environment at greater than annual timescales, it may prove possible to provide the information so urgently required by the modelling community for such regions. This study will assess the potential of such an approach through analysis of the IWUE developed using ringless trees from the aseasonal lowland dipterocarp rainforests of Sabah, Malaysia.

## 2. MATERIAL AND METHODS

### (a) *Site description, sample collection and analysis*

Four trees were sampled from two lowland rainforest locations in Sabah, Malaysian Borneo. At Danum Valley Conservation Area (04°57' N, 117°48' E; figure 1), one of the largest remaining regions of pristine lowland dipterocarp forest, disc samples of *Shorea superba* and *Shorea johorensis* trees were collected from sites about 300 m altitude on gently sloping terrain.

The *S. superba* (a mature tree outside the conservation area) and the *S. johorensis* (a canopy tree within a long-term forest ecology plot inside the conservation area) had both been blown over immediately prior to sampling during storms in March 2001 (*S. superba*) and June 2009 (*S. johorensis*). The climate of Danum is equatorial with a mean annual rainfall (1985–2009) of 2873 mm. There is no dry season and monthly rainfall means range from 313 mm in January to 159 mm in April. During El Niño/Southern Oscillation events, however, dry periods with two to four successive months with less than 100 mm of rain occur, as in 1992 and 1998. The geology is a heterogeneous melange of Miocene siltstones, sandstones, cherts, spillites and tuffs and soils are mainly FAO haplic alisols (Alh), comprising 38–57% sand, 20–35% silt and 13–27% clay [40,41]. At Imbak (05°53' N, 117°05' E) two samples of *Eusideroxylon zwageri* (local name Belian) trees were collected in an area of moderate relief (200–400 m altitude) and sandstone geology east of Imbak Canyon Conservation Area. Limited meteorological data suggest a climate broadly similar to that of Danum Valley, although somewhat wetter (over 3000 mm per annum). Borneo represents an important study region because its ever-wet climate is affected by the Indo-Australian Monsoon System and is strongly affected by El Niño/Southern Oscillation events. Few high-resolution terrestrial palaeoenvironmental records exist for this region.

The four trees sampled were mature canopy trees and apparently healthy (with intact wood across the entire discs) prior to felling. With the exception of the *S. johorensis*, samples were from near-ground stem avoiding buttresses and rotten timber. All tree sections exhibited asymmetrical growth, nevertheless it was possible to select a single radius from each sample for both radiometric dating and stable isotopic analysis to avoid disturbance/reaction tissues, and represented, as far as was possible, the natural annular growth form.

Samples were prepared for radiocarbon dating using a scalpel and microdrill to obtain the greater than 0.6 g of wood powder required for cellulose extraction. Care was taken to ensure that the wood powder did not char, which could cause isotopic fractionation. Each dated radius was sub-sampled for stable carbon isotope analysis. Samples were divided into contiguous 1 mm sections and further subdivided into thin slivers using a scalpel prior to isolation of cellulose [42–44]. The *S. superba* prepared independently by Robertson *et al.* [45] was also cut radially and  $\alpha$ -cellulose prepared. Owing to its greater size, for practical reasons, sequential wholewood rather than cellulose samples were analysed for the *S. johorensis*. These two components preserve similar signals, but an adjustment of 1 per mille is made to the wholewood carbon isotope ratios prior to calculation of IWUE to account for the isotopic offset between wholewood and cellulose [46].

Between 300 and 350  $\mu\text{g}$  of dry homogenized  $\alpha$ -cellulose or dry wood powder were weighed into tin-foil capsules and combusted quantitatively to carbon dioxide over chromium(III) oxide at 1000°C using a Sercon GSL Elemental Analyser interfaced

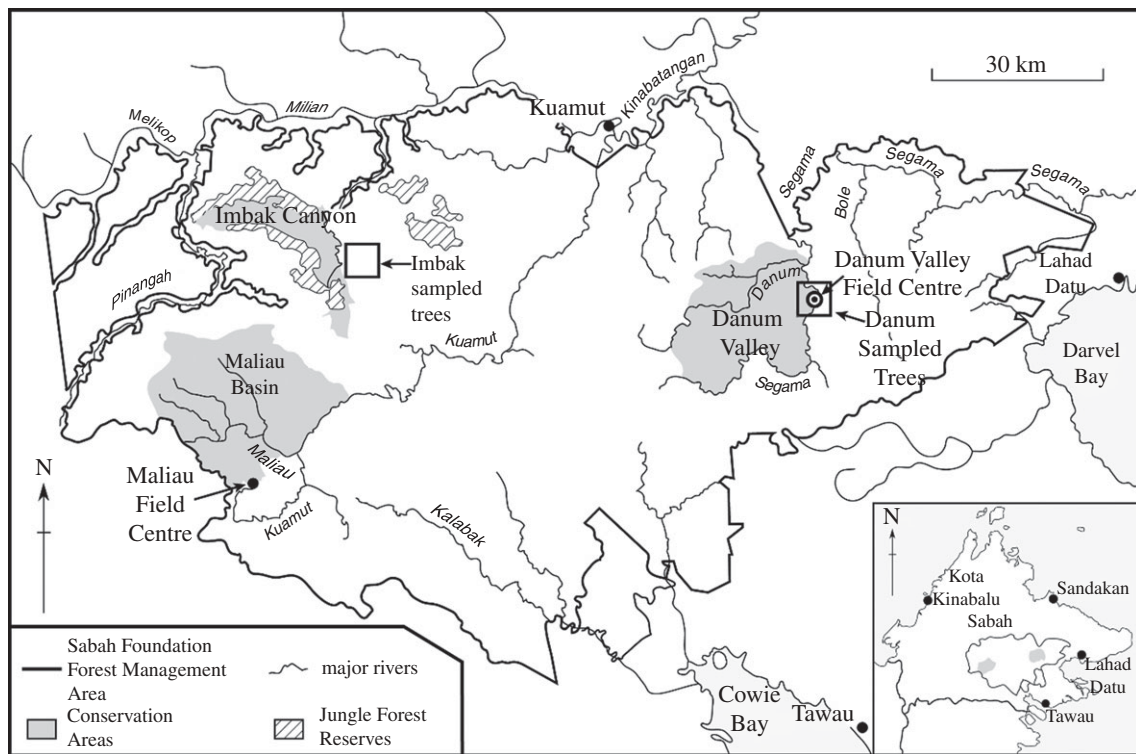


Figure 1. Location map of Sabah, Borneo, identifying the study site locations at Danum Valley and Imbak Canyon.

with a 20/20 isotope ratio mass spectrometer. Results are expressed using the delta ( $\delta$ ) notation relative to the Vienna Pee Dee Belemnite (VPDB) standard [4]. Precision, based on replicate analyses of an in-house standard, is typically 0.1 per mille ( $\sigma_{n-1}$ ,  $n = 10$ ). The *S. superba* series developed by Robertson *et al.* [45] was analysed in a similar manner.

#### (b) Chronology and age-growth modelling

A simple age-growth model was produced by fitting a negative exponential curve between radiocarbon dating control points. The radiocarbon method can provide several possible dates for a sample depending on the points at which it intercepts the radiocarbon calibration curve, but the use of multiple dates can reduce significantly this uncertainty [45]. The modelled  $\delta^{13}\text{C}$  series were annualized to facilitate calculation of the IWUE by interpolation and/or combination depending on the temporal resolution of the growth model. The temporal uncertainty in the radiocarbon dates ( $\pm 40$  years, 1 sigma) was expressed in terms of  $\delta^{13}\text{C}$  and IWUE as one standard deviation in the range of values falling within the dating uncertainty for each annualized point. While this approach simplifies a relatively complex and non-normally distributed error structure, it does permit a conservative calculation of uncertainty point-for-point in each series.

### 3. RESULTS AND DISCUSSION

#### (a) Chronology

The radiocarbon dating results ( $\pm 1$  sigma) of near-pith wood of the two *E. zwageri* trees (AD 1330 and AD 1250) suggest ages of  $670 \pm 40$  and  $759 \pm 40$

years, respectively, which given the relatively small radial growth sampled (18 and 29 cm, respectively) indicates very slow growth rates averaging *ca* 0.27 and 0.38 mm yr<sup>-1</sup>. By contrast, the ages of the *S. superba* and *S. johorensis* trees are much lower at  $330 \pm 40$  and  $240 \pm 40$  years, respectively, indicating faster mean growth rates for these dipterocarps (sampled radii 44 and 65 cm, respectively). In the Imbak Canyon Conservation Area, many *E. zwageri* specimens exceed 2 m in diameter, suggesting ages of at least 1500 years. The dates support the findings of Kurokawa *et al.* [33] who also identified the long-lived and slow-growing nature of this species through analysis of stump and remnant samples in neighbouring Sarawak. Such long-lived trees offer potential for developing millennial length records in the everwet tropics for palaeoclimatic research. They highlight also the considerable age of the trees growing in the primary forest and thus demonstrate how rapidly felling can remove the carbon fixed into an ecosystem over many centuries. It is important that such slow-cycling is taken into account when developing conservation and management strategies [47].

#### (b) Stable carbon isotopes and intrinsic water-use efficiency

The annualized stable carbon isotope data (figure 2a,b) all exhibit a characteristic declining trend, which can be related to parallel changes in the carbon isotopic composition of atmospheric carbon dioxide following the onset of global industrialization (*ca* AD 1850). At Danum Valley in particular, the *S. superba* and *S. johorensis* display an encouraging degree of coherence. The *E. zwageri* records show slightly lower coherence, and a smoother trend reflecting the lower

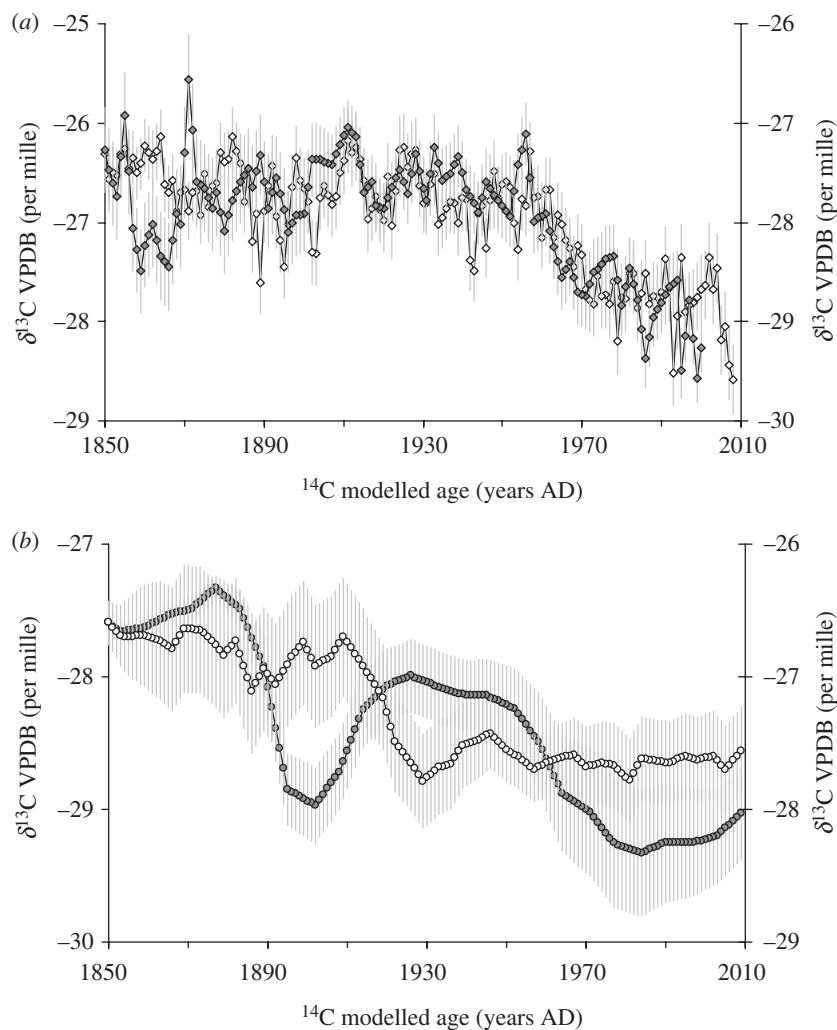


Figure 2. Graphs showing the raw stable isotope series for the four trees studied at (a) Danum (*S. superba* and *S. johorensis*) and (b) Imbak (*E. zwageri*). Filled diamonds, *S. superba*; open diamonds, *S. johorensis*; filled circles, *E. zwageri* (Tree T6B2); open circles, *E. zwageri* (Tree T5B1). Grey error bars represent the uncertainty in age–growth model (1 sigma  $\approx \pm 40$  years) expressed as the running standard deviation of tree response over a 40 year period.

temporal resolution of each millimetre increment analysed (approx. 2 and 5 years growth, respectively).

Calculation of the IWUE for each of the trees (figure 3*a,b*) also reveals a common response over time, although a slight decline in the IWUE of the naturally felled *S. johorensis* immediately prior to death is observed, which may indicate a change in the health of the tree making it more susceptible to windthrow or the onset of a plateau in IWUE. At both sites, absolute values are in a range similar to those reported for tropical and boreal species [6,14,21–25]. All series remain relatively stable until *ca* AD 1960–1970, after which the trees improve their IWUE coincident with a change in the rate of increase of atmospheric CO<sub>2</sub> concentration. Increasing IWUE can result from either (or a combination of) an increase in assimilation rate (which affects carbon sequestration) or a decrease in stomatal conductance (which more directly influences the hydrological cycle and climate system), and it is currently not possible to determine which factor dominates the response observed here as the trends observed may be driven by either changing carbon dioxide concentrations or a change in climate, or both.

Gagen *et al.* [6] were able to deconvolve the IWUE signal through paired analysis of tree-rings and sub-fossil leaves. They identified stomatal conductance as the dominant driver of changing IWUE at a boreal site in northern Fennoscandia. In the tropics, historic leaf material (albeit limited) available in herbaria and large tracts of forest and riverine peat deposits make the preservation and development of an equivalent archive of sub-fossil leaf material an exciting possibility and potential source of additional information on past forest response.

While there are few inland meteorological records available for this region, a record of relative humidity at the Danum site (AD 1985–to date) exhibits no trend in monthly relative humidity recorded at 08.00 h but a slight positive trend in data recorded at 14.00 h over the past 25 years. Through its effect on stomatal conductance, however, an increase in relative humidity should logically have the effect of reducing IWUE (assuming assimilation rate remains constant), so it is unlikely that humidity alone is driving the changes observed. Sunshine (photon flux) directly controls the rate of photosynthesis and the amount of carbon sequestered. At Danum, analysed records

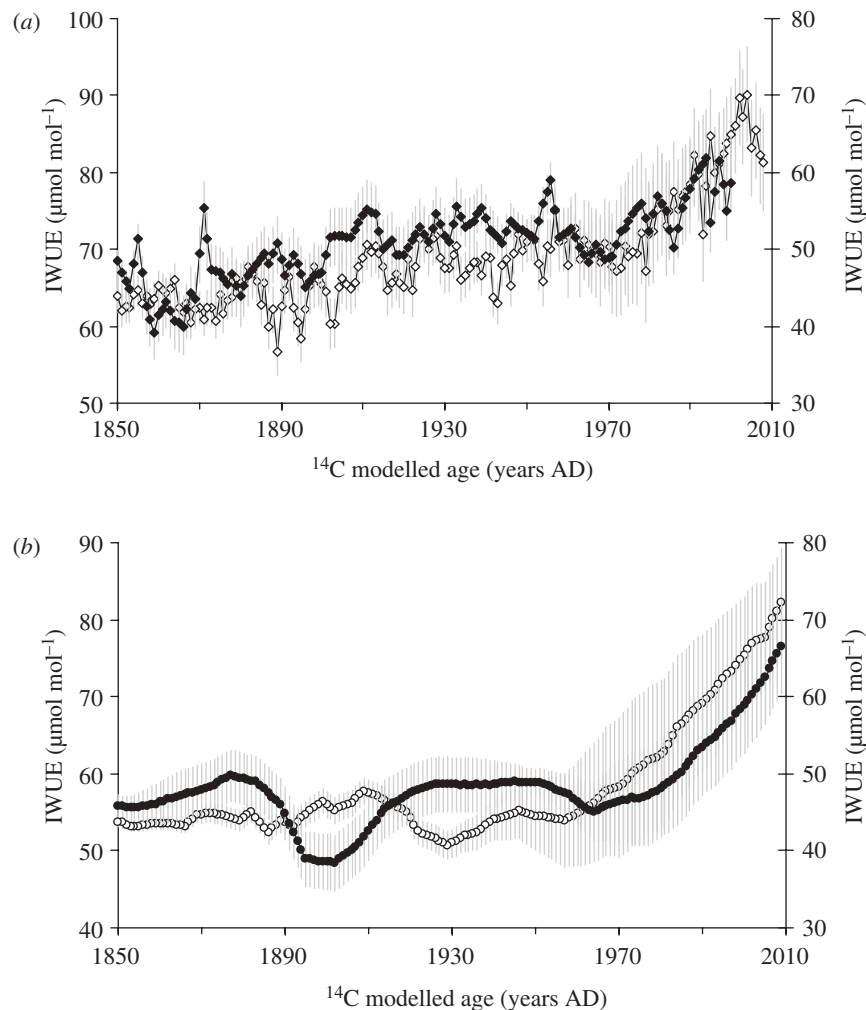


Figure 3. Intrinsic water-use efficiency (IWUE) calculated for each tree and plotted against time for (a) Danum and (b) Imbak. Filled diamonds, *S. superba*; open diamonds, *S. johorensis*; filled circles, *E. zwageri* (Tree T6B2); open circles, *E. zwageri* (Tree T5B1). Grey error bars represent the uncertainty in age–growth model (1 sigma  $\approx \pm 40$  years) expressed as the running standard deviation of tree response over a 40 year period. Note: a 1 per mille adjustment to account for the wholewood/cellulose isotopic difference has been made to the *S. johorensis* series prior to calculation of IWUE.

of sunshine are only available since AD 2009, so it is impossible to discern whether the increase in the IWUE is the result of increased photosynthetic rate. Several studies have reported an increase in wood formation in tropical trees during recent decades [47,48] and have related this to increased levels of atmospheric carbon dioxide. Such direct evidence of a possible CO<sub>2</sub> fertilization effect on trees growing under natural environmental conditions worldwide remains, however, limited and difficult to detect [49–51]. It is not possible to determine from these data alone whether or not a similar increase in carbon sequestration and allocation in woody biomass are also occurring across Sabah.

Figure 4 illustrates the individual series expressed as departures in IWUE from the mean of the four trees calculated for the common period (AD 1850–2000) and plotted against both time and atmospheric CO<sub>2</sub> concentration. The magnitude of the mean response observed (29%) between AD 1850 and AD 2000 is broadly in line with values (23–48%) obtained from tree-based studies of the IWUE from high-latitude regions and only slightly less than that reported by Hietz *et al.* [25] for Brazilian trees. In contrast to Waterhouse *et al.* [14] and McCarroll *et al.* [52],

there is no conclusive evidence for a sustained leveling-off in the response of the trees to increasing carbon dioxide concentrations at either Danum Valley or Imbak Canyon and it would appear, within the limitations of the approach, that at present trees are maintaining a stable level of  $c_i/c_a$  as levels of atmospheric CO<sub>2</sub> increase. What is not known is whether and for how long this trend will continue in the future. These findings, therefore, provide a unique and timely motivation for integrating modelling and monitoring initiatives across the South East Asian rainforest to permit more accurate prediction, detection and attribution of future changes in water–plant–carbon interactions.

#### 4. CONCLUSIONS

##### (a) *Conclusions, recommendations and future opportunities*

This study has analysed the stable carbon isotopic composition of primary rainforest trees over the last 160 years for a region where aseasonal climate prevents the formation of annual growth bands and the application of conventional dendrochronological techniques. Radiocarbon dating was used to develop a timescale

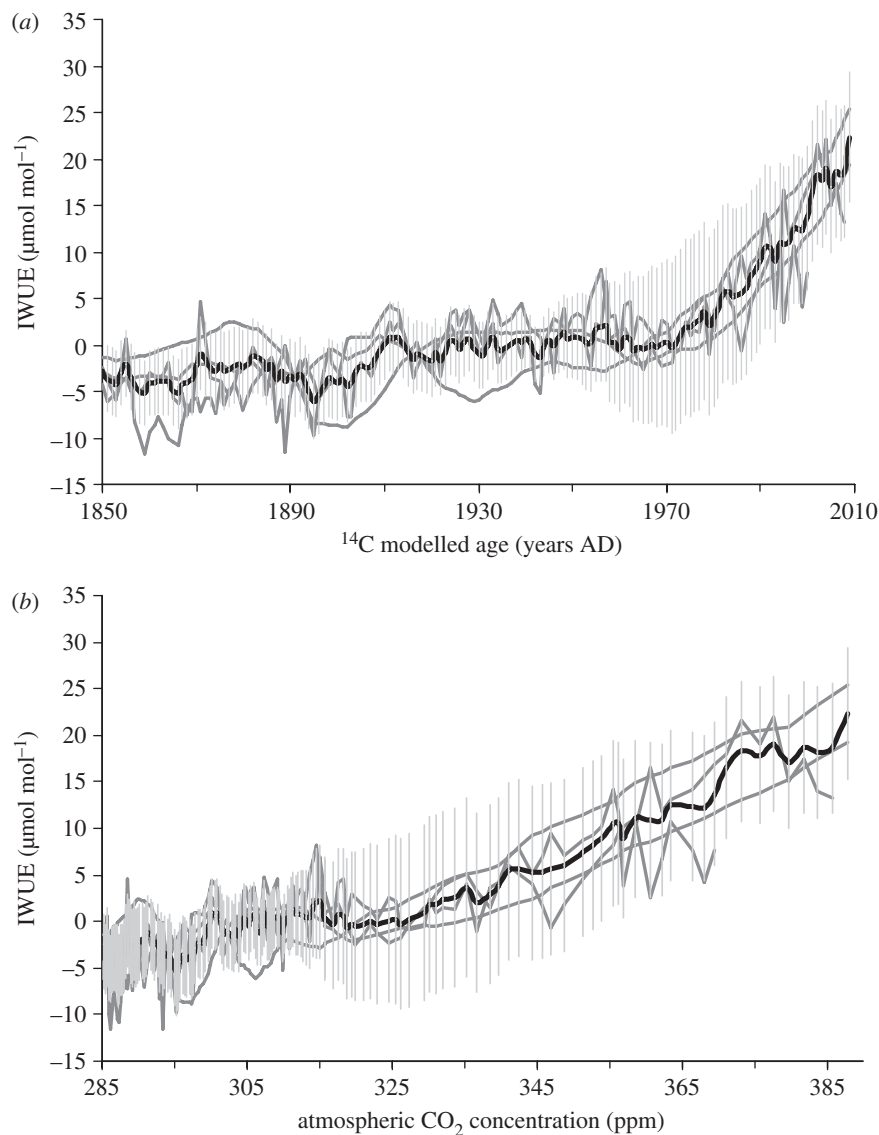


Figure 4. Composite graphs showing the relationship between the individual tree IWUE series plotted against (a) time and (b) atmospheric CO<sub>2</sub> concentration. All series are expressed as deviations from the mean calculated for the common period (AD 1850–2000). Thick grey lines represent the individual series, the thick black line represents the mean response (four trees) and the vertical error bars fitted to the mean (fine grey lines) represent the maximum annual uncertainty associated with the age–growth modelling of the four trees (1 sigma  $\approx \pm 40$  years).

for dendrochemical analyses and to demonstrate how coherent (palaeo)environmental information can be successfully extracted from this archive.

Further to the development of high-resolution dendrochemical studies conducted in the tropics thus far, this study proposes an alternative, *non-annual* approach for extracting palaeoenvironmental data from ringless tropical trees. Although such an approach is also resource-intensive, this change of emphasis will facilitate development of the data urgently required for climate model evaluation in this region. A non-annual approach also permits increased replication and greater temporal coverage than is typically achieved using high-resolution methods alone. Further improvements can be made to the age–growth modelling component most easily through (i) the addition of further radiocarbon dates, (ii) incorporation of probabilistic (Bayesian or Monte Carlo) methods and (iii) combining the low-frequency signals with high-resolution elemental

(Ca or stable C,H,O isotopic) analyses, where these are known to be temporally reliable.

Calculation of the IWUE for each of the trees demonstrated that at both sites the trees increased their IWUE by *ca* 29 per cent over the last 160 years (from a mean IWUE = 52 μmol mol<sup>-1</sup> in AD 1851–1860 to 67 μmol mol<sup>-1</sup> in AD 1991–2000). The change is similar in magnitude to that found in other climatic and vegetational zones and represents an active/plastic response of the trees to rising atmospheric CO<sub>2</sub>. Were the trees insensitive to rising CO<sub>2</sub>, one would expect to see the amount of CO<sub>2</sub> within the leaves (*c<sub>i</sub>*) increase in parallel with levels outside the leaves (*c<sub>a</sub>*), so that *c<sub>a</sub> - c<sub>i</sub>* (and thus IWUE) would remain stable through time. Instead the trees appear to have maintained a near constant *c<sub>i</sub>/c<sub>a</sub>* ratio despite rising ambient levels of CO<sub>2</sub>, so that the increase in *c<sub>i</sub>* is less than the increase in *c<sub>a</sub>*. It is this ‘active response’ [52] that produces the ‘physiological

forcing' via a reduction in stomatal conductance, and therefore evapotranspiration, and/or an increase in carbon assimilation rate. A similar active response to increases in atmospheric CO<sub>2</sub>, reflected in increasing IWUE, has been identified in several tree-ring isotope chronologies from temperate areas, but in most cases there is evidence that the limits to plasticity have already been reached [14,20,21,52]. The change from an active to a passive response to increasing CO<sub>2</sub> has important implications for climatic and carbon-cycle modelling. As described above, the calculation of IWUE from tree-ring data has limitations, and although only based on four trees, these results confirm that there is no *universal* level of CO<sub>2</sub> beyond which trees cannot continue to respond actively, but that the limits to plasticity are likely to be species- and site-specific. Nevertheless, it seems unlikely that there are no limits to the plasticity of tropical trees, as anthropogenic activities in the near future appear likely to drive atmospheric CO<sub>2</sub> concentrations beyond those experienced during the evolution of most living tree species [52].

The *E. zwageri* trees sampled were by no means the largest growing at Imbak Canyon, yet radiocarbon dating revealed these to be remarkably long-lived (*ca* 700 years old). The presence of many living *E. zwageri* trees of well over twice the breast height diameter of the sampled trees in the Imbak Canyon Conservation Area implies that tree ages well in excess of this age are likely. The identification of such ancient trees in the South East Asian rainforest supports the vision of resource management organizations such as Yayasan Sabah in identifying and designating primary forest conservation areas. It highlights the timescales that must be considered when replanting and regenerating tropical forests and in the development of carbon-offsetting schemes (through the United Nations Collaborative Programme on Reducing Emissions from Deforestation and Forest Degradation in Developing Countries; REDD). The immense ecological value and fragility of these ancient ecosystems, their palaeo-ecological potential and value for monitoring the impacts of CO<sub>2</sub> and climatic change emphasize the urgent need for their further conservation.

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