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Annual cyclicity in high-resolution stable carbon and oxygen isotope ratios in the wood of the mangrove tree *Rhizophora mucronata*

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ABSTRACT

In the present study, the high-resolution stable carbon ($^{13}C/^{12}C$) and oxygen ($^{18}O/^{16}O$) isotope ratio profiles in the wood of the mangrove *Rhizophora mucronata* Lam., a tropical tree species lacking distinct growth rings, were investigated. Variations of both isotope ratios revealed a remarkable annual cyclicity with lowest values occurring at the latewood/earlywood boundary (April–May) and highest values during the transition from earlywood to latewood (October–November). Based on the current knowledge of the physiology of this mangrove species, as well as on the current literature available on high-resolution profiles of stable isotope ratios in tree rings, possible driving forces responsible for this seasonal pattern are discussed. The annual cyclicity, together with a conspicuous isotope pattern appearing in the El-Niño year 1997, promises great potential for tropical dendrochronology.

Key-words: $\delta^{13}C$; $\delta^{18}O$; dendrochronology; El-Niño; East Africa; high-resolution sampling; seasonal pattern; tree rings; tropical tree.

INTRODUCTION

Mangroves are tropical forests inhabiting the inter-tidal areas of estuaries, lagoons and beaches protected from wave action. Worldwide, mangroves are disappearing at a rate of 1% per year (FAO 2003). In addition, a sudden die-off and an increased structural degradation of the forests have been reported for several countries (Spalding, Blasco & Field 1997). It has been hypothesized that the degradation results from a reduction in the freshwater input caused by drought, reduction of the groundwater outflow and/or redirection of rivers (Cardona & Botero 1998; Elster *et al.* 1999; Kovacs, Wang & Blanco-Correa 2001). However, the lack of long-term environmental data makes it difficult to prove causal relationships. In order to protect this valuable ecosystem, there is an urgent need for identifying adequate proxies, which can provide information on past changes in environmental conditions (Diop 2003).

Stable oxygen and carbon isotope ratios of tree rings have been proven on several occasions to be valuable proxies for environmental (including climatic) conditions (e.g. Dupouey *et al.* 1993; Schleser *et al.* 1999; Edwards *et al.* 2000 and references therein). In mangroves, the $\delta^{13}C$ of leaves has been found to mainly reflect the soil water salinity. Increased soil salinity causes a reduction of the CO_2 assimilation rate, as well as a decrease of the stomatal conductance resulting in a lower ratio of the intercellular to atmospheric CO_2 concentration (c_i/c_a) and consequently in a higher $\delta^{13}C$ value (Farquhar *et al.* 1982; Lin & Sternberg 1992a; Medina & Francisco 1997; Kao, Tsai & Tsai 2001). In general, a decrease in soil water potential will lead to an increase in the $\delta^{13}C$ value (Guy, Reid & Krouse 1980). Several studies have further demonstrated a relationship between mangrove growth forms (expressed in tree height) and carbon isotopic composition of leaves, with tall mangrove stands showing lower $\delta^{13}C$ values than scrub mangroves (Lin & Sternberg 1992b; Kao & Chang 1998; McKee *et al.* 2002). Although, the growth forms are the result of a synergistic effect of different stress factors, such as soil salinity, sulphide concentration and nutrient levels (Lin & Sternberg 1992a; McKee *et al.* 2002), only salinity affected the c_i/c_a. of culture grown mangrove seedlings (Lin & Sternberg 1992a).

The oxygen isotopic composition of the xylem sap has been used to trace the water source utilized by mangrove trees (Sternberg & Swart 1987; Lin & Sternberg 1994). This is possible since no fractionation of oxygen isotopes occurs during water uptake by the roots (Förstel 1978; Lin & Sternberg 1993). Lin & Sternberg (1994) showed that mangrove trees depend on surface soil water as their sole water source. In estuarine systems, this surface soil water is a mixture of freshwater (precipitation, ground- or river water) and seawater, each with its own isotopic signal. Consequently, the $\delta^{18}O$ of mangrove stem cellulose

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has been suggested as a proxy for the isotopic composition of the source water used by the plant, and hence for the freshwater input into the mangrove ecosystem (Sternberg & Swart 1987; Ish-Shalom-Gordon, Lin & Sternberg 1992).

In light of the above findings, both $\delta^{13}C$ and $\delta^{18}O$ should be valuable proxies to study changes in salinity caused by drought or reduction in freshwater input and provide possible insight in the forest dynamics and the observed degradation when time-series based on tree rings are analysed. However, recent evidence indicates that our understanding of stable isotope fractionation by plants is far from satisfactory and that the observed variation in isotopic composition cannot fully be explained by earlier models (e.g. Terwilliger & Huang 1996; Damesin & Lelarge 2003). This becomes especially evident when high-resolution isotope profiles of tree rings are investigated. Helle & Schleser (2004a) found that the intra-annual fluctuations of carbon isotope ratios in temperate deciduous trees showed a clear annual cyclicity, which they related primarily to seasonal changes in post-photosynthetic fractionation processes, such as, for example, accumulation and remobilization of storage material (e.g. starch). A better understanding of the processes influencing the isotopic composition of the wood will eventually lead to an increase in the reliability of tree rings as proxies for environmental conditions.

This study investigates, for the first time, the profiles of stable carbon and oxygen isotope ratios in the wood of the mangrove *Rhizophora mucronata* Lam. by using ultra-high temporal resolution. More specifically this study is aimed at: (1) determining whether a seasonal pattern, comparable to the cyclicity observed in temperate trees, is present in the isotope profiles of this tropical tree species; (2) identifying the possible factors (physiological and/or environmental) influencing the isotopic composition of the wood in this species; and (3) evaluate the potential of the isotopic composition of *R. mucronata* tree rings for dendrochronology.

Carbon isotope theory and model predictions

Farquhar *et al.* (1982) described the $\delta^{13}C$ value of C_3 plant material ($\delta^{13}C_c$) as a function of the fractionations caused by diffusion of CO_2 through the stomata (a = constant, 4.4‰) and by enzymatic CO_2 fixation (b = constant, 29‰), the ratio of the intercellular to atmospheric CO_2 concentration (c_i/c_a) and the source value, given by the isotopic composition of atmospheric CO_2 ($\delta^{13}C_{atm}$).

$$\delta^{13}C_c = \delta^{13}C_{atm} - a - (b - a)c_i/c_a \qquad (1)$$

Strictly speaking, Eqn 1 is only applicable to the first triose sugars formed by photosynthesis. Additional fractionations occur during further metabolic processes and need to be taken into account when predicting $\delta^{13}C$ values of plant bulk material. These additional fractionations are responsible for a ^{13}C enrichment of usually less than 5‰ in stem cellulose, as compared to leaf cellulose (e.g. Leavitt & Long 1982; Schleser 1992). Furthermore, the presence of other substances in bulk wood, in particular various amounts of ^{13}C depleted lignin, also influences the $\delta^{13}C$ value of bulk wood. Verheyden *et al.* (2005) found that bulk wood of *R. mucronata* is on average 0.97‰ (SD = 0.17‰, range 0.64–1.29‰) lighter than the corresponding extracted α-cellulose. Despite the factors mentioned above, leaf and stem isotopic signals were found to correlate well showing very similar seasonal trends (cf. Leavitt & Long 1982; Damesin & Lelarge 2003; Helle & Schleser 2004a). For the prediction of $\delta^{13}C_{wood}$ from Eqn 1, 4‰ were subtracted to account for the fractionations occurring during wood synthesis and the difference between bulk wood and α-cellulose.

To predict intra-annual variation in $\delta^{13}C_{wood}$ values, data on intra-annual c_i/c_a are needed. In mangroves c_i has been found to decrease with increasing soil water salinity (Farquhar *et al.* 1982; Lin & Sternberg 1992a; Medina & Francisco 1997; Kao *et al.* 2001). Soil water salinity fluctuates with fresh water input and therefore with precipitation (cf. Semeniuk 1983; Naidoo 1989). Monthly c_i values were constructed by assuming a linear relationship between precipitation and soil water salinity on the one hand and salinity and c_i, on the other. Maximum c_i values are therefore expected in May, the month with the highest rainfall, while minimum values are expected in February, the month with the lowest rainfall (see Fig. 2). Maximum and minimum c_i values were set to 200 and 100 p.p.m., respectively, which is the range reported in literature for several mangrove species over a wide range of salinities (e.g. Ball & Farquhar 1984; Andrews & Muller 1985), however, lower and higher values have also been reported (Clough & Sim 1989; Mwangi Theuri, Kinyamario & Van Speybroeck 1999). Since a linear relationship between precipitation, salinity and c_i can be debated, a second set of monthly c_i values was constructed by including a time-lag between increase in salinity and reduction of rainfall, as well as by including a buffering for short-term rainfall events, such as those occurring during the short rainy season (October–November, see Materials and Methods). The $\delta^{13}C_{wood}$ profile predicted from a linear and a non-linear relationship with salinity is given in Fig. 1. The amplitude of the isotopic signal as well as the absolute $\delta^{13}C$ values should be considered as rough estimates, as these depend on the entered data, which are only an approximation. However, in this study we are primarily interested in the shape of the $\delta^{13}C_{wood}$ profile. Relative humidity has also been shown to influence the $\delta^{13}C$ value of mangroves through a closure of the stomata at low relative humidity, resulting in a low c_i (Farquhar *et al.* 1982). In Kenya, the relative humidity follows a similar trend as the precipitation (Fig. 2) and should therefore further accentuate the increase in $\delta^{13}C_{wood}$ during the dry season (Fig. 1). Finally, in this model we assume a constant intra-annual $\delta^{13}C_{atm}$ (–8‰). The variability in $\delta^{13}C_{atm}$ may be as high as 1‰ in the Northern Hemisphere (Farquhar & Lloyd 1993; Helle & Schleser 2004a), however, it is usually much smaller in tropical regions. Buchmann *et al.* (1997), for example, found no seasonal difference in the below canopy atmosphere of a tropical rainforest.

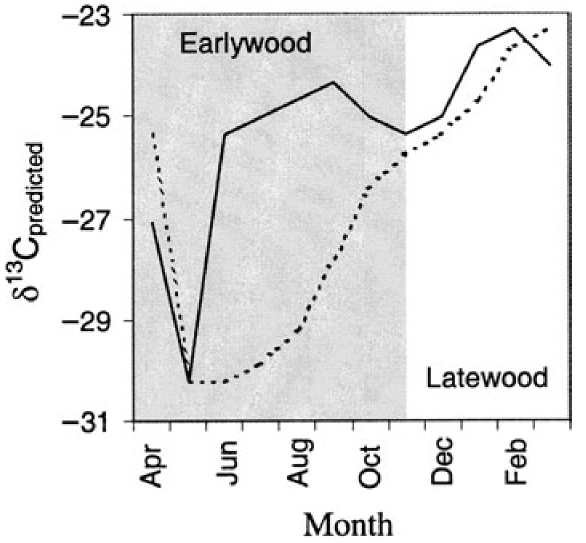

Figure 1. Predicted intra-annual $\delta^{13}C$ values in the tree rings of *Rhizophora mucronata* assuming a linear relationship between precipitation, soil water salinity and intercellular CO_2 concentration (c_i) (solid line) and assuming a non-linear relationship (dashed line).

Oxygen isotope theory and model predictions

The oxygen isotopic composition of tree ring cellulose ($\delta^{18}O_c$) is described as a function of the isotopic composition of the source water ($\delta^{18}O_s$), the ^{18}O enrichment of the leaf water due to evaporation, the ^{18}O enrichment during biosynthesis of glucose ($\varepsilon_{wc} = 27‰$) and a post-photosynthetic exchange of carbonyl oxygen with the xylem water (see Saurer, Aellen & Siegwolf 1997; Anderson *et al.* 2002; Barbour, Walcroft & Farquhar 2002):

$$\delta^{18}O_c = \delta^{18}O_s + f\,[\varepsilon_k + \varepsilon^* + (\delta^{18}O_v - \delta^{18}O_s - \varepsilon_k)e_a/e_i] + \varepsilon_{wc} \quad (2)$$

where ε_k is the kinetic fractionation factor associated with diffusion through the leaf boundary layer and stomata, ε^* is the proportional depression of equilibrium vapour pressure by $H_2{}^{18}O$, e_a/e_i is the ratio of the water vapour pressure in the air to the water vapour pressure in the leaf, the subscripts c, s and v stand for cellulose, source water and atmospheric water vapour, respectively, and the factor f is a dampening factor which summarizes the effects of the heterogeneity in the leaf water enrichment and the post-photosynthetic exchange of carbonyl oxygen with stem water. Similar to the carbon isotopic composition, the $\delta^{18}O$ value of wood has also been found to correlate well with the $\delta^{18}O$ of α-cellulose, however, bulk wood is about 5‰ lighter than cellulose (Borella, Leuenberger & Saurer 1999; Saurer, Cherubini & Siegwolf 2000).

Since the evaporative enrichment at the leaf is controlled by the vapour pressure ratio of the air to the leaf (e_a/e_i), the $\delta^{18}O_c$ shares the dependence on the stomatal conductance with the $\delta^{13}C_c$. However, only the $\delta^{13}C_c$ value is dependent on the photosynthetic activity. Simultaneous measurements of stable carbon and oxygen isotope ratios therefore may help to separate both signals (cf. Scheidegger *et al.* 2000). In the carbon isotope model, we argued that the stomatal conductance displayed the largest variation, resulting in the decrease in c_i from May onwards. Therefore, we should obtain a similar profile for the $\delta^{18}O_{wood}$ value, with an abrupt decrease at the onset of the long rainy season in April–May and a gradual increase with the decrease in relative humidity and the decrease in stomatal conductance as the rainy season and dry season progresses. In addition, seasonal changes in the $\delta^{18}O$ of the source water may further accentuate this trend. Data obtained from the Global Network of Isotopes in Precipitation (GNIP) database for Dar Es

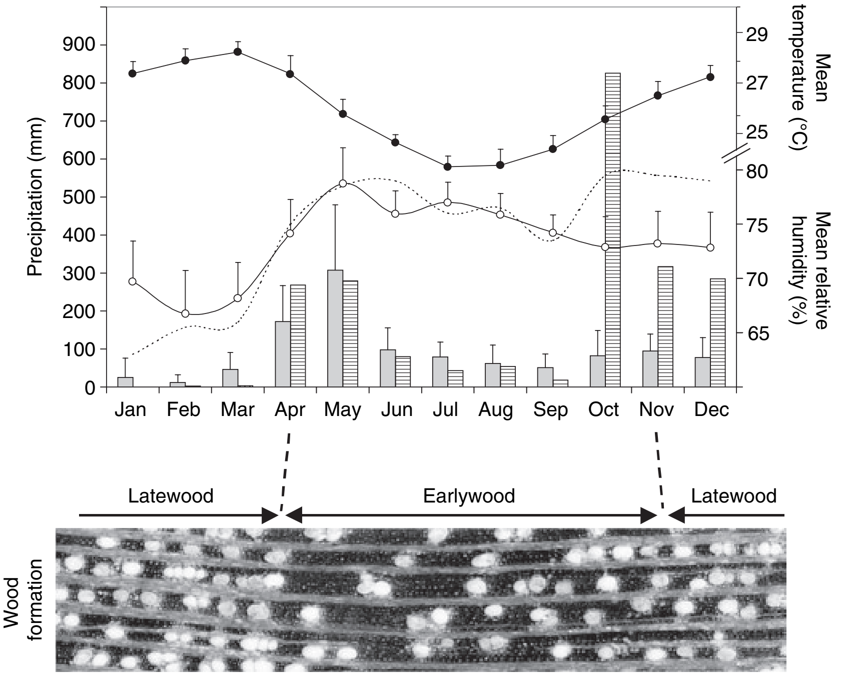

Figure 2. Mean monthly precipitation (grey bars), mean relative humidity (○) and mean temperature (●) (± SD) for the Mombasa region, Kenya for the period between 1982 and 1999, excluding the year 1997. The extreme events of 1997 are shown for the precipitation (dark grey shaded bars) and for the relative humidity (dashed line). Temperature data in 1997 did not show any significant deviation from the average pattern. Data obtained from the Mombasa meteorological station, Mombasa, Kenya. The timing of earlywood (low vessel density wood) and latewood (high vessel density wood) formation as observed by Verheyden *et al.* (2004) is also shown.

Salaam, Zanzibar (39.2° E, 6.88° S), show that May precipitation δ^{18}O is, on average, –2.23 ± 0.68‰ (IAEA/WMO 2001). Therefore, high amounts of precipitation in this region potentially lower the δ^{18}O value of the source water.

MATERIALS AND METHODS

Site description and plant material

Three stem discs collected in 1999 from Gazi Bay, Kenya (39.50° E, 4.42 S) were selected for the purpose of this study. All stem discs were collected 30 cm above the highest stilt root. The discs are now part of the xylarium of the Royal Museum for Central Africa, Tervuren, Belgium (accession numbers Tw55880, Tw55885 and Tw55891). Stems Tw55880 and Tw55891 both originate from a basin forest (*sensu* Lugo & Snedaker 1974), that occurs in a topographical depression. The site is flooded by high tide approximately 50 times a month, which corresponds to inundation class two (cf. Tomlinson 1986). Due to the basin topography, the water often stagnates, especially during the rainy season, when land run-off is high. Stem Tw55885 was collected in a fringe forest (*sensu* Lugo & Snedaker 1974), about 15 m from the river Kidogoweni. Due to its low topographical position, this area is flooded by almost all high tides (twice daily), which corresponds to inundation class one.

Climate description

The rainfall along the Kenyan coast shows a bimodal distribution, which is locally expressed in terms of the long rains (from April to July) and the short rains (from October to November) (McClanahan 1988), with a mean annual precipitation of 1144 mm (data for Mombasa, 1890–1985) (Lieth *et al.* 1999). The monthly average temperature ranges from 23.3 to 29.9 °C with a mean annual temperature of 26.4 °C (1931–1990) (Lieth *et al.* 1999). Mean monthly precipitation, mean relative humidity and mean temperature are shown in Fig. 2 for the Mombasa region, 40 km North of Gazi (data obtained from the Mombasa Meteorological Station, Mombasa, Kenya).

Defining the growth rings

Despite early reports of the absence of growth rings in *R. mucronata* (Panshin 1932; van Vliet 1976), we have recently proven the presence of annual growth rings in this mangrove species in Kenya (Verheyden *et al.* 2004). In short, the annual growth rings in *R. mucronata* consist of a low vessel-density earlywood layer (which visually appears as a dark ring) and a high vessel-density latewood layer (which appears as a light ring). The boundaries between the annual rings and between the earlywood and latewood are indistinct and are characterized by a gradual transition in vessel density (see Fig. 2). The earlywood formation starts in April, while the latewood formation starts in November (Verheyden *et al.* 2004; see also Fig. 2). The dating of the growth rings in each graph corresponds to the year in which their earlywood started forming. The growth ring dated as 1998, for example, started its earlywood formation during April 1998 and continued its latewood formation until March 1999. However, for practical reasons the whole ring will be referred to as the ring of 1998, despite the fact that part of the ring was formed in 1999.

Sampling and sample preparation

A radial wood section of approximately 1 cm height and 0.5 cm width was sawn out of each stem disc. The transversal sides were slightly sanded to allow for visual recognition of the annual growth rings. Tangential sections of 20 μm thickness were collected using a sliding microtome (Microm, HM 440 E; Microm, Walldorf, Garmany) with a tungsten carbide D-profile knife. Notes were taken as to which growth ring each sample belonged to, as well as its position in earlywood or latewood. However, due to the indistinct nature of the boundaries (Verheyden *et al.* 2004), an error on the exact location is unavoidable. In a preliminary stage, every contiguous 20 μm wood sample collected from a growth ring was analysed. However, contiguous samples did not show large variations in their δ^{13}C value (mean difference between two contiguous samples = 0.09‰, max = 0.3‰, $n = 20$), resulting in a rather smooth signal. This is consistent with observations on the high-resolution profiles in temperate trees (Schleser *et al.* 1999; Helle & Schleser 2004a). Therefore, the resolution was decreased and adapted to the ring width, which means that the analysed samples still originate from 20 μm sections, but not every single contiguous sample was analysed. Based on ring width measurements and assuming a constant intra-annual growth rate, a rough estimate of the temporal resolution achieved in this study was calculated. In stem disc Tw55891, each 20 μm wood section represents 2 d of growth in the years with highest growth rate. For the years 1994 to 1998, every fourth sample was analysed, therefore corresponding to a measurement every 8 days. However it is very unlikely that the growth rate remains constant throughout the year. Therefore, the temporal resolution achieved in this study is probably higher than estimated during the rainy season, but lower during the dry season.

Two additional radial sections of stem disc Tw55891 were also analysed over 5 years of growth to investigate the circumferential variation of the high-resolution stable carbon isotope profile. Due to the different growth rates along the three axes, a normalization was applied by using the boundaries between earlywood and latewood as reference points. Low-resolution (seasonal) samples were obtained for the 10 most recent annual rings of sample Tw55891 for comparison with the data obtained from the high-resolution samples. Earlywood and latewood of each year were carefully separated over the whole circumference of the stem disc and homogenized using a modified coffee mill (see Verheyden *et al.* 2005).

The samples (low- and high-resolution) were not processed for cellulose extraction since many authors observed that the isotopic composition of bulk wood and cellulose correlates well and that the slope of the linear regression is not significantly different from one. This has been reported for stable carbon isotope ratios analysed with annual resolution (Livingston & Spittlehouse 1996; Borella *et al*. 1998; MacFarlane *et al*. 1999) and with ultra-high resolution (Helle & Schleser 2004a). In addition, Loader, Robertson & McCarroll (2003) found that bulk wood is a better climate proxy than α-cellulose. A high correlation between the $\delta^{13}C$ value of α-cellulose and bulk wood, based on a seasonal (earlywood/latewood) resolution, was also shown for *R. mucronata* (Verheyden *et al*. 2005). The similarity of bulk wood and α-cellulose has also been observed for stable oxygen isotopes (Borella *et al*. 1999; Saurer *et al*. 2000). The samples for oxygen isotopes were dried for three days in a vacuum oven at 60 °C, to remove as much adsorbed water as possible, since this influences the measured isotopic signal.

Stable isotope measurements

Stable carbon isotopic composition of the wood samples were measured at the Laboratory of Analytical and Environmental Chemistry (Vrije Universiteit Brussel, Brussels, Belgium) using an elemental analyser (Flash 1112 Series EA; Thermo Finnigan, Bremen, Germany) coupled via a CONFLO III to an isotope ratio mass spectrometer (IRMS) (DELTA$^{\text{plus}}$XL; Thermo Finnigan). From each sample, 500 μg of wood material was analysed for stable carbon isotopic composition. Sucrose was used as a reference material (IAEA-C6, $\delta^{13}C = -10.4 \pm 0.1‰$). Results are reported using the conventional δ notation relative to the VPDB standard. The long-term reproducibility of the instrument, as calculated from repeated measurements on sucrose, is 0.08‰ ($n = 214$).

Stable oxygen isotope composition was measured on-line at the Institute for Chemistry and Dynamics of the Geosphere (ICG-V) at the Forschungszentrum, Jülich, Germany. Samples (300 μg) were pyrolysed to CO at 1080 °C in a Carlo-Erba 1500 elemental analyser (CE Instruments, Milan, Italy) coupled on-line to an OPTIMA IRMS (Micromass Ltd, Manchester, UK). The pyrolysis tube setup follows the description of Werner *et al*. (1996). The $\delta^{18}O$ values are referred to VSMOW and the overall analytical uncertainty is ±0.3‰. Replicates were measured occasionally to test the reproducibility.

Statistical analysis

Correlation between climate variables and isotope data were calculated using the Pearson Product-Moment correlation (Statistica 4.3; Statsoft Inc., USA). Only significant correlations at the level $P < 0.01$ were considered. Climate data for comparison with the isotope profiles were obtained from the Mombasa Meteorological Station, Mombasa, Kenya. Climate variables included in the statistical investigation are monthly precipitation, monthly mean temperature and monthly mean relative humidity. Data of 1997 and 1998 were not included in the analysis, since they were clearly recognized as outliers.

RESULTS

The high-resolution stable carbon isotope profile of stem disc Tw55891 shows a clear annual cyclicity (Fig. 3). The lowest $\delta^{13}C$ values occur in the vicinity of the latewood/earlywood boundary (further referred to as annual ring boundary). The $\delta^{13}C$ values steadily increase in the earlywood to reach a maximum at the earlywood/latewood boundary (further referred to as seasonal ring boundary) (Figs 4a, 5, 6a & b). In the latewood, the $\delta^{13}C$ value steadily decreases. In addition, in some years, the increase of the stable carbon isotopic value in the earlywood is further characterized by a slight decrease, prior to reaching the maximum $\delta^{13}C$ value, resulting in the presence of a 'dent' in the $\delta^{13}C$ peak (see arrows Fig. 4a). Although, it seems that the lowest $\delta^{13}C$ values usually occur slightly later than the onset of the earlywood and the highest values tend to occur on the seasonal ring boundary, it is at this stage impossible to confirm this tendency. Due to the indistinct nature of the growth rings, the exact position of the bound-

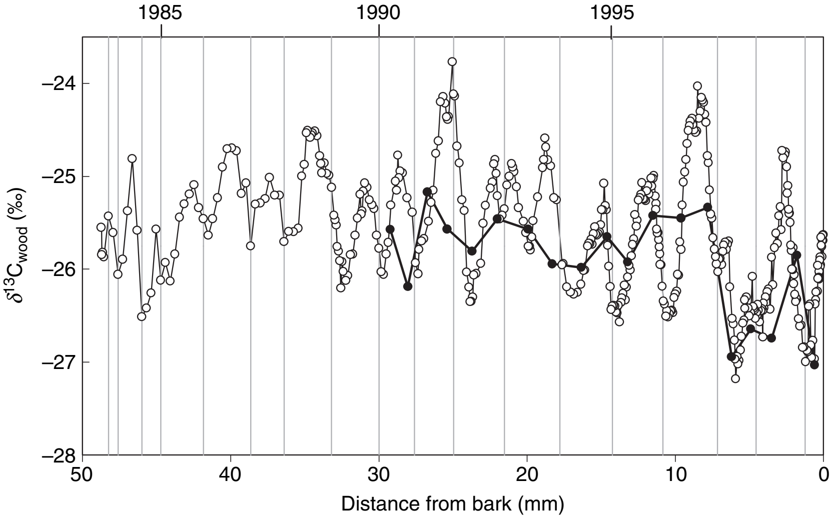

Figure 3. Annual cyclicity in the high-resolution (20 μm thick wood sections, ○) stable carbon isotope profile of *R. mucronata* (stem Tw55891). The vertical bars represent the annual growth ring boundaries (latewood/earlywood). The low-resolution profile (●) was obtained from measurements on homogenized earlywood and latewood blocks for the 10 most recent years.

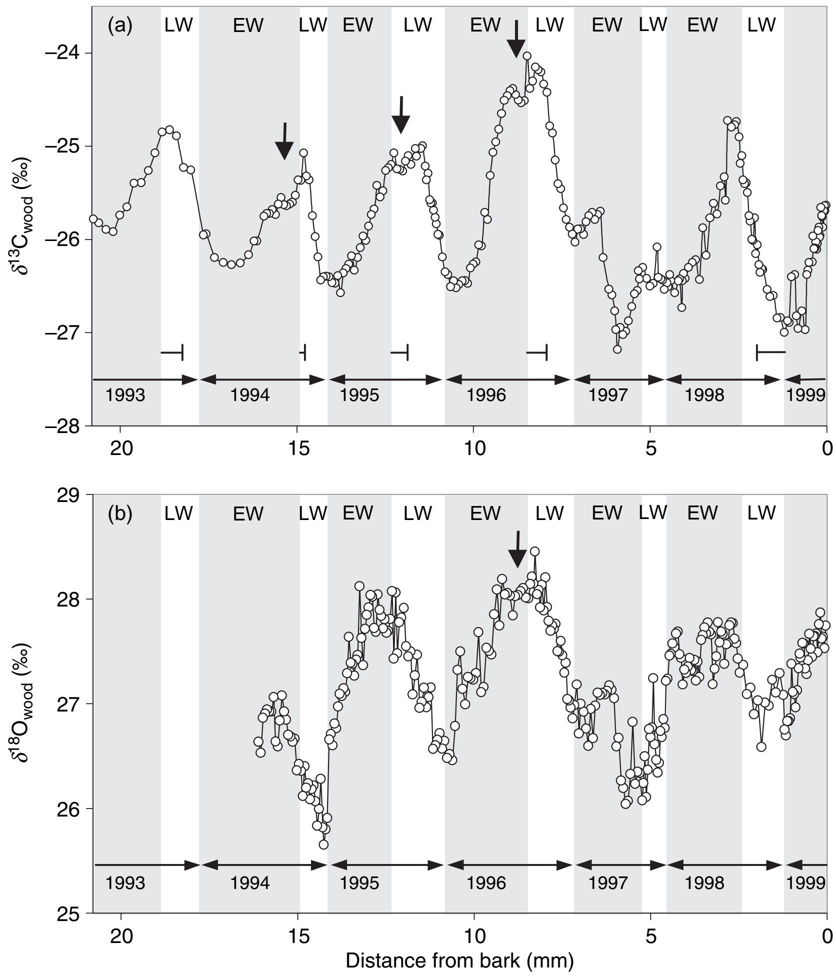

Figure 4. Annual cyclicity in the stable carbon (a) and oxygen (b) isotope ratios across the tree rings of *R. mucronata* (Tw55891, basin forest, in relation to the earlywood (EW, shaded background) and latewood (LW, white background) boundaries. The uncertainty on the position of earlywood and latewood boundaries is shown as horizontal error bars in Fig 4a. Arrows indicate the presence of a 'dent' in the peaks, which is characterized by a slight decrease, prior to reaching the maximum value.

aries is uncertain (see error bars in Fig. 4a and discussion in Verheyden *et al*. 2004). An exception on the annual cyclicity in the $\delta^{13}C$ profile occurs in the year 1997, which is characterized by a sharp drop in the $\delta^{13}C$ value in the earlywood (Fig. 4a). The cyclicity seems to be restored in 1998. The lowest $\delta^{13}C$ values (at the annual ring boundaries) did not show any correlation with climatic data from April or May The highest $\delta^{13}C$ values (at the seasonal ring boundaries) could only be correlated to October relative humidity ($r = -0.65$, $n = 15$, $P < 0.01$).

Low-resolution measurements, obtained from earlywood and latewood samples for the 19 most recent seasonal rings,

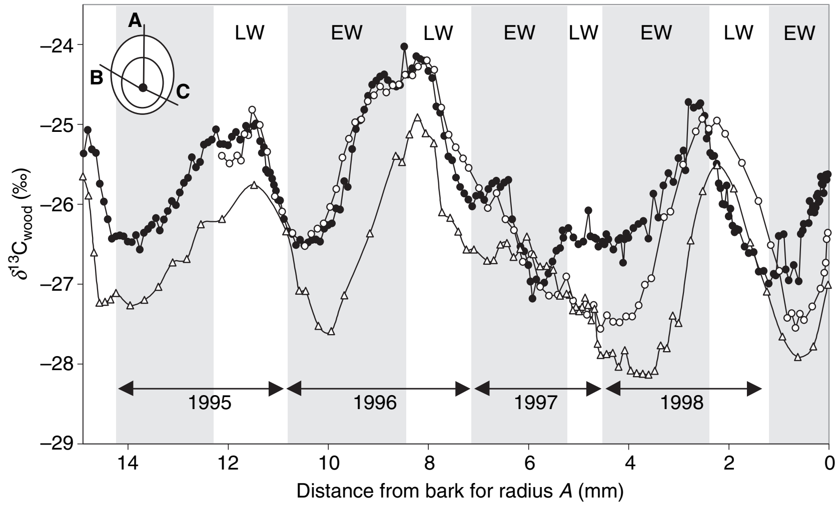

Figure 5. High-resolution stable carbon isotopic composition along three cross sections of stem Tw55891: A (●), B (○) and C (△). The radial section A is also shown in Figs 3 and 4. The three axes were normalized for differences in growth rates by using the visual boundaries as reference points (Section B and C were fitted to section A). Shaded background represents earlywood, white background represents latewood.

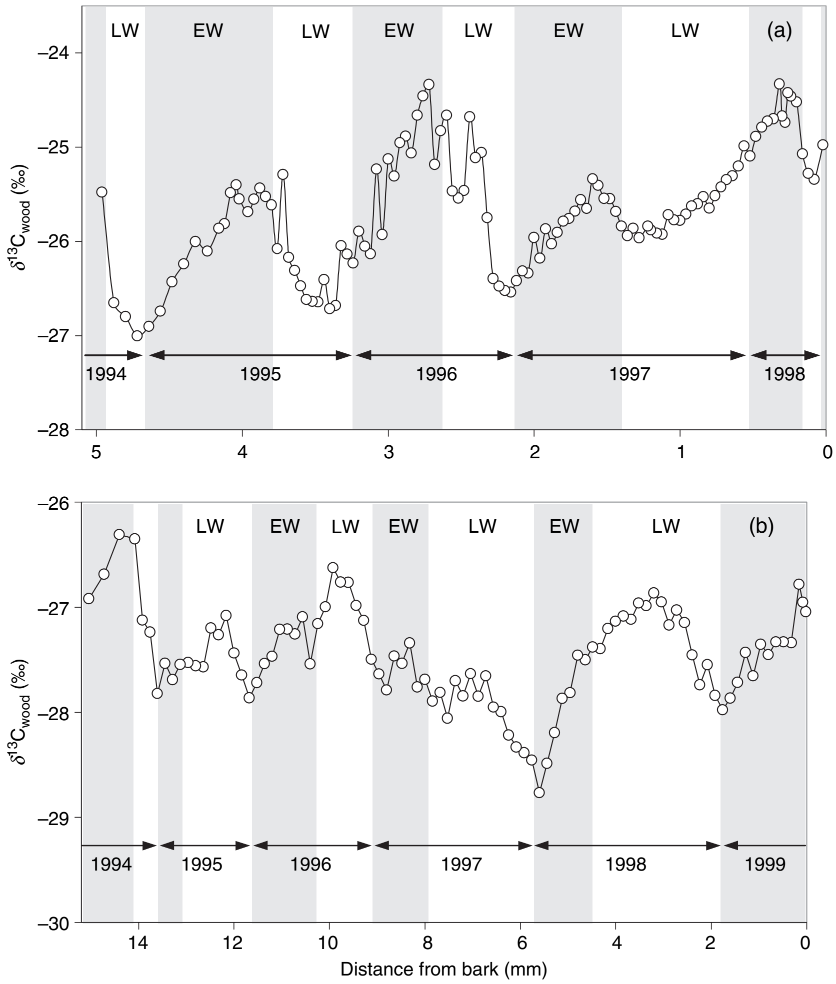

Figure 6. High-resolution profiles of the δ^{13}C values (over a period of 5 years) for two additional samples collected from Gazi Bay, Kenya: stem Tw55880 (a) (basin forest) and stem Tw55885 (b) (fringe forest). Shaded background represents earlywood, white background represents latewood.

are shown in Fig. 3. Within-year variability obtained from high-resolution δ^{13}C measurements (as the difference between highest and lowest δ^{13}C values) ranged from 0.95 to 2.36‰, and was considerably larger than the within-year variability obtained from the low-resolution data for the same time period (range =0.11–0.89‰). Between-year variability was also higher for the latewood values obtained from the high-resolution (SD = 0.96‰, $n = 9$) than from the low-resolution (SD = 0.42‰), but lower for the earlywood (SD = 0.26‰ versus 0.60‰, respectively).

The annual cyclicity in the δ^{13}C profile as well as the exceptional observations of the year 1997 were also detected in the two additional radial sections of stem disc Tw55891 (Fig. 5). The position of the lowest and highest values in relation to the annual and seasonal ring boundaries were comparable in all three radial sections. Shifts in the relative position of these values on the three radial sections occur in some years (e.g. 1998). However, it has to be kept in mind that the annual and seasonal ring boundaries, which were used as reference points for the normalization of the three growth axes, are themselves prone to errors. The absolute values of δ^{13}C were similar in the radial sections A and B, but were, on average, 0.78 ± 0.15‰ ($n = 10$, based on lowest and highest δ^{13}C values) lower in the radial section C. This is within the range reported for circumferential variation based on low-resolution measurements (e.g. Tans & Mook 1980; Sheu & Chiu 1995). Despite the difference in absolute values, the three radial sections showed similar relative values. For example, of the 5 years analysed, the latewood of 1996 showed the highest value in all three sections. The presence of the dent in the peak of the δ^{13}C value, is visible in radial section A and B, but is indistinct in radial section C. The low resolution, resulting from a slow growth rate along the axis of C, may explain the absence of this dent. The overall good match between the three radial sections indicates that the seasonal pattern is present over the full circumference of the tree, and that the positions of the highest and lowest δ^{13}C values, as well as the relative values (in comparison to other years) are indicative and can be discussed as such. The absolute values, however, should be used with caution, when comparing different trees, since considerable circumferential variation was observed within one tree.

The high-resolution profile obtained for the stable oxygen isotope ratios shows a remarkably similar pattern as the one observed in the δ^{13}C values (Fig. 4b). Lowest and highest δ^{18}O values occur in the vicinity of the annual and seasonal ring boundaries, respectively. Furthermore, an exception on the annual cyclicity is observed in 1997, similar to the one in the δ^{13}C profile, with a sharp drop in the δ^{18}O value occurring in the earlywood. In the δ^{18}O peak of the year 1996 a dent is discernible, similar to the dents in the peaks of the δ^{13}C series (see arrow, Fig. 4b). Although, the seasonal pattern is very similar, the δ^{18}O value in the earlywood seems to climb to a maximum at a faster rate than the δ^{13}C value.

The annual cyclicity was also confirmed in the δ^{13}C variations of two additional stem discs: Tw55880 and Tw55885 (Fig. 6a & b). The stem disc Tw55880 (Fig. 6a) shows a similar pattern as Tw55891 (Fig. 4a), with lowest and highest δ^{13}C values occurring in the vicinity of the annual and seasonal ring boundaries, respectively. Both stem discs, Tw55880 and Tw55891 were collected in a basin forest (see Materials and Methods). However, the δ^{13}C profile of Tw55885 (Fig. 6b), which was collected in a fringe forest, slightly differs. First, the amplitude of the signal is much more reduced than in the stems from the basin forest. Second, the highest δ^{13}C value does not occur at the seasonal ring boundary, but is shifted towards the centre of the latewood. As in Tw55891, a deviation from the normal δ^{13}C pattern is also observed in Tw55880 and Tw55885 for the year 1997. In Tw55880, the peak in the δ^{13}C value is lower than in other years, while in Tw55885 the peak is absent.

DISCUSSION

Several recent studies have reported a distinct seasonal pattern in the intra-annual variability of δ^{13}C and δ^{18}O values in the leaves and wood of temperate tree species (e.g. Barbour *et al*. 2002; Jäggi *et al*. 2002; Damesin & Lelarge 2003; Keitel *et al*. 2003; Helle & Schleser 2004b; Helle & Schleser 2004a). This seasonal pattern has mainly been attributed to post-photosynthetic processes related to the use and storage of reserve material (starches) for bud break and leaf development. Therefore, distinct seasonality in the carbon and oxygen isotopic signals of tropical evergreen trees from sites near the equator is unexpected. However, the high-resolution profiles of the stable carbon and oxygen isotope ratios obtained for the stem discs of *R. mucronata* reveal a remarkable annual cyclicity (Figs 3, 4, 5 & 6). In this study we investigate whether the observed pattern reflects a response to environmental factors or whether additional (physiological) processes should also be considered. Furthermore, the potential of the isotopic composition for the dendrochronology of this species is evaluated.

Annual cyclicity in δ^{13}C

In the high-resolution profiles of the stem discs collected from the basin forest (Tw55880 and Tw55891), the lowest δ^{13}C value is observed at the annual ring boundary, which corresponds to wood formed during April–May and coincides with the onset of the long rainy season (Verheyden *et al*. 2004; see Fig. 2). The isotopic signal of the earlywood displays a gradual increase in the δ^{13}C value. These results are consistent with the prediction from the model, which considers the influence of soil water salinity on the internal CO_2 concentration (c_i) and consequently on the δ^{13}C value (see Fig. 1). The δ^{13}C value reaches a maximum at the seasonal ring boundary, which corresponds to wood formed in October–November (Verheyden *et al*. 2004; see Fig. 2). The presence of a 'dent', characterized by a slight decrease in the δ^{13}C, prior to reaching the maximum value, is a very peculiar and specific feature in the seasonal pattern of the profile of Tw55891 (arrows, Fig. 4a). Although only occurring in certain years, this dent appeared in at least 5 years, between 1990 and 1998. The lower resolution utilized in the tree rings prior to 1990 does, however, not allow verification of the presence of this 'dent' in earlier years. Comparison with the model predictions reveals that a dent in the δ^{13}C profile is expected at the onset of the short rainy season in October (Fig. 1), which causes a short-lived increase in the soil water potential and therefore a slight decrease in the δ^{13}C value. Stem disc Tw55885, collected from the fringe forest showed a slightly different seasonal pattern than observed in the stems from the basin forest. In this sample, the amplitude of the signal is reduced and the δ^{13}C peak is shifted towards the centre of the latewood. The fringe forest is in close proximity to the river Kidogoweni and experiences a daily tidal inundation frequency. Seasonal fluctuations in soil water salinity are expected to be much smaller in this site in comparison with the basin forest and therefore reduce the amplitude of the δ^{13}C profile. This is further supported by the fact that the absolute δ^{13}C values of Tw55885 are lower than those of the two stem discs from the basin forest. However, absolute values should be compared with caution due to the circumferential variation (Fig. 5). Due to the buffering of the seasonal differences, the increase in salinity may be delayed and occur only in the dry season. This probably causes the shift in the δ^{13}C peak to the latewood.

The overall good similarity with the model predictions, as well as the presence of a dent coinciding with the onset of the short rainy season and a reduced amplitude in δ^{13}C values from a site where fluctuations in salinity are expected to be low, offers strong evidence for an environmental driving force (soil water salinity) controlling the δ^{13}C value in the earlywood of *R. mucronata*. However, other observations made during this study fail to conform to the model's predictions and suggest that additional processes should be considered. First, no correlation was found between the lowest δ^{13}C values (at the annual ring boundaries) and the climatic conditions in April–May. Second, the seasonal pattern in the δ^{13}C value appears as a smooth continuous signal, almost resembling a sinusoidal function (see Fig. 4a). With the exception of the short rainy season and perhaps the El-Niño rains of 1997 (see below), no short-term rainfall anomalies are observed in the isotopic signal. However, the monthly precipitation in Kenya shows considerable between-year variation (see error bars in Fig. 2). For example, the amount of rainfall in June and July of 1995 and 1996 is very similar, but, August 1995 received seven times more rainfall than August 1996 (180 versus 24 mm, Mombasa Meteorological Station, Mombasa, Kenya). In stem disc Tw55891, a temporal resolution of about one sample every 8 d was obtained between 1994 and 1998, which allows the monitoring of short-term changes in the environmental conditions. However, the carbon isotopic signal in the earlywood of 1995 does not noticeably differ from the signal in 1996. Finally, the decrease of the δ^{13}C value during the dry season (latewood) is not consistent with the constraints of the model. The dry season (December to March) is characterized by a further decrease in rainfall and a lower relative humidity than during the long and short rainy season (Fig. 2). Soil water salinity considerably increases (cf. Semeniuk 1983; Naidoo 1989) causing a reduction in c_i (Farquhar *et al.* 1982; Lin & Sternberg 1992a; Medina & Francisco 1997; Kao *et al.* 2001). Therefore, according to the Farquhar *et al.* (1982) model, we should expect a further increase in the δ^{13}C value, whereas the contrary is observed. These results strongly suggest that additional environmental or physiological processes influence the δ^{13}C value in the dry season.

One possible explanation is that the extreme conditions or the combination of stress factors during the dry season cause an increase in c_i, such as has been reported in several studies. Ball & Farquhar (1984) found that a low relative humidity causes a reduction in c_i in low salinity conditions, but an increase in c_i in high salinity conditions. Increased c_i was also observed as a result of thermal stress under field conditions (Andrews & Muller 1985; Ball, Cowan & Farquhar 1988) and attributed to an inhibition of the carbon assimilation (Andrews & Muller 1985). Low relative humidity, increased salinity and high temperatures are characteristic for the dry season in Kenya. Therefore, the decrease in the δ^{13}C value in the latewood of *R. mucronata* may be caused by an increase in the c_i as a result from the inhibition of carbon assimilation. This hypothesis is further supported by the fact that Sobrado (1999), in a study on the mangrove *Avicennia germinans* (L) Stearn., found higher c_i values during the dry season.

In addition to environmental factors, post-photosynthetic physiological processes may also considerably influence the intra-annual variability of δ^{13}C and may be responsible for the rather smooth isotopic signal and the lack of a significant correlation with climate data. In fact, the main driving force behind the seasonal pattern observed in δ^{13}C of tree rings and leaves of temperate trees is now believed to originate from post-photosynthetic physiological processes rather than from environmentally controlled c_i/c_a ratios (Jäggi *et al.* 2002; Damesin & Lelarge 2003; Helle & Schleser 2004a). The similarity in the seasonal pattern of δ^{13}C in *R. mucronata* and the pattern observed in temperate trees, in particular evergreen (coniferous) trees (Helle 1997), lead us to conclude that similar driving forces might act in both, tropical and temperate tree species and merits further investigation. In temperate tree species the remobilization of ^{13}C enriched starches at the beginning of the growth season causes an increase in the δ^{13}C value of wood and leaves (cf. Damesin & Lelarge 2003; Helle & Schleser 2004a). An increased δ^{13}C value during leaf emergence in the leaves of neotropical tree species has also been observed and is considered to be related to the use of imported enriched carbon or to an increased proportion of CO_2 fixed by the enzyme phospho-enol-pyruvate carboxylase (Terwilliger *et al.* 2001). Furthermore, significant seasonal variation was found in the total non-structural carbohydrate concentrations in branch, trunk and root tissue of deciduous, as well as evergreen tropical tree species, with lowest trunk-carbohydrate concentrations occurring during canopy rebuilding (Newell, Mulkey & Wright 2002). In *R. mucronata*, leaf production peaks in the rainy season (Kairo 1993) therefore the increase in δ^{13}C in the earlywood is consistent with a remobilization and use of ^{13}C enriched starches. In addition to the remobilization of storage material, Damesin & Lelarge (2003) concluded that part of the within-year δ^{13}C variations in beech trees (*Fagus sylvatica* L.) is caused by stem respiration. Dark respiration and photorespiration were shown to cause ^{13}C enrichment of respired CO_2 relative to the plant material (cf. Ivlev, Bykova & Igamberdiev 1996; Duranceau *et al.* 1999; Ghashghaie *et al.* 2001), which, depending on the respiration rate, may significantly deplete the plant material. Duranceau *et al.* (1999) found a depletion of 1‰ in the plant material, which was attributed to dark respiration. Increased (photo)respiration rates during the dry season, as suggested by Sobrado (1999) therefore, also seems a possible explanation for the observed decrease in the δ^{13}C value in the latewood, however, no data on seasonal rates of respiratory processes for *R. mucronata* could be found in the literature. In conclusion, both environmental and physiological processes may offer potential explanations for the observed seasonal pattern in the stable carbon isotope profile, however, high-resolution time-series of c_i values, starch content and isotopic composition of the starch are needed to determine which of these processes prevails and whether the intra-annual composition of *R. mucronata* tree rings can provide information on environmental conditions.

δ^{13}C values in 1997

All three stem discs investigated in this study, showed a clear anomaly in the seasonal pattern of the stable carbon isotope ratios in 1997. The deviation from the normal seasonal pattern was largest in stem disc Tw55891, where δ^{13}C sharply dropped after the onset of the rainy season. The year 1997 was an El-Niño year, characterized by extremely high rainfall during the short rainy season, which continued during the dry season of 1998 (Webster *et al.* 1999). In October and November 1997 five times more rainfall fell in these 2 months as compared to other years for the same time period (Fig. 2). This unusually high amount of fresh water input may cause a low δ^{13}C value in the latewood of these mangrove trees. Strangely enough, the sharp drop in the δ^{13}C value occurs prior to the onset of the earlywood/latewood boundary. Extreme El-Niño years have distinct precursors in the Indian Ocean (anomalous atmospheric pressure, sea surface temperature, wind speed and direction), which appear as early as July (Webster *et al.* 1999;

Mutai & Ward 2000; Hastenrath & Polzin 2003). The sharp drop in the δ^{13}C value appearing in the middle of the earlywood may be caused by changes in meteorological conditions, preceding the short rainy season. Other (less extreme) El-Niño events were, however, not recorded in the isotope profiles of the wood.

Annual cyclicity in δ^{18}O

From the model presented in the introduction (Eqn 2), we predicted that the δ^{18}O profile in the wood of *R. mucronata* should display a similar trend as the δ^{13}C profile. However, this hypothesis is valid if we assume that the changes in δ^{13}C are primarily caused by changes in the stomatal conductance. From the results of the δ^{13}C profile, we argued that this assumption may have been violated. If, as we propose above, the decrease in δ^{13}C during the dry season is caused by an inhibition of the carbon assimilation, this should not be recorded in the δ^{18}O profile. However, the δ^{18}O shows a decline during the dry season, which is surprisingly similar to the decline in the δ^{13}C profile. Correlation coefficients between δ^{13}C and δ^{18}O values are similar to those reported in other studies (Fig. 7) (cf. Barbour *et al.* 2002). At low δ^{18}O values, there seems to be a deviation from the linear relationship. The low δ^{18}O values are characteristic for the rainy season, during which high stomatal conductance results in high evapotranspiration rates, despite the high relative humidity (Mwangi Theuri *et al.* 1999). However, high transpiration rates have been suggested to reduce the back diffusion of enriched ^{18}O water from the sites of evaporation to other sites in the leaf (Farquhar & Lloyd 1993; Barbour *et al.* 2000). This process is usually referred to as the Péclet effect and results in a heterogeneous isotopic composition of the leaf water and a lower δ^{18}O of the cellulose than predicted.

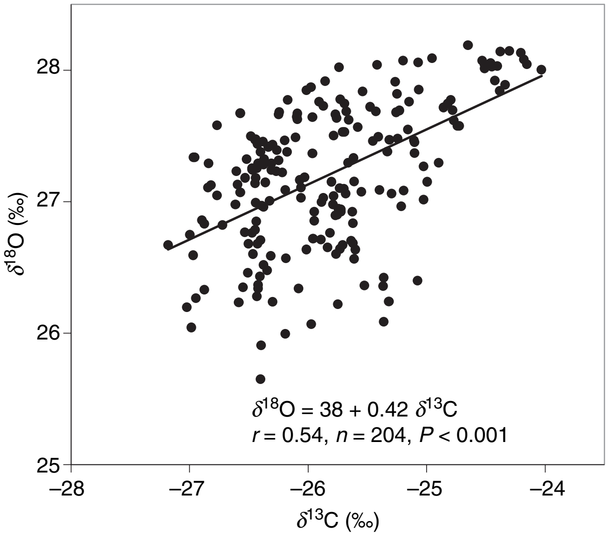

Figure 7. Relationship between δ^{13}C and δ^{18}O of *R. mucronata* wood for stem Tw55891.

With our current knowledge on mangrove physiology and oxygen isotope fractionation in plants, we cannot explain the observed decline in δ^{18}O during the dry season. The remarkable similarity between the seasonal patterns of δ^{13}C and δ^{18}O suggests that similar driving forces are responsible for the carbon and oxygen isotope discrimination in these trees. Barbour *et al.* (2002) found a negative correlation between the δ^{18}O in the cellulose and the modelled δ^{18}O of the precipitation, which is the reverse of the predicted relationship and is also observed in our data. They suggest important physiological effects on the δ^{18}O, which is supported by our results.

Evaluation of stable carbon and oxygen isotope ratios for dendrochronology

In the present study, we found a remarkable annual cyclicity in the high-resolution profiles of the stable carbon and oxygen isotope ratios of *R. mucronata*, a tropical tree lacking distinct growth rings. This annual cyclicity, together with the conspicuous isotope pattern within a group of trees appearing in the event year 1997, promises a high potential for tropical dendrochronology. The method of cross-dating, namely the procedure of matching ring characteristics among different trees, allows the identification of the exact year in which each ring was formed. Trees from temperate and boreal zones can be dated by means of cross-dating ring width sequences. However, cross-dating normally fails when applied to tropical trees. Suitable ring width sequences cannot be established due to indistinct tree ring boundaries or individual sequences do not show any common features or pointer years. Nonetheless, cross-dating might be successfully applied to high-resolution isotope sequences of tropical timbers if our results can be confirmed in other tropical tree species. During the revision of this manuscript, Poussart, Evans & Schrag (2004) found similar seasonal patterns in the δ^{13}C and δ^{18}O profiles of tropical Asian tree species, which reinforces our conclusion. However, additional research is needed to determine the driving force(s) responsible for the seasonal pattern in *R. mucronata* wood before environmental information can be extracted. Finally, it is worth noting that although, the high-resolution approach is costly, a low-resolution sampling is not encouraged, as this results in a serious artificial dampening of the isotopic signal and an artificial within-year and between-year variability (Fig. 3).

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