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UV-laser-based microscopic dissection of tree rings – a novel sampling tool for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ studies

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Summary

- UV-laser-based microscopic systems were utilized to dissect and sample organic tissue for stable isotope measurements from thin wood cross-sections.
- We tested UV-laser-based microscopic tissue dissection in practice for high-resolution isotopic analyses ($\delta^{13}\text{C}/\delta^{18}\text{O}$) on thin cross-sections from different tree species. The method allows serial isolation of tissue of any shape and from millimeter down to micrometer scale. On-screen pre-defined areas of interest were automatically dissected and collected for mass spectrometric analysis.
- Three examples of high-resolution isotopic analyses revealed that: in comparison to $\delta^{13}\text{C}$ of xylem cells, woody ray parenchyma of deciduous trees have the same year-to-year variability, but reveal offsets that are opposite in sign depending on whether wholewood or cellulose is considered; high-resolution tree-ring $\delta^{18}\text{O}$ profiles of Indonesian teak reflect monsoonal rainfall patterns and are sensitive to rainfall extremes caused by ENSO; and seasonal moisture signals in intra-annual tree-ring $\delta^{18}\text{O}$ of white pine are weighted by non-linear intra-annual growth dynamics.
- The applications demonstrate that the use of the UV laser-based microdissection allows for sampling plant tissue at ultrahigh resolution and unprecedented precision. This new technique facilitates sampling for stable isotope analysis of anatomical plant traits like combined tree eco-physiological, wood anatomical and dendroclimatological studies.

Keywords:

dendroclimatology, high-precision sampling, high-resolution stable isotope analysis, plant physiology, tree rings, UV laser microdissection, wood anatomy

Introduction

Stable isotope analyses (hydrogen (H), nitrogen (N), carbon (C), oxygen (O)) on organic tissue are widely used in plant physiological, ecological and climatological research (e.g. Heinrich et al. 2013, Brien et al. 2012, Schleser et al. 1999, Dawson et al. 2002, Werner et al. 2012, Ehleringer, Hall and Farquhar 1993, West et al. 2010, Ziegler 1995, Helle and Schleser 2004b, Treydte et al. 2006, Hietz et al. 2011). State of the art isotope ratio mass spectrometry (IRMS) and cavity ring-down spectroscopy (CRDS) allow precise determination of stable isotope ratios on minimal sample amounts and large numbers of samples. However, accurate preparation of small samples in large numbers is challenging, as this still has to be done manually in most of the research areas. Despite the high precision and accuracy of modern analytical devices, data quality very much depends on the skills and motivation of the person responsible for sample preparation. This applies in particular to inter- and intra-annual stable isotope studies of woody tissue attempting to extract valuable seasonal climatic and environmental information (e.g. Leavitt and Long 1982, Loader, Switsur and Field 1995, Treydte et al. 2006, Schollaen et al. 2013) or assessing plant eco-physiological processes (e.g. Gessler et al. 2009, Helle and Schleser 2004a, Schubert and Jahren 2011, Krepkowski et al. 2013) from annual growth rings or parts thereof. Furthermore, high-precision dissection is crucial in studies using high-resolution stable isotope data for the identification of anatomically non-distinct annual rings in tropical trees (e.g. Pons and Helle 2011, Evans and Schrag 2004, Verheyden et al. 2004).

Different methods exist for the dissection of wood tissue from tree rings for stable isotope measurements. One common method has been to divide wood segments or cores by cutting tangential slices, utilizing fixed-blade sledge and rotary microtomes (Helle and Schleser 2004a, Loader et al. 1995, Pons and Helle 2011, Verheyden et al. 2004, Ogée et al. 2009, Poussart, Evans and Schrag 2004, Barbour, Walcroft and Farquhar 2002, Ogle and McCormac 1994) or scalpel/razor blades (e.g. Managave et al. 2010b, Roden, Johnstone and Dawson 2009). With a microtome, wood slices down to $\sim 10 \mu\text{m}$ thickness can be achieved (Helle and Schleser 2004a). Another high-resolution sampling method is by collecting wood dust from a series of small wood holes in radial direction utilizing twist drills (Fichtler, Helle and Worbes 2010, Gebrekirstos et al. 2009, Walcroft et al. 1997), robotic micromilling techniques (Dodd et al. 2008, Wurster, Patterson and Cheatham 1999) or an UV-laser ablation system in combination with isotope ratio mass spectrometry (LA-C-GC-IRMS), first

described in Schulze et al. (2004). Accurate sample adjustment, as well as unambiguous identification of tree rings is normally provided by visual inspection using a microscope or digital camera. After dissection, wood samples are ground to a fine powder and either cellulose is extracted or bulk wood samples are used for stable isotope analysis. The latter is the case for the UV-laser ablation method where dust is ablated from a wood core or segment in an airtight chamber flushed with helium. From the ablation chamber wood dust is directly transferred into a combustion oven and converted to CO₂. Subsequently, $\delta^{13}\text{C}$ is measured by an isotope ratio mass spectrometer that is coupled online to the UV-laser ablation and preparation system. Various studies used this method to analyse the intra-annual $\delta^{13}\text{C}$ in tree rings responding to carbohydrate storage and remobilization, as well as short-term climatic effects in conjunction with wood density variability (Intra-annual Density Fluctuations, IADFs) (e.g. Battipaglia et al. 2010, De Micco et al. 2012, Skomarkova et al. 2006). The spatial resolution of a UV-laser shot is 40 μm (Schulze et al. 2004) to 150 μm (De Micco et al. 2012). The widths of the holes caused by the mechanical drills can vary from 500 μm (Gebrekirstos et al. 2009) to 800 μm (Fichtler et al. 2010) up to 1.5 mm (Walcroft et al. 1997) depending on the diameter of the drill. Robotic micromilling generally requires a minimum path width of 400 μm for increment core samples, whereas individual path widths of <100 μm are possible (Dodd et al. 2008).

Different drawbacks exist for the dissection methods mentioned before. For the methods using blades or microtomes, wood samples must have straight-line ring structure boundaries in tangential and transversal direction, as samples are cut in parallel linear segments. However, ring width and curvature of growth rings are rarely strictly parallel and fibre or vessel angles vary under natural conditions as well as depend on species-specific wood anatomical characteristics. Thus, often only a limited number of consecutive tree rings per sample is suitable for high-precision intra-annual stable isotope measurements when applying these dissection methods. Generally, cross-contamination during cutting, milling and other preparatory steps can not be excluded especially when quality control by electronic image documentation is not implemented. For the methods drilling wood holes, straight-line boundaries in transversal direction are less important, but differences in vertical direction can be problematic, depending on the drill depth and the deviation of wood fibre or vessel orientation from the vertical direction (Fig. 1b). LA-C-GC-IRMS systems are presently confined to carbon isotope analysis. Aerosol transfer of wood dust between the ablation

chamber and the combustion furnace requires a rather high helium consumption and transfer capillaries may clog up sometimes.

Fig. 1 Possible steps of wood sample preparation and high-precision sampling using UV-laser-based microscopic dissection systems. (a) Top view and profile of a metal frame slide with wood cross-sections used for gravity-assisted collection (LMD7000, Leica). Up to three cross-sections of 59-mm length can be mounted between two metal frames. For stable isotope analyses it is possible to cut serial sections at inter- to intra-annual resolution or even to pool sample material, e.g. for cellulose extraction. To avoid material loss the dissection of tissue can be alternately shifted. (b) Schematic view of sample preparation with a wood segment in radial and cross-section. The use of cross-sections diminishes difficulties of cross-contamination when sampling tree rings with tight curvatures, non-parallel boundaries, varying fibre and vessel angles and/or changing tree-ring width in radial direction. The dissection of wood cross-sections ranging in thickness from 100 μm to a maximal 1000 μm is possible. Remaining wood sample can be used for multidisciplinary approaches, e.g. combining high-resolution stable isotope analysis, quantitative wood anatomy, ring width or wood density analysis. The need for methodological improvements to facilitate fine dissection of irregular and narrow shapes has emerged lately, we assessed a novel method to dissect wood tissue from tree rings using UV-laser microdissection microscopes of Leica (LMD7000, Leica Microsystems GmbH, Wetzlar, Germany) and Zeiss (PALM MicroBeam, Carl Zeiss

Microscopy GmbH, Jena, Germany). UV-laser-based microscopic dissection systems are being applied widely in biomedical (e.g. Fend and Raffeld 2000), animal (e.g. Bayona-Bafaluy et al. 2005) and also in plant research (e.g. Abbott et al. 2010, Nelson et al. 2006). However, to our knowledge, this is the first time it has been utilized to precisely dissect plant tissue for stable isotope research. Laser-based microscopic dissection uses an UV-laser beam to isolate tissues of interest from thin sections of samples. We describe the design and handling of the two different UV-laser microdissection microscopes. Advantages and constraints are discussed on the basis of high-resolution stable isotope analyses on woody plant material of deciduous and coniferous trees from temperate and tropical climate zones. Variability of the new sub-seasonal C and O stable isotope data was evaluated with respect to seasonal changes of climatic conditions and wood growth dynamics. Furthermore, we tested whether $\delta^{13}\text{C}$ in the woody ray parenchyma and in xylem cells of a deciduous tree is different.

Material and Methods

UV-laser-based microscopic dissection

UV-laser microdissection microscopes were used as a precise tool for dissecting different wood samples in stable isotope studies. We tested two different UV-laser microdissection microscopes of Leica (LMD7000, software v6.7.1.3952, Leica Microsystems GmbH) and Zeiss (PALM MicroBeam, software v4.5.0.9, Carl Zeiss Microscopy GmbH). We followed a preparation scheme containing five steps:

1. manual preparation of thin wood cross-sections (max. 1000 μm thickness) with a microtome or a high precision saw,
2. microscopic identification and pen-screen selection of tissues of interest,
3. automatic UV-laser-based microscopic dissection of inter-or intra-annual wood sections,
4. semi-automatic sample collection into tin or silver capsules by gravity or forceps, optional chemical treatment (e.g. cellulose extraction) and
5. stable carbon (C) and oxygen (O) isotope analysis via conventional Isotope Ratio Mass Spectrometry (IRMS) coupled online to a combustion or pyrolysis furnace.

UV-laser-based microscopic dissection enables selection of relevant plant cells/tissues on screen by pen, while nonrelevant tissues (e.g. resin ducts, wood rays) may not be selected or

be removed (Fig. 2a,d) prior to sampling. Any size and area can be dissected, which is important for precise dissection of asymmetric tree rings or parts thereof as shown for example by lobate growth (Fig. 2a), intra-ring density fluctuations (Fig. 2b) or wedging tree rings (Fig. 2c). Furthermore, it is possible to cut serial sections or even to pool sample material, for example if the weight of the dissected tissue from one thin section is not sufficient for a stable isotope measurement. Sample material from the same array of wood cells can be identified unambiguously on a second or third cross-section and may be pooled for chemical treatment, like cellulose extraction prior to IRMS analysis (Fig. 1a).

In general, wood samples of 100 μm to 1000 μm thickness can be dissected without any major constraints (Fig. 1b). Dissection samples of up to 1000 μm thickness are achieved by cutting iterations and adjustment of the z-focus of the UV-laser beam. The use of cross-sections thinner than 100 μm normally results in insufficient amount of material for present-day combustion/pyrolysis systems normally coupled to IRMS. Taking thin sections, however, has the advantage that part of the wood sample is left intact, providing the opportunity for reanalysis of ring widths or allowing other investigations as for example quantitative wood anatomy or wood density measurements. Furthermore, the risk of cross contamination of various tissues is diminished, for example when studying narrow tree rings that have non-parallel boundaries and/or have varying fibre and vessel angles (Fig. 1b).

Fig. 2 Schematic examples of different dissected wood tissues from several tree species for cutting process with an UV-laser microscopic dissection system. Nonrelevant tissues (e.g. resin ducts, wood rays) can be removed before stable isotope analysis and any size and area can be dissected. (a) Cross-section of *Pinus sylvestris*. Resin ducts and asymmetric growth rings can be easily dissected; (b) Cross-section of *Pinus sylvestris*. Density fluctuations within tree rings are dissected (nos 2 and 5); (c) Cross-section of *Juniperus excelsa*. Tree rings are marked, including wedging rings; (d) Cross-section of *Quercus robur*. Wood ray (no 1) is marked together with tree rings.

Sample material

In order to test the UV-laser-based microscopic dissection method in practice we focused on three applications. In the first experiment, we tested whether the $\delta^{13}\text{C}$ values in xylem cells of an oak sample (*Quercus robur* L.) differ from the $\delta^{13}\text{C}$ values in ray cells in order to assess potential influences of ray parenchyma on the isotope variability of tree-ring sequences. Therefore, an oak tree was sampled at the Telegrafenberg Hill in Potsdam, northeastern Germany (52°23'N, 13°04'E; 94 m above sea level (a.s.l.)). Germany is characterized by a

warm, temperate and humid climate of the mid-latitudes with weakening oceanic influences from the northwest to the southeast. In the second and third example, we dissected wood tissues to analyse high-resolution intra-annual $\delta^{18}\text{O}$, $\delta^{13}\text{C}$ and wood density data. The material selected comprises wood of coniferous and broad-leaf trees from two study sites differing in climatic conditions. The sampling site of the coniferous tree (*Pinus strobus* L.) is located in St. Arnold, northwestern Germany (52°13'N, 7°23'E; 56 m a.s.l.), having similar climatic conditions as the site of the first example. The sampling site of the broad-leaf tree is located in lowland rain forest in the eastern part of Central Java, Indonesia (07°52'S, 111°11'E; 380 m a.s.l.). An increment core of 5 mm in diameter of a living teak tree (*Tectona grandis* L.) was chosen from a collection that was gathered during a field campaign in 2008 (Schollaen et al. 2013). At this study site, the climate is characterized by a distinct dry season from June to September and a rainy season from October to May (Fig. 5b). The growing season for teak in this region generally lasts from the beginning of October to the end of May. During the dry season the trees are leafless and produce no wood as they are in a state of cambial dormancy (Coster 1928).

Sample preparation

Firstly, increment cores were cut into segments of 5 cm length and secondly, transverse or cross-sections of approximately 500 μm thickness for the teak sample, 350- μm for the oak sample and 1000- μm for the pine sample were cut with a high-precision diamond saw (ISOMED5000, ITW Test & Measurement GmbH, Düsseldorf, Germany) or a core microtome (Gärtner and Nievergelt 2010), respectively. Extractives, such as resins and oils, were removed from the wood cross-sections by boiling them in de-ionized water and ethanol. For further treatment, the cross-sections of resin-extracted wood were fixed between two stainless metal frame slides (Fig. 1a) for use with the Leica system and between two conventional microscope slides (26 x 76 mm, Thermo Scientific, Menzel GmbH, Braunschweig, Germany) for the Zeiss system. Three of these slides, each carrying up to three cross-sections of maximum 6 cm length can be mounted onto the slide holder of each microscopic dissection system. Hence, in total cross-sections from a wood core measuring c. 54 cm in length can be processed by a UV-laser-based dissection system in one operation.

Sample collection

Depending on the thickness of wood cross-sections, samples are viewed under the microscopes in transmitted- or reflected-light mode. Series of tissues of interest are first marked with mouse or screen-pen (Figs 2, 3). Every segment drawn was dissected with the UV-laser beam and collected for the Leica system by gravity into single silver ($\delta^{18}\text{O}$) or tin ($\delta^{13}\text{C}$) capsules standing in a collection holder. For the Zeiss system tissue was taken up with a forceps and put into silver/tin capsules.

In the first experiment on an oak sample (*Q. robur*), resin-extracted wood and cellulose of ray parenchyma and xylem tissue from six (cellulose) and eight (wood) consecutive tree rings were cut, respectively (Fig. 4a). Cellulose extraction was performed according to the standard method as described by Wieloch et al. (2011).

In the second and third experiment, tree-ring parts of interest were graphically subdivided on a pen-screen in radial direction into equidistant parts of 150 μm in width for the teak sample (Fig. 3) and 20 μm in width for the pine sample. Cutting lines were drawn parallel to wood anatomical structures and tree-ring boundaries independent from their shape. The number of segments per year varied depending on the tree-ring width.

Stable isotope analyses

Oxygen isotope ratios were measured using a high temperature TC/EA pyrolysis oven (at 1340°C) coupled online to an Isotope Ratio Mass Spectrometer (IRMS; Delta V Advantage, Thermo Scientific, Bremen, Germany). The carbon isotope ratios were measured by combustion (at 1080°C) using an elemental analyser (Model NA 1500; Carlo Erba, Milan, Italy) coupled online to an IRMS (Isoprime Ltd. Cheadle Hulme, UK). Sample masses of 130-220 μg of resin-extracted wood or cellulose were used for IRMS analyses. Sample replication resulted in a reproducibility of better than $\pm 0.1\text{‰}$ for $\delta^{13}\text{C}$ values and up to $\pm 0.3\text{‰}$ for $\delta^{18}\text{O}$ values. The isotope ratios are given in the delta (δ) notation, relative to the standards VPDB for $\delta^{13}\text{C}$ and VSMOW for $\delta^{18}\text{O}$ (Craig 1957).

Determination of wood density

Wood density of the intra-annually resolved pine sample was precisely calculated from the masses of equidistant subsections of equal volume (20 x 1000 x 4000 μm) that were weighed by a micro balance (AX26 DeltaRange, Mettler Toledo GmbH, Greifensee, Switzerland).

Fig. 3 Microscopic image of a teak (*Tectona grandis*) cross-section (500 μm thickness, magnification x5). Consecutive sections of intra-annual tree-ring parenchyma and xylem tissue marked with numbers for the cutting process with an UV-laser microdissection microscope (LMD7000; Leica). Screen shots before, during and after UV-laser-based microscopic dissection of wood parenchyma band.

Results

Influence of ray parenchyma on the $\delta^{13}\text{C}$ signature in tree rings of oak (*Quercus robur*)

All $\delta^{13}\text{C}$ values from resin-extracted wood ($\delta^{13}\text{C}_{\text{wd}}$, Fig. 4b) of the ray parenchyma were found lower than those of the associated xylem tissue with a mean difference of 0.21‰. This is well above the analytical precision of ± 0.08 ‰. Despite the significant general offset, $\delta^{13}\text{C}$ signals of ray and xylem tissue follow the same year-to-year variability and trend as confirmed by a R^2 of 0.99. Likewise, cellulose $\delta^{13}\text{C}$ values ($\delta^{13}\text{C}_{\text{cel}}$, Fig. 4c) from ray and xylem tissue show the same year-to-year variability. However, the $\delta^{13}\text{C}_{\text{cel}}$ values of ray parenchyma are slightly higher compared to the $\delta^{13}\text{C}_{\text{cel}}$ values of xylem tissue. The mean difference is 0.26‰ with R^2 of 0.96 and analytical precision of ± 0.08 ‰.

Fig. 4 Comparison between $\delta^{13}\text{C}$ values from resin-extracted wood ($\delta^{13}\text{C}_{\text{wd}}$) and cellulose ($\delta^{13}\text{C}_{\text{cel}}$) of ray parenchyma and xylem tissue in tree rings of an oak sample (*Quercus robur*). (a) Microscopic image of an oak cross-section (350-μm thickness) with schematic example of dissected tree-ring xylem and ray tissues for cutting process with the UV-laser-based microscopic dissection method. (b) Tree-ring $\delta^{13}\text{C}$ values ($\delta^{13}\text{C}_{\text{wd}}$) of ray parenchyma and xylem cells from six consecutive tree rings dissected with the UV-laser microscope. (c) Cellulose $\delta^{13}\text{C}$ values ($\delta^{13}\text{C}_{\text{cel}}$) of ray parenchyma and xylem cells from eight consecutive tree rings. The analytical precisions of ± 0.08 ‰ are shown as error bars. The $\delta^{13}\text{C}$ signals of ray and xylem tissue follow the same year-to-year variability and trend, but reveal offsets that are opposite in sign depending on whether wholewood or cellulose is analysed.

High-resolution intra-annual $\delta^{18}\text{O}$ results from teak (*Tectona grandis*)

The intra-annual $\delta^{18}\text{O}$ profiles of a teak tree ($\delta^{18}\text{O}_{\text{wd}}$) from Indonesia show a clear annual cycle (Fig. 5a). Annual wood formation starts with a parenchyma band showing $\delta^{18}\text{O}_{\text{wd}}$ values that are similar to the $\delta^{18}\text{O}_{\text{wd}}$ values at the end of the previous tree ring. Wood formed directly after the parenchyma band is characterized by rapidly rising $\delta^{18}\text{O}_{\text{wd}}$ values up to the seasonal maximum appearing early in the growing season. This $\delta^{18}\text{O}_{\text{wd}}$ maximum is followed by a decline to a seasonal minimum typically in the 2nd third of each tree ring before $\delta^{18}\text{O}_{\text{wd}}$ is marginally rising again in the last third of the growing season. The pattern described is rather consistent in spite of the different numbers of sub-sections per year and follows the annual cycle in rainfall amount and its corresponding isotope signature (Fig. 5b). The high-resolution pattern of the year 1985 does not show distinct high values at the beginning of the growing season and together with the year 1984 two minima during a growing season are revealed. Note, that for tree-ring dating, we followed the convention for the southern hemisphere, which assigns to each tree ring the year in which radial growth begins (Schulman 1956).

Fig. 5 Intra-annual $\delta^{18}\text{O}_{\text{wd}}$ variations from teak (*Tectona grandis*) collected in the eastern part of Central Java, Indonesia and corresponding precipitation data. (a) High-resolution isotope data are plotted vs monthly precipitation sums at the study site (GPCC V6, mean of 9 nearest grid-points, Schneider et al. 2011) and $\delta^{18}\text{O}$ values in precipitation ($\delta^{18}\text{O}_{\text{Pre}}$) of the corresponding year from Jakarta/ Indonesia (IAEA/WMO 2006). Dissected part of parenchyma band is marked as a dashed line. El Niño and La Niña events are highlighted in yellow and blue, respectively (NOAA 2013). (b) Average monthly precipitation sums (bars) for the eastern part of Central Java (GPCC V6, 1901-2010, mean of 9 nearest grid-points, Schneider et al., 2011) and mean $\delta^{18}\text{O}$ in precipitation (line) for Jakarta/ Indonesia (1962-

1998) (IAEA/WMO 2006) are shown. The seasonal $\delta^{18}\text{O}_{\text{wd}}$ pattern reflect the annual cycle of $\delta^{18}\text{O}$ in precipitation and corresponding seasonal changes in the amount of precipitation at the study site (Schollaen *et al.*, 2013). $\delta^{18}\text{O}_{\text{wd}}$ pattern is sensitive to rainfall extremes caused by ENSO events.

High-resolution intra-annual $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and wood density of white pine (*Pinus strobus*)

The $\delta^{13}\text{C}_{\text{wd}}$ profile begins with a downward trend of c. 1‰ during the first third of the tree ring (Fig. 6b). $\delta^{13}\text{C}_{\text{wd}}$ values rise again in the middle part before they increase strongly by >2‰ to a seasonal maximum followed by a sharp and drastic decrease at the very end of the latewood. In contrast, the $\delta^{18}\text{O}_{\text{wd}}$ profile is characterized by a broad peak in the first third of the tree ring, reaching $\delta^{18}\text{O}_{\text{wd}}$ values of >30‰. A second increase, but less pronounced, is indicated for the last part of the ring, with a maximum that is synchronous to the peak in $\delta^{13}\text{C}_{\text{wd}}$. The wood density profile starts with low values and follows synchronously, although attenuated, the increase and decline of $\delta^{18}\text{O}_{\text{wd}}$ within the first half of the tree ring. Starting from a minimum in the middle of the tree ring, wood density increases towards the end of the tree ring. Beside the general increase in density, a few intra-annual density fluctuations (IADFs) are visible. Increasing $\delta^{13}\text{C}_{\text{wd}}$ values are related to these short-term intra-ring density fluctuations with a short time lag. At the end of the growing season all parameters ($\delta^{13}\text{C}_{\text{wd}}$, $\delta^{18}\text{O}_{\text{wd}}$, wood density) reach their seasonal maxima.

The corresponding seasonal courses of temperature, precipitation and relative air humidity for the year 1977 do not deviate strongly from the long-term mean, except for a conspicuous dry period of 3 wk in May (Fig. 6a). Only one strong rainfall event was recorded during this period and relative humidity was persistently below 60%.

Fig. 6 Seasonal variations of wood density and stable isotope ratios ($\delta^{13}\text{C}_{\text{wd}}$, $\delta^{18}\text{O}_{\text{wd}}$) of a pine tree (*Pinus strobus*) from northwestern Germany for 1977 (b) and the corresponding daily temperature, precipitation and relative humidity data (a). Intra-annual density fluctuations (IADFs) are marked with arrows in the photomicrograph. Seasonal moisture signals in $\delta^{18}\text{O}_{\text{wd}}$ are weighted by non-linear intra-annual growth dynamics. DOY, day of year.

Discussion

Ray parenchyma and xylem tissue of deciduous trees show offsets in $\delta^{13}\text{C}$, but same year-to-year variability

Our results exhibit the same year-to-year variability between the $\delta^{13}\text{C}$ signals of ray parenchyma and xylem tissue over 6 and 8 yr, respectively (Fig. 4). The similarity of data from the different woody tissues may indicate that they result from the same physiological and biochemical processes. Of course, during the vegetation period assimilates are used at the same time for vessel-, fibre- and ray parenchyma-formation. However, ray cells live longer, up to several years and thus their structure and chemical composition may change over longer time intervals. Furthermore, woody rays are the main storage organs for non-structural carbohydrates, for example sugars and starch. Accumulation and mobilization of non-structural carbohydrates may be accompanied by increased metabolic activity and respiration of parenchymatic tissue as compared to xylem tissue. Indeed, the offsets found between $\delta^{13}\text{C}$ of ray parenchyma and xylem tissue seem to reflect differences in chemical composition and metabolic activity. Ray parenchyma dissected from resin-extracted wood is depleted in ^{13}C as compared to xylem tissue (Fig. 4b). Our finding confirms a study by Vaganov et al. (2009) using LA-C-GC-IRMS on wood tissue of four consecutive tree rings from a Norway spruce (*Picea abies*) sample. Data revealed a similar ^{13}C -depletion of ray parenchyma of up to 0.23‰ in comparison to tracheids. Vaganov et al. (2009) explain the lower $\delta^{13}\text{C}_{\text{wd}}$ values as a result of continuing incorporation of lignin into the long-lived parenchyma cells. Lignin is generally depleted in ^{13}C by up to 3‰ as compared to cellulose (e.g. Wilson and Grinstead 1977, Loader, Robertson and McCarroll 2003, Harlow, Marshall and Robinson 2006). Hence, a slightly higher content of lignin in ray cells may well cause the observed ^{13}C depletion over xylem tissue in coniferous wood (Vaganov et al. 2009) and in broad-leaf deciduous oak of this study. However, UV-laser-based microscopic tissue dissection allows chemical treatment, like cellulose extraction, prior to IRMS analysis. In contrast to a depletion in ^{13}C of resin-extracted wood we found cellulose of ray parenchyma ($\delta^{13}\text{C}_{\text{cel}}$) to be slightly enriched in ^{13}C when compared to cellulose from xylem tissue. This minor, but apparent enrichment of cellulose of woody rays may be promoted by slightly enhanced catabolic metabolism, i.e. enhanced respiration making ATP available for the biochemical processes of accumulation and mobilization of starch. Gleixner et al. (1993) showed that ‘lighter’ sugar molecules are

preferentially used in catabolic, or respiratory reactions, whereas ‘heavier’ ones are involved in polymerization of cellulose, for example. Hence, an increased carbon isotope partitioning between anabolic and catabolic metabolism in ray parenchyma may result in the observed slight enrichment in $\delta^{13}\text{C}_{\text{Cel}}$ in comparison with xylem tissue. Note, that the differences in $\delta^{13}\text{C}$ between woody rays and xylem tissue may not be constant (Fig.6, Vaganov et al. 2009). Varying cellulose to lignin ratios or respiration rates may cause the observed differences in the offsets in $\delta^{13}\text{C}$ and may also act on longer timescales.

A recent study on ray parenchyma from Spanish juniper (*Juniperus thurifera*) revealed that time series based on the abundance of ray cells reveal complementary climatic signals to those derived from tree-ring width chronologies (Olano et al. 2013). Our results demonstrate that the $\delta^{13}\text{C}$ values of ray parenchyma and xylem tissue contain the same year-to-year variability. Hence, we can assume from this pilot study that the $\delta^{13}\text{C}$ signals of ray cells may not provide additional information in dendroclimatic studies. However, the offsets in $\delta^{13}\text{C}$ between ray cells and xylem are dependent on whether wood or cellulose is analysed. This result indicates the crucial value of the new dissection method as it allows for new approaches towards a better understanding of the physiological processes controlling ray parenchyma and xylem cell formation.

High-resolution $\delta^{18}\text{O}$ values in tree rings of Indonesian teak are sensitive to rainfall extremes caused by ENSO

The seasonal tree-ring $\delta^{18}\text{O}_{\text{wd}}$ pattern in Indonesian teak is hypothesized to reflect the annual cycle of $\delta^{18}\text{O}$ in precipitation and corresponding seasonal changes in the amount of precipitation at the site (Fig. 5). It could be shown, that high $\delta^{18}\text{O}_{\text{wd}}$ values during the start of the growing season represent the $\delta^{18}\text{O}_{\text{Pre}}$ signature of the prior dry season, while lowest tree-ring $\delta^{18}\text{O}$ values reveal the $\delta^{18}\text{O}_{\text{Pre}}$ signature of the main rainy season (Schollaen et al. 2013). Towards the end of the growing season tree-ring $\delta^{18}\text{O}$ values increase again following the $\delta^{18}\text{O}$ -trend of precipitation. The described pattern fits nicely during the years of 1983 and 1986. The low rainfall amount during the dry season of 1983 is caused by an El Niño event (El Niño 1982-83). The two minima in tree-ring $\delta^{18}\text{O}$ values during the year 1984, as well as the missing upward trend at the beginning of the growing season in year 1985 can be explained by rather high rainfall amounts due to an ongoing La Niña phase (blue shaded

periods). This demonstrates that sub-seasonal tree-ring $\delta^{18}\text{O}$ records of Indonesian teak are very sensitive to rainfall extremes caused by ENSO, with high $\delta^{18}\text{O}_{\text{wd}}$ values during El Niño events and low $\delta^{18}\text{O}_{\text{wd}}$ values during La Niña events.

Correlations between $\delta^{18}\text{O}_{\text{wd}}$ and rainfall amount were also found in several other studies on tropical or subtropical trees (e.g. Poussart et al. 2004, Brienen et al. 2012, Sano, Xu and Nakatsuka 2012, Managave et al. 2010b). However, to our knowledge, this is the first time that intra-annual $\delta^{18}\text{O}_{\text{wd}}$ values in tropical trees are shown to reflect the rainfall pattern over an entire year with distinct rainy and dry season signals. This underlines the value of high-resolution intra-seasonal isotope measurements of tropical wood, especially in the light of extreme rainfall events often associated with El Niño and La Niña.

High-resolution stable isotope variations in white pine reflect seasonal changes of climatic conditions weighted by nonlinear growth dynamics

Tree rings are integrating environmental information over the vegetation period. The intra-annual variations in $\delta^{13}\text{C}_{\text{wd}}$, $\delta^{18}\text{O}_{\text{wd}}$ and wood density are mainly driven by interactions between seasonal variation in meteorological conditions, soil water availability and plant response. Specific weather events, such as dry periods are clearly recorded in the seasonal pattern of $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and wood density (Eilmann et al. 2010, e.g. Barbour et al. 2002, Sarris, Siegwolf and Körner 2013). In this study a detailed view of the weather conditions of the year 1977 at our site in northwestern Germany indicates several time periods with no or less rainfall, so called ‘dry’ days (Fig. 6a). The corresponding intra-annual pattern of $\delta^{13}\text{C}_{\text{wd}}$ (Fig. 6b) follows a typical course observed for coniferous species with a gradual increase in $\delta^{13}\text{C}_{\text{wd}}$ to a maximum in latewood followed by a sharp decline at the very end of the tree ring (Schulze et al. 2004, Barbour et al. 2002, Walcroft et al. 1997, cf. Leavitt 1993). Short-term increases in $\delta^{13}\text{C}_{\text{wd}}$ values of the second half of the tree ring were observed after dry periods and are in agreement with the wood density pattern. In contrast, the first dry period in May is not well observed in the $\delta^{13}\text{C}_{\text{wd}}$ pattern. However, this dry period in late spring, characterized by low rainfall as well as low relative humidity, is well represented in the $\delta^{18}\text{O}_{\text{wd}}$ record by a conspicuous broad peak. It seems that $\delta^{18}\text{O}_{\text{wd}}$ is much more sensitive to changes in moisture conditions than $\delta^{13}\text{C}_{\text{wd}}$ and wood density at the beginning of the growing season. During the

second half of the growing season it appears to be *vice versa*, i.e. $\delta^{13}\text{C}_{\text{wd}}$ and wood density reflect moisture deficits better than $\delta^{18}\text{O}_{\text{wd}}$ does.

Furthermore, environmental information seems to be integrated in the tree ring in a non-linear way. The ‘dry’ period of 21 d in May represents approximately only one eighth of the vegetation period, while the $\delta^{18}\text{O}_{\text{wd}}$ peak represents one third of the whole tree-ring width. The ability of a tree ring to integrate environmental information depends on the rate of wood cell formation and the longevity of cells. Within the vegetation period these two features are inversely related (Fig. 7). While thin-walled earlywood cells are formed at a high rate, they may only live for days up to a few weeks. Thick-walled latewood cells are built at very slow rates, but may live up to several months and integrate environmental information over a much longer period of time (Fig. 7a). Hence, environmental conditions reflected by intra-annual tree-ring parameters are weighted by seasonal dynamics of wood formation. Additionally, other seasonal changes related to tree physiological processes such as kind and size of carbohydrate pools (amount of C and O), partitioning between catabolic and anabolic metabolism, as well as metabolic flux rates in conjunction with corresponding isotope fractionations have to be considered (Fig. 7b and e.g. Werner et al. 2012, Gessler et al. 2009, Helle and Schleser 2004a).

Fig. 7 Non-linear seasonal growth increments and longevity of cells during the vegetation period for conifers at temperate sites (a; DOY, day of year). (b) Seasonal dynamics of major factors influencing tree-ring stable isotope ratios of wood cell formation is characterized by increasing cell wall thickness, cell longevity and amount of C/O per cell row (elemental mass/wood density), while cell diameter is decreasing.

Evaluation and comparison of UV-laser-based microscopic dissection systems

The results presented suggest that UV-laser-based microscopic dissection is a very useful method for sampling woody tissue in stable isotope studies. It can be used likewise for dissecting whole tree rings and intra-annual sampling at very high spatial resolution. The two UV-laser microdissection microscopes of Leica (LMD7000, Software Version 6.7.1.3952, Leica Microsystems GmbH, Wetzlar, Germany) and Zeiss (PALM MicroBeam, Software Version 4.5.0.9, Carl Zeiss Microscopy GmbH, Jena, Germany), we tested in these studies, differ concerning their practical implementations and applications (Table 1). The laser from Leica is being moved via optics and the cross-section samples are mounted on a fixed stage. The Leica system uses high-precision optics to steer the laser beam by means of prisms along the desired cut lines on the tissue. As a side effect, the laser can only cut drawn lines or areas marked within the actual microscopic field of view. If larger areas, for example whole tree rings, need to be dissected additional shapes must be defined and additional dissections are required for gathering a single large sample. The dissected sample tissues principally fall down by gravity into collection vessels, e.g. tin or silver capsules. Thus, samples can be prepared directly for conventional autosampler systems coupled to isotope ratio mass spectrometers. An important limitation of the Leica system is the lack of an automatic z-focus adjustment that would allow repeated laser cutting of thicker cross-sections.

Compared to the Leica microscope, the objectives of the Zeiss microscope are installed inversely, which means underneath the sample holder. Hence, tissues of interests are marked via mouse or screen pen on the lower sample side and can be selected beyond the visible screen. Thus, tissues of interests can be marked as big as necessary, since the laser stays fixed while the sample is moved by the high-precision stage during the dissection process. The UV-laser passes through the glass slide and the dissected sample tissues remain in position. After all marked tissue has been dissected specimens are being picked up manually with a forceps and transferred into tin or silver capsules for stable isotope measurements. Dissected sample tissues were too heavy for laser-induced sample transfer that comes with the Zeiss system. The Zeiss system has an automatic z-focus feature that allows for defining the number of automatic cutting iterations. For each cutting cycle the focus of the UV-laser beam will be adjusted by a pre-defined z-focus delta value. As the Zeiss laser is less powerful than the laser from Leica more cutting iterations are generally required. Together with the manual collection of dissected specimens, the overall sampling process with the Zeiss system requires more time

than with the Leica system. On the other hand, the dissection process with the Zeiss can be operated automatically, for example over night. The Leica system requires fulltime presence of the user. Firstly, the adjustment of z-focus of the laser beam has to be done manually. Secondly dissected specimens sometimes tilt, stick and do not fall down into the collection vessels as the cutting line of the laser beam is much narrower than the thickness of the sample.

The use of the UV-laser microdissection microscopes is not necessarily faster than traditional methods for the dissection of wood tissue. In general, the cutting process of selected tissue lasts between 1 to 2 minutes, depending on the size of the selected area, the thickness of the cross section, density of the wood material and the UV-laser settings. If further cutting iterations are required, more time is needed. The average sample throughput per 8-hour day may vary between 20 to 120 samples. This includes the on-screen selection of area, the automatic UV-laser-based microscopic dissection and the collection of specimens, as well as considering unpredictable interferences (e.g. stuck specimens (Leica LMD7000)). With some modification of the current sample collection methods the UV-laser microdissection could also be run automatically over night, which would increase sample throughput drastically.

At this stage, the key advantage using the novel technique is on-screen selection of areas and ultra-high resolution sampling of plant tissue at an unprecedented precision. Furthermore, both systems provide electronic documentation of the dissection processes by photo or video sequences, as well as a report of labeled and dissected elements.

Table 1 Comparison of the characteristics of the two UV-laser-based microscopic dissection systems tested.

Zeiss microdissection microscope (PALM MicroBeam /software v4.5.0.9)	Leica microdissection microscope (LMD7000 /software v6.7.1.3952)
General characteristics	
Laser specifications: Wavelength: 355 nm Max. pulse energy: 100 μ J Laser beam stays fixed, stage is moved	Laser specifications: Wavelength: 349 nm Max. pulse energy: 120 μ J Laser beam is moved, stage stays fixed
Cutting process with different magnifications (e.g. x2.5, x5 or x10)	Cutting process with different magnifications (e.g. x5, x10 or x20)
Best range of sample thickness: 100-1000 μ m	Best range of sample thickness: 100-1000 μ m
Advantages	
Selection of tissue/shape beyond the visible screen	Laser has more power and less cutting iteration needed
Automatic z-focus adjustment/ cutting iterations possible	Fast sample collection process by semi-automatic sample collection into tin or silver capsules by gravity
Better software handling and multifarious choice of drawing tools	Automatic image documentation before and after each dissection by image-database (IM500)
Limitations	
Nonautomatic collection of selected tissues of interests in tin/silver capsules (specimens are being picked up manually with a forceps and transferred into tin/silver capsules)	Lack of automatic z-focus
Laser has less power and frequently cutting iteration needed	Drawing area of interests larger than the field of view is not possible
Nonautomatic image documentation of the laser dissection process	Control check of dissected samples needed after every cutting process as samples may stick and do not fall down automatically

Conclusions

We presented a novel method for dissecting plant tissue in stable isotope studies by using UV-laser-based microscopic dissection. Using two commercially available UV-laser microdissection microscopes, we prepared high-resolution intra-annual wood samples from various tree species of different climate zones for carbon and oxygen isotope analyses with conventional IRMS systems.

The UV-laser-based microscopic dissection enables precise selection of relevant plant cells/tissues on video screen by pen. This procedure allowed us to detect that $\delta^{13}\text{C}$ of xylem cells and woody ray parenchyma of the deciduous species (*Q. robur*) have the same year-to-year variability, but reveal offsets that are opposite in sign depending on whether wholewood or cellulose is analysed. Furthermore, we showed that UV-laser-based microscopic dissection facilitates electronically documented sub-seasonal sampling of tree rings with irregular shapes or narrow ring widths. This is a prerequisite for establishing long and continuous high-resolution isotope chronologies for high-quality climate reconstructions. Single cell rows can be sampled and thus short-term climatic events such as droughts or extensive rainfall events (e.g. caused by ENSO) may be related directly. The three applications demonstrate that the use of this new technique enables the user to sample plant tissue at ultra-high resolution and unprecedented precision.

This new technique opens new ways for studying environmental information in tree rings non-linearly integrated over the vegetation period. The combination of direct growth measurements, which provide exact growth rates of tree rings (e.g. punching method (Rossi, Anfodillo and Menardi 2006, Forster, Schweingruber and Denneker 2000), dendrometers) and precisely selected and dissected specimens may provide a better time-match between high-resolution stable isotope ratios and real-time climate data. Comprehensive eco-physiological process studies on stable isotope signal transfer in the arboreal system can be combined with qualitative and quantitative microscopic investigations on anatomical properties. As wood anatomical properties are climate sensitive (e.g. Olano et al. 2013, Fonti et al. 2010, Liang et al. 2013), more and more studies are attempting to combine quantitative wood anatomy and stable isotope analysis (e.g. Rossi et al. 2013, Ponton et al. 2001, De Micco et al. 2012, Battipaglia et al. 2010). Such investigations may explicitly profit from UV-laser-based microscopic dissection. For instance, detailed assessment of carbon and oxygen isotope

variability in woody parts such as resin ducts, rays, fibres, vessels or parenchyma cells is possible, since they can now be precisely targeted on thin sections by UV the novel method.

The use of UV-laser-based microscopic dissection systems offers new possibilities in view of relating plant structure with plant functioning (derived from isotope ratios) in studies on vulnerability, resilience and adaption of plants to past and present global change. Multidisciplinary analyses (cell structure, wood density and wood chemistry analyses) on the same wood sample are now possible with a complete recovery of the sample due to the use of thin sections.

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References

- Abbott E, Hall D, Hamberger B, Bohlmann J. 2010.** Laser microdissection of conifer stem tissues: Isolation and analysis of high quality RNA, terpene synthase enzyme activity and terpenoid metabolites from resin ducts and cambial zone tissue of white spruce (*Picea glauca*). *BMC Plant Biology* **10**(1): 106.
- Barbour MM, Walcroft AS, Farquhar GD. 2002.** Seasonal variation in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of cellulose from growth rings of *Pinus radiata*. *Plant Cell and Environment* **25**(11): 1483-1499.
- Battipaglia G, De Micco V, Brand WA, Linke P, Aronne G, Saurer M, Cherubini P. 2010.** Variations of vessel diameter and $\delta^{13}\text{C}$ in false rings of *Arbutus unedo* L. reflect different environmental conditions. *New Phytologist* **188**(4): 1099-1112.
- Bayona-Bafaluy M, Blits B, Battersby BJ, Shoubridge EA, Moraes CT. 2005.** Rapid directional shift of mitochondrial DNA heteroplasmy in animal tissues by a mitochondrially targeted restriction endonuclease. *Proceedings of the National Academy of Sciences of the United States of America* **102**(40): 14392-14397.

- Brienen RJW, Helle G, Pons TL, Guyot JL, Gloor M. 2012.** Oxygen isotopes in tree rings are a good proxy for Amazon precipitation and El Niño-Southern Oscillation variability. *Proceedings of the National Academy of Sciences* **109**(42): 16957-16962.
- Coster C. 1928.** Zur Anatomie und Physiologie der Zuwachszonen- und Jahresringbildung in den Tropen. *Ann. Jard. Bot. Buitenzong* **38**: 1-114.
- Craig H. 1957.** Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. *Geochimica et Cosmochimica Acta* **12**: 133-149.
- Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP. 2002.** Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics* **33**: 507-559.
- De Micco V, Battipaglia G, Brand W, Linke P, Saurer M, Aronne G, Cherubini P. 2012.** Discrete versus continuous analysis of anatomical and $\delta^{13}\text{C}$ variability in tree rings with intra-annual density fluctuations. *Trees* **26**(2): 513-524.
- Dodd JP, Patterson WP, Holmden C, Brasseur JM. 2008.** Robotic micromilling of tree-rings: A new tool for obtaining subseasonal environmental isotope records. *Chemical Geology* **252**(1-2): 21-30.
- Ehleringer J, Hall AE, Farquhar G. 1993.** *Stable Isotopes and Plant Carbon-Water Relations*. London: Academic Press.
- Eilmann B, Buchmann N, Siegwolf R, Saurer M, Cherubini P, Rigling A. 2010.** Fast response of Scots pine to improved water availability reflected in tree-ring width and $\delta^{13}\text{C}$. *Plant, Cell and Environment* **33**(8): 1351-1360.
- Evans MN, Schrag DP. 2004.** A stable isotope-based approach to tropical dendroclimatology. *Geochimica et Cosmochimica Acta* **68**(16): 3295-3305.
- Fend F, Raffeld M. 2000.** Laser capture microdissection in pathology. *Journal of Clinical Pathology* **53**(9): 666-672.
- Fichtler E, Helle G, Worbes M. 2010.** Stable-Carbon Isotope Time Series from Tropical Tree Rings Indicate a Precipitation Signal. *Tree-Ring Research* **66**(1): 35-49.
- Fonti P, von Arx G, García-González I, Eilmann B, Sass-Klaassen U, Gärtner H, Eckstein D. 2010.** Studying global change through investigation of the plastic responses of xylem anatomy in tree rings. *New Phytologist* **185**(1): 42-53.
- Forster T, Schweingruber FH, Denneler B. 2000.** Increment puncher - A tool for extracting small cores of wood and bark from living trees. *Iawa Journal* **21**(2): 169-180.
- Gärtner H, Nievergelt D. 2010.** The core-microtome: A new tool for surface preparation on cores and time series analysis of varying cell parameters. *Dendrochronologia* **28**(2): 85-92.
- Gebrekirstos A, Worbes M, Teketay D, Fetene M, Mitlöhner R. 2009.** Stable carbon isotope ratios in tree rings of co-occurring species from semi-arid tropics in Africa: Patterns and climatic signals. *Global and Planetary Change* **66**(3-4): 253-260.

- Gessler A, Brandes E, Buchmann N, Helle G, Rennenberg H, Barnard RL. 2009.** Tracing carbon and oxygen isotope signals from newly assimilated sugars in the leaves to the tree-ring archive. *Plant, Cell & Environment* **32**(7): 780-795.
- Gleixner G, Danier HJ, Werner RA, Schmidt HL. 1993.** Correlations between the ^{13}C content of primary and secondary plant products in different cell compartments and that in decomposing basidiomycetes. *Plant Physiology* **102**: 1287-1290.
- Harlow BA, Marshall JD, Robinson AP. 2006.** A multi-species comparison of $\delta^{13}\text{C}$ from whole wood, extractive-free wood and holocellulose. *Tree Physiology* **26**(6): 767-774.
- Heinrich I, Touchan R, Dorado Liñán I, Vos H, Helle G. 2013.** Winter-to-spring temperature dynamics in Turkey derived from tree rings since AD 1125. *Climate Dynamics* **41**(7-8): 1685-1701.
- Helle G, Schleser GH. 2004a.** Beyond CO_2 -fixation by Rubisco – an interpretation of $^{13}\text{C}/^{12}\text{C}$ variations in tree rings from novel intra-seasonal studies on broad-leaf trees. *Plant, Cell and Environment* **27**(3): 367-380.
- Helle G, Schleser GH 2004b.** Interpreting climate proxies from tree-rings. In: Fischer H, Floeser, G., Kumke, T., Lohmann, G., Miller, H., Negendank, J.F.W., von Storch, H. ed. *The KIHZ project: Towards a synthesis of Holocene proxy data and climate models*, 129-148.
- Hietz P, Turner BL, Wanek W, Richter A, Nock CA, Wright SJ. 2011.** Long-Term Change in the Nitrogen Cycle of Tropical Forests. *Science* **334**(6056): 664-666.
- IAEA/WMO 2006.** Global Network of Isotopes in Precipitation. The GNIP Database. [WWW document] URL <http://www.iaea.org/water>. [accessed 5 August 2013].
- Krepkowski J, Gebrekirstos A, Shibistova O, Bräuning A. 2013.** Stable carbon isotope labeling reveals different carry-over effects between functional types of tropical trees in an Ethiopian mountain forest. *New Phytologist* **199**(2): 431-440.
- Leavitt SW. 1993.** Seasonal $^{13}\text{C}/^{12}\text{C}$ changes in tree rings: species and site coherence, and a possible drought influence. *Canadian Journal of Forest Research* **23**(2): 210-218.
- Leavitt SW, Long A. 1982.** Stable carbon isotopes as a potential supplemental tool in dendrochronology. *Tree-Ring Bulletin* **42**: 49-55.
- Liang W, Heinrich I, Simard S, Helle G, Dorado Liñán I, Heinken T. 2013.** Climate signals derived from cell anatomy of Scots pine in NE-Germany. *Tree Physiology*.
- Loader NJ, Robertson I, McCarroll D. 2003.** Comparison of stable carbon isotope ratios in the whole wood, cellulose and lignin of oak tree-rings. *Palaeogeography, Palaeoclimatology, Palaeoecology* **196**(3-4): 395-407.
- Loader NJ, Switsur VR, Field EM. 1995.** High-resolution stable isotope analysis of tree rings: implications of "microdendroclimatology" for palaeoenvironmental research. *The Holocene* **5**(4): 457-460.

- Managave SR, Sheshshayee MS, Borgaonkar HP, Ramesh R. 2010.** Past break-monsoon conditions detectable by high resolution intra-annual $\delta^{18}\text{O}$ analysis of teak rings. *Geophysical Research Letters* **37**(5): L05702.
- Nelson T, Tausta SL, Gandotra N, Liu T. 2006.** Laser microdissection of plant tissue: What You See Is What You Get. *Annual Review of Plant Biology* **57**(1): 181-201.
- NOAA 2013.** National Weather Service. Historical El Niño/ La Niña episodes (1950-present). [WWW document] URL http://www.cpc.ncep.noaa.gov/products/analysis_monitoring/ensostuff/ensoyears.shtml. [accessed 5 August 2013].
- Ogée J, Barbour MM, Wingate L, Bert D, Bosc A, Stievenard M, Lambrot C, Pierre M, Bariac T, Loustau D, Dewar RC. 2009.** A single-substrate model to interpret intra-annual stable isotope signals in tree-ring cellulose. *Plant, Cell & Environment* **32**(8): 1071-1090.
- Ogle N, McCormac FG. 1994.** High-resolution $\delta^{13}\text{C}$ measurements of oak show a previously unobserved spring depletion. *Geophysical Research Letters* **21**(22): 2373-2375.
- Olano JM, Arzac A, García-Cervigón AI, von Arx G, Rozas V. 2013.** New star on the stage: amount of ray parenchyma in tree rings shows a link to climate. *New Phytologist* **198**(2): 486-495.
- Pons T, Helle G. 2011.** Identification of anatomically non-distinct annual rings in tropical trees using stable isotopes. *Trees - Structure and Function* **25**(1): 83-93.
- Ponton S, Dupouey JL, Breda N, Feuillat F, Bodenes C, Dreyer E. 2001.** Carbon isotope discrimination and wood anatomy variations in mixed stands of *Quercus robur* and *Quercus petraea*. *Plant Cell and Environment* **24**(8): 861-868.
- Poussart PF, Evans MN, Schrag DP. 2004.** Resolving seasonality in tropical trees: multi-decade, high-resolution oxygen and carbon isotope records from Indonesia and Thailand. *Earth and Planetary Science Letters* **218**(3-4): 301-316.
- Roden JS, Johnstone JA, Dawson TE. 2009.** Intra-annual variation in the stable oxygen and carbon isotope ratios of cellulose in tree rings of coast redwood (*Sequoia sempervirens*). *The Holocene* **19**(2): 189-197.
- Rossi L, Sebastiani L, Tognetti R, d'Andria R, Morelli G, Cherubini P. 2013.** Tree-ring wood anatomy and stable isotopes show structural and functional adjustments in olive trees under different water availability. *Plant and Soil* **372**(1-2): 567-579.
- Rossi S, Anfodillo T, Menardi R. 2006.** Trephor: A new tool for sampling microcores from tree stems. *Iawa Journal* **27**(1): 89-97.
- Sano M, Xu C, Nakatsuka T. 2012.** A 300-year Vietnam hydroclimate and ENSO variability record reconstructed from tree ring $\delta^{18}\text{O}$. *Journal of Geophysical Research* **117**(D12): D12115.

- Sarris D, Siegwolf R, Körner C. 2013.** Inter- and intra-annual stable carbon and oxygen isotope signals in response to drought in Mediterranean pines. *Agricultural and Forest Meteorology* **168**(0): 59-68.
- Schleser GH, Helle G, Lucke A, Vos H. 1999.** Isotope signals as climate proxies: the role of transfer functions in the study of terrestrial archives. *Quaternary Science Reviews* **18**(7): 927-943.
- Schneider U, Becker A, Finger P, Meyer-Christoffer A, Rudolf B, Ziese M. 2011.** GPCC Full Data Reanalysis Version 6.0 at 0.5°: Monthly Land-Surface Precipitation from Rain-Gauges built on GTS-based and Historic Data.
- Schollaen K, Heinrich I, Neuwirth B, Krusic PJ, D'Arrigo RD, Karyanto O, Helle G. 2013.** Multiple tree-ring chronologies (ring width, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) reveal dry and rainy season signals of rainfall in Indonesia. *Quaternary Science Reviews* **73**(0): 170-181.
- Schubert BA, Jahren AH. 2011.** Quantifying seasonal precipitation using high-resolution carbon isotope analyses in evergreen wood. *Geochimica et Cosmochimica Acta* **75**(22): 7291-7303.
- Schulman E. 1956.** *Dendroclimatic Change in Semiarid America*. Tuscon, Arizona: University of Arizona Press.
- Schulze B, Wirth C, Linke P, Brand WA, Kuhlmann I, Horna V, Schulze ED. 2004.** Laser ablation-combustion-GC-IRMS - a new method for online analysis of intra-annual variation of delta ^{13}C in tree rings. *Tree Physiology* **24**(11): 1193-1201.
- Sheppard PR, Witten ML. 2005.** Laser Trimming Tree-Ring Cores for Dendrochemistry of Metals. *Tree-Ring Research* **61**(2): 87-92.
- Skomarkova MV, Vaganov EA, Mund M, Knohl A, Linke P, Boerner A, Schulze ED. 2006.** Inter-annual and seasonal variability of radial growth, wood density and carbon isotope ratios in tree rings of beech (*Fagus sylvatica*) growing in Germany and Italy. *Trees-Structure and Function* **20**(5): 571-586.
- Treydte KS, Schleser GH, Helle G, Frank DC, Winiger M, Haug GH, Esper J. 2006.** The twentieth century was the wettest period in northern Pakistan over the past millennium. *Nature* **440**(7088): 1179-1182.
- Vaganov E, Schulze E-D, Skomarkova M, Knohl A, Brand W, Roscher C. 2009.** Intra-annual variability of anatomical structure and $\delta^{13}\text{C}$ values within tree rings of spruce and pine in alpine, temperate and boreal Europe. *Oecologia* **161**(4): 729-745.
- Verheyden A, Helle G, Schleser GH, Dehairs F, Beeckman H, Koedam N. 2004.** Annual cyclicity in high-resolution stable carbon and oxygen isotope ratios in the wood of the mangrove tree *Rhizophora mucronata*. *Plant Cell and Environment* **27**(12): 1525-1536.
- Walcroft AS, Silvester WB, Whitehead D, Kelliher FM. 1997.** Seasonal Changes in Stable Carbon Isotope Ratios within Annual Rings of *Pinus radiata* Reflect Environmental Regulation of Growth Processes. *Functional Plant Biology* **24**(1): 57-68.

- Werner C, Schnyder H, Cuntz M, Keitel C, Zeeman MJ, Dawson TE, Badeck F-W, Brugnoli E, Ghashghaie J, Grams TEE, Kayler ZE, Lakatos M, Lee X, M'aguas C, Ogée J, Rascher KG, Siegwolf RTW, Unger S, Welker J, L.Wingate, Gessler A. 2012.** Progress and challenges in using stable isotopes to trace plant carbon and water relations across scales. *Biogeosciences* **9**: 3083–3111.
- West JB, Bowen GJ, Dawson TE, Tu KP. 2010.** *Isoscapes: Understanding movement, pattern, and process on Earth through isotope mapping* London: Springer Science+Business Media B.V.
- Wieloch T, Helle G, Heinrich I, Voigt M, Schyma P. 2011.** A novel device for batch-wise isolation of α -cellulose from small-amount wholewood samples. *Dendrochronologia* **29**(2): 115-117.
- Wilson AT, Grinsted MJ. 1977.** $^{12}\text{C}/^{13}\text{C}$ in cellulose and lignin as palaeothermometers. *Nature* **265**: 133-135.
- Wurster CM, Patterson WP, Cheatham MM. 1999.** Advances in micromilling techniques: a new apparatus for acquiring high-resolution oxygen and carbon stable isotope values and major/minor elemental ratios from accretionary carbonate. *Computers & Geosciences* **25**(10): 1159-1166.
- Ziegler H 1995.** Stable Isotopes in Plant Physiology and Ecology. In: Behnke HD, Lüttge U, Esser K, Kadereit J, Runge M eds. *Progress in Botany*: Springer Berlin Heidelberg, 1-24.