

EXAMINATION OF STABLE CARBON AND OXYGEN ISOTOPIC VARIABILITY IN THE CELLULOSE AND WHOLEWOOD OF *Castanea sativa* Mill., *Fagus sylvatica* L., *Pinus sylvestris* L. AND *Quercus robur* L.

ANALIZA VARIABILNOSTI STABILNIH IZOTOPOV OGLJIKA IN KISIKA V CELULOZI IN LESU BRANIK PRAVEGA KOSTANJA (*Castanea sativa* Mill.), BUKVE (*Fagus sylvatica* L.), RDEČEGA BORA (*Pinus sylvestris* L.) IN DOBA (*Quercus robur* L.)

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ABSTRACT

This paper presents results from a comparison of wholewood and cellulose carbon and oxygen isotope ratios for four UK tree species. These species occur within the historic buildings archive as both primary and supplementary construction materials and have been used to reconstruct the climate of the past. New advances in the application of stable isotopes have widened the scope of the isotope approach, but require the time-consuming purification of cellulose. Comparison of the oxygen and carbon isotope signals preserved in the wood and cellulose components confirms and builds upon previous research in this field and provides additional insight into the covariance of these two sample types between species, and the potential to employ wood isotope analysis for both pre-screening trees for palaeoclimatology and chronology research.

Key words: stable isotopes, tree ring, dendrochronology, dendroclimatology

IZVLEČEK

V prispevku so predstavljeni rezultati primerjave analize stabilnih izotopov ogljika in kisika v lesu in celulozi drevesnih branik štirih najpogostejših drevesnih vrst v Veliki Britaniji: pravega kostanja, bukve, doba in rdečega bora. Les teh drevesnih vrst pogosto najdemo tudi v različnih lesenih konstrukcijah, kar nam, v kombinaciji z lesom iz živih dreves, omogoča rekonstrukcijo klime v čas pred instrumentalnimi meritvami. Razvoj detekcijskih tehnik za stabilne izotope je omogočil uporabo stabilnih izotopov tudi tam, kjer to tradicionalno ni bilo možno (npr. za datiranje starih stavb), še vedno pa je glavna ovira za širšo uporabo teh metod zahtevna in zamudna ekstrakcija celuloze iz posameznih lesnih branik. Primerjava stabilnih izotopov kisika in ogljika v lesu in (alfa)-celulozi je potrdila dosedanje vedenje in dodala nova spoznanja o primerljivosti razmerij stabilnih izotopov, analiziranih v lesu in celulozi, kar nam bi lahko omogočilo izločitev zamudne tehnike ekstrakcije celuloze in neposredno uporabo homogeniziranega lesa branik za analizo stabilnih izotopov ogljika in kisika v branikah za potrebe paleoklimatologije in historične dendrokronologije.

Ključne besede: stabilni izotopi, drevesne branike, dendrokronologija, dendroclimatologija, Velika Britanija

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1 INTRODUCTION

1 UVOD

Choosing the most appropriate wood component for isotopic analysis in tree-ring research is not a trivial matter. Factors such as research objective, cost, preparation time, sample size, intra- and inter-tree variability and the stability of the signal through time (i.e. the propensity for diagenesis to affect inter-annual variability) all play a role in the sampling protocols adopted and influence experimental design (Borella et al., 1999; McCarroll & Loader 2004; Loader et al., 2003).

Recent developments in the use of stable isotopes for dating historic building timbers in Europe (Loader et al. in review) offer the potential for dating samples previously considered undateable by dendrochronology alone (Caitling 2018; Loader et al. in review). At present this work is conducted using cellulose extracted from the wholewood of each tree ring. However, cellulose extraction can be time consuming and requires specialist equipment (McCarroll et al. 2004). If the isotopic signal contained within wholewood can be demonstrated to be similar enough to that of cellulose to

preserve a sufficiently useful signal, then this may provide a means for rapid testing or pre-screening of cores for isotope dating or dendroclimatology prior to more labour intensive cellulose-based analyses (Loader et al. 2008).

Several early studies utilized unprocessed wholewood (e.g. Farmer et al., 1974); however, Wilson and Grinsted (1977) demonstrated that different wood components differed significantly in their isotopic value within a single ring. This observation, coupled with the clearer mechanistic understanding of the timing and controls of cellulose formation in tree rings, resulted in a move away from wholewood analysis for palaeoclimate and plant physiological analyses. However, over the past few decades the benefits of using cellulose over wholewood have been periodically revisited, as the task of cellulose extraction is somewhat time consuming and labour intensive (Alessandro et al., 2004; Borella et al., 1999; McCarroll & Loader 2004; Loader et al., 2003). Even advances in batch processing, and more recently, the cellulose extraction of intact cores/laths is far from straightforward and may only be appropriate for specific tree types (Loader et al., 1997; 2002; Kagawa et al., 2015; Helle et al., 2004). Studies addressing the issues of wholewood and cellulose have generally found a strong correlation between wholewood and cellulose in both angiosperm and gymnosperm trees (Borella et al., 1998, 1999; Barbour et al., 2001; Loader et al., 2003, 2017; Alessandro et al., 2004; McCarroll et al., 2004; Cullen et al., 2006; Sidorova et al., 2008; Szymczak et al., 2011; Schleser et al., 2015). However, some studies have highlighted isotopic variability on both short and long time scales, which may represent cellulose recording some aspects of climate which wholewood analysis obscures. This could have implications for the veracity of any resulting climate reconstructions, based upon the isotopic analysis of wholewood rather than cellulose (Barbour et al. 2001, Borella et al., 1999; Cullen et al., 2005; Szymczak et al., 2011; Schleser et al., 2015). These results have been questioned by papers suggesting that the extraction of cellulose is an unnecessary step, and also by those suggesting that wholewood may hold additional, or different, environmental information than that attainable from cellulose alone (Barbour et al. 2001; Borella et al., 1998; Loader et al., 2003; Alessandro et al., 2004; McCarroll and Loader 2004).

There is little doubt that, for successful dendroclimatology, it is essential that the sample signal is stable through time and unambiguously linked to the tree ring under analysis (McCarroll and Loader 2004). This is most unambiguously achieved, by analysing a

single component (typically α -cellulose) from dated tree rings. However, more recent developments, which capitalise upon the inter-annual coherence in stable oxygen isotopes to date tree ring sequences, provide new scope for re-evaluation of wholewood/cellulose variability with this specific application in mind (Loader et al. *In review*). This pilot study, which builds upon an existing body of similar studies, examines four European tree species found in the UK dendrochronological (historic building) arena; English oak (*Quercus robur* L.), European beech (*Fagus sylvatica* L.), Sweet Chestnut (*Castanea sativa* Mill.), and Scots pine (*Pinus sylvestris* L.). The study will investigate the extent to which observed isotopic differences between cellulose and wholewood are present and consistent in the tree species analysed. If both the cellulose and wholewood oxygen holds the same, or a very similar signal, as have been reported elsewhere for carbon isotopes (Borella et al. 1998, 1999; Loader et al. 2008), then this may widen the scope of the isotopic dating technique and provide a means for pre-screening samples, prior to more resource-intensive cellulose preparation.

2 MATERIALS AND METHODS

2 MATERIALS IN METODE

Wood samples were collected from living trees growing in Broadmoor woods, southern England, UK (51.38°N, -0.74°E) (Figure 1). This woodland contains a mosaic of broadleaved stands and conifer plantations, allowing for the collection of the *Castanea sativa* (sweet chestnut), *Fagus sylvatica* (European beech), *Pinus sylvestris* (Scots pine), and *Quercus robur* (English oak) used in this study. All trees were located on sandy, moderately well-draining soils overlaying free-draining Eocene gravels of the Camberley Formation (Bracklesham group). Sampled trees were located towards the base of shallow slopes. 12 mm diameter cores were obtained using an increment borer at a height of c. 1.1 m. One core was taken per species, samples were air-dried and sanded to enable clear ring identification. Each sample was measured to an accuracy of 0.01 mm using a horizontally-travelling Velmex™ measuring stage, under binocular magnification. The tree ring width data were statistically cross-dated (synchronised) using the Time Series Analysis and Presentation (TSAP™) software. Ring separation for isotopic analysis was conducted using scalpel and microscope. Earlywood and latewood were separated in oak and chestnut, due to their ring-porous nature, and therefore the potential for signal carry-over from the previous year in the earlywood (Switsur et al., 1995). The separation of earlywood and latewood was

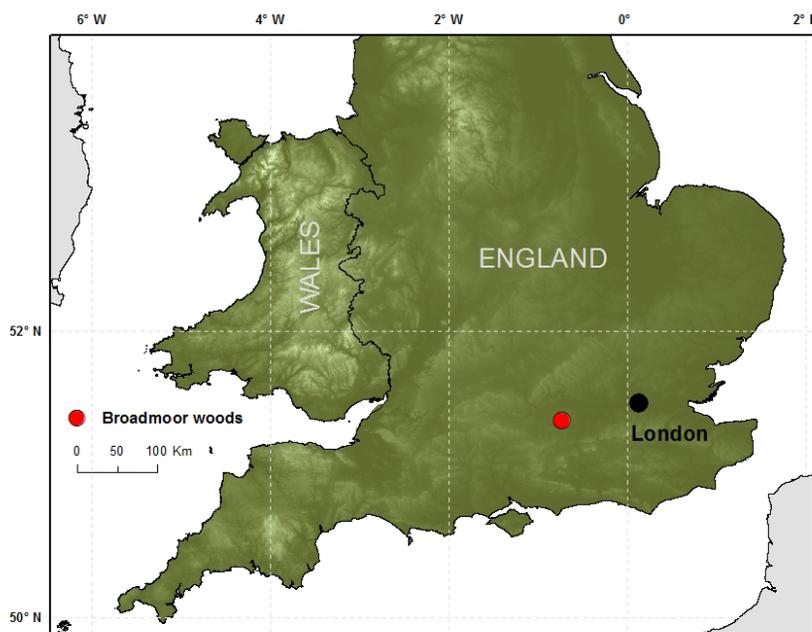


Fig. 1: Map of the southern UK showing the location of the Broadmoor woods (red circle) from where samples were collected for this study.

deemed unnecessary in pine due to the coherence of their isotopic ratios (Kress et al., 2009). Due to the diffuse porous wood anatomy of beech, separation was also considered unnecessary (Schleser et al., 2015). Sample material was limited and so it was not possible to cut and mill the wholewood and to have sufficient material remaining for cellulose analysis. To overcome this limitation a small number of wholewood slivers were removed from the cut sample for wholewood isotope analysis. Wood samples 0.30–0.35 mg were then sub-sampled from these wood slivers and weighed into silver capsules. Significant intra-ring variability has been previously reported and we recognise that this sub-sampling strategy, forced by material availability is sub-optimal. A conscious effort was therefore made, to include parts of different shavings for each year to obtain a value more broadly representing the isotopic ratio of the whole ring. In addition, ten sub-samples were taken from a single year from both the wholewood and cellulose samples to demonstrate the intra-annual variability of the wholewood and allow for the error of this sub-sampling strategy to be assessed.

Cellulose samples were prepared using standard batch methods (after Loader et al., 1997), followed by homogenisation using an ultrasonic probe and freeze drying. Cellulose samples were weighed using an identical protocol to the wood sub-samples, including replication to demonstrate isotopic variability. Both

Slika 1: Karta južnega dela Velike Britanije z vrisano lokacijo mesta vzorčenja – Broadmoor woods (rdeči krog)

cellulose and wholewood samples were converted to carbon monoxide (CO) by high temperature pyrolysis over glassy carbon at 1400 °C using a Flash HT elemental analyser and the stable carbon and oxygen isotopic ratios measured simultaneously using a Thermo Delta V isotope ratio mass spectrometer. Results are expressed using the delta notation as per mille deviations from the VPDB and VSMOW standards (Coplen, 1995). Analytical precision for repeat analysis of standard (Sigma) cellulose for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ is typically ± 0.1 ‰, and ± 0.3 ‰ (σ_{n-1}) (Sigma Aldrich, UK. No. C-8002 Lot. 92F-0243) (Woodley et al., 2012, Loader et al. 2015). This “standard” analytical uncertainty should be considered when comparing standard deviations in carbon and oxygen isotopes, in the context of the results and may play a role in the lower than expected r values for the $\delta^{18}\text{O}$ series of the less-homogenous wood chips (see Tables 1 & 2). Although not a climate study, carbon isotopes were corrected (after McCarroll and Loader 2004) for changes in the carbon isotopic value of the CO_2 used by the plant for photosynthesis, as this has been progressively modified through time since the onset of global industrialisation (the atmospheric $\delta^{13}\text{C}$ “Suess” effect). Employing this adjustment reduces the risk of artificial inflation of correlation arising from the industrial $\delta^{13}\text{C}$ decline in atmospheric CO_2 post-industrialisation which dominates the stable carbon isotope signal.

3 RESULTS AND DISCUSSION

3 REZULTATI IN RAZPRAVA

3.1 Intra-annual isotopic variability

3.1 Intraannualna (znotrajletna) variabilnost stabilnih izotopov

A possible consequence of the wholewood sampling strategy which measured only a small part of the tree-ring rather than an homogenous mix from the entire ring, is that the resulting isotopic value would likely exhibit greater variability, relative to the cellulose samples. This in turn could influence the degree of coherence between the two sampled media. Table 1 displays intra-annual variability for a single year of each species. Values differ between species and without additional trees; it is not possible to determine whether intra-annual isotopic variability differs systematically between tree species, or whether these results are indicative of specific tree or ring properties. Results do however demonstrate that, as anticipated, the unhomogenized (wood chip) samples show significantly higher variability in all cases. This is most clearly demonstrated in chestnut, with $\delta^{18}\text{O}$ having a standard deviation of 1.14 ‰ in wholewood compared to 0.35 ‰ in $\delta^{18}\text{O}$ cellulose (Table 1)(Borella et al. 1998). Furthermore, all samples displayed at least double the variability in wholewood compared to cellulose in $\delta^{18}\text{O}$, indicating that the lack of homogenization has a clear

effect on accuracy. This relationship is similar for $\delta^{13}\text{C}$, however, to a significantly lesser extent, with the difference between wholewood and cellulose variability in chestnut only 0.12 ‰ compared to 0.78 ‰ in $\delta^{18}\text{O}$.

The intra-annual variability found in the $\delta^{18}\text{O}$ of this study are comparable to those found in other works such as; 0.8–3 ‰ (Roden et al., 2009) and 0.5–1.8 ‰ (Helle et al., 2004). However, $\delta^{13}\text{C}$ intra-annual variability of 0.23–0.36 ‰ is markedly lower than the results reported by Roden et al. (2009), which showed an increased variability of 2 ‰ compared to the $\delta^{18}\text{O}$ of the same tree.

3.2 Wholewood and cellulose analyses

3.2 Analiza lesa in celuloze

For all four species studied, a significant Pearson's r correlation of $p < 0.01$ was demonstrated between cellulose and wholewood for both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, with average offsets varying considerably between isotopes and species. Table 2 displays average offset between cellulose and wholewood, as well as the Pearson's r -values and the significance of correlation between cellulose and wholewood. We observed that for $\delta^{18}\text{O}$ wholewood is isotopically depleted (less of the heavier isotope) by 5.46–4.33 ‰ in comparison to cellulose, these results are similar to those established by the literature, with offsets of 4.3 ‰ (Borella et al., 1999),

Table 1: Results obtained from the repeat isotopic of manually-sampled wood chips and alpha-cellulose prepared from a single tree-ring and run to explore intra-annual isotopic variability in $\delta^{18}\text{O}$ & $\delta^{13}\text{C}$ wholewood and cellulose analysis (Standard deviation (σ_{n-1}), $n=10$ per species and sample type are presented).

Species Drevesna vrsta	$\delta^{18}\text{O}$ σ_{n-1} (‰) Wholewood / Les	$\delta^{18}\text{O}$ σ_{n-1} (‰) Cellulose / Celuloza	$\delta^{13}\text{C}$ σ_{n-1} (‰) Wholewood / Les	$\delta^{13}\text{C}$ σ_{n-1} (‰) Cellulose / Celuloza
Oak / <i>dob</i>	0.67	0.29	0.23	0.12
Chestnut / <i>pravi kostanj</i>	1.14	0.35	0.36	0.14
Pine / <i>rdeči bor</i>	0.51	0.25	0.29	0.14
Beech / <i>bukev</i>	0.72	0.26	0.28	0.10

Preglednica 1: Rezultati ponovljenih meritev stabilnih izotopov vzorcev lesa in alfa celuloze iz ene drevesne branike z namenom preučiti intra-annualno variabilnost stabilnih izotopov ogljika $\delta^{13}\text{C}$ in kisika $\delta^{18}\text{O}$ v lesu in celulozi (standardni odklon (σ_{n-1}), $n=10$ vzorcev na drevesno vrsto in tip vzorca (les / celuloza)).

Table 2: Pearson's correlation coefficients (r) between wholewood and cellulose and average offset between the two (wholewood and cellulose) constituents for each tree species.

Species Drevesna vrsta	$\delta^{18}\text{O}$ (r)	Average Offset (‰) Povprečni odklon (‰)	$\delta^{13}\text{C}$ (r)	Average Offset (‰) Povprečni odklon (‰)
Oak / <i>dob</i>	0.60**	4.50	0.81**	-0.62
Chestnut / <i>pravi kostanj</i>	0.42**	5.46	0.89**	-0.46
Pine / <i>rdeči bor</i>	0.51**	4.54	0.82**	+0.30
Beech / <i>bukev</i>	0.74**	4.33	0.90**	-0.34

Preglednica 2: Pearsonovi korelacijski koeficienti med razmerjem stabilnih izotopov ogljika in kisika v lesu in celulozi glede na drevesno vrsto

** Significance / Značilnost 99%, *Significance / Značilnost 95%

3.6 ‰ for oak, 3.9 ‰ pine (Barbour et al., 2001) and 4.8 ± 0.72 ‰ (Szymczak et al., 2011). Although slightly higher levels of isotopic depletion were observed in this study, they are in general agreement with the literature.

Average offsets are markedly lower for $\delta^{13}\text{C}$; -0.62 to -0.34 ‰, when compared with results from the literature and our $\delta^{18}\text{O}$ results, with the offset for pine at $+0.3$ relative to (non-resin extracted) wholewood. Previous work has demonstrated $\delta^{13}\text{C}$ offsets with $\delta^{13}\text{C}$ wholewood depletion of 1.17 ± 0.4 ‰ (Cullen et al., 2006), 1.20 ± 0.50 ‰ (Szymczak et al., 2011), 1.50 ± 0.36 ‰ (Schleser et al., 2015) and c. 1.2 ‰ (Loader et al., 2003). These studies examined several species of pine and oak, as well as the tropical wood *Cariniana micrantha* and produced considerably higher offsets than those obtained in this study. The isotopic enrichment (more of the heavier isotope) between wood and cellulose in pine could be attributed to the lack of resin extraction for the *Pinus* samples or/and high intra-annual isotopic variability in the wholewood of the rings subsampled in this study. Pine would be especially affected due to its resinous nature, and this may be the reason for the carbon isotopic enrichment of cellulose in its result (Rinne et al., 2005).

Across all species and isotopes, significant correlations ($p < 0.01$) were found between wholewood and

cellulose, with especially high r values of between r 0.81–0.90 found for $\delta^{13}\text{C}$. The results of this study are generally comparable with the existing literature, with $\delta^{13}\text{C}$ values: $r = 0.98$, $p < 0.001$ (Loader et al., 2003); $r = 0.64$, $p < 0.001$ (Cullen et al., 2006); $r = 0.71$, $p < 0.01$ (Szymczak et al., 2011); and $r = 0.96$, $p < 0.01$ (Schleser et al., 2015). These findings were split over several species including oak, *Cariniana micrantha* and pine, agreeing with our data in showing that the correlation between cellulose and wholewood is not restrained by species. Furthermore, the significance of correlations found in this studies $\delta^{13}\text{C}$ series and their comparability to the literature suggest that even using a simple wholewood preparation techniques, cogent results can be produced. Pearson's correlation (r) values for $\delta^{18}\text{O}$ calculated in this study were found to be between $r = 0.42$ and 0.74 ($p < 0.01$), with all species showing significant correlations between cellulose and wholewood. Our results are comparable with those found in the existing literature: (Cullen et al., 2006) $r = 0.68$, $p < 0.001$; (Szymczak et al., 2011) $r = 0.77$, $p < 0.01$; (Barbour et al., 2001) $r = 0.79$, $p < 0.001$. These studies were conducted on oak and chestnut series, but as earlier suggested, the wholewood and cellulose relationship does not seem to be significantly impacted by differences in species. The isotopic timeseries for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ are displayed in Figures 2 and 3, which demon-

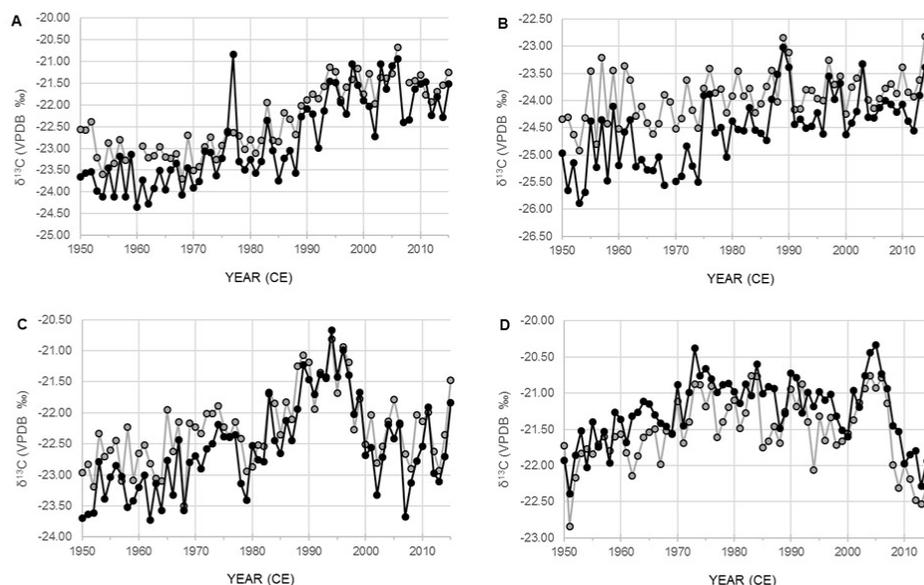


Fig. 2: Stable carbon isotope time series ($\delta^{13}\text{C}$ VPDB) 1950 – 2015. (Cellulose: black line filled circles, wholewood: grey line and open circles). Chestnut (A), Oak (B), Beech (C) and Pine (D)

Slika 2: Časovne vrste stabilnega izotopa ogljika ($\delta^{13}\text{C}$ VPDB) za obdobje 1950 – 2015 (celuloza – črna črta, črni krogi, les – siva črta, beli krogi), pravi kostanj (A), dob (B), bukev (C) in rdeči bor (D)

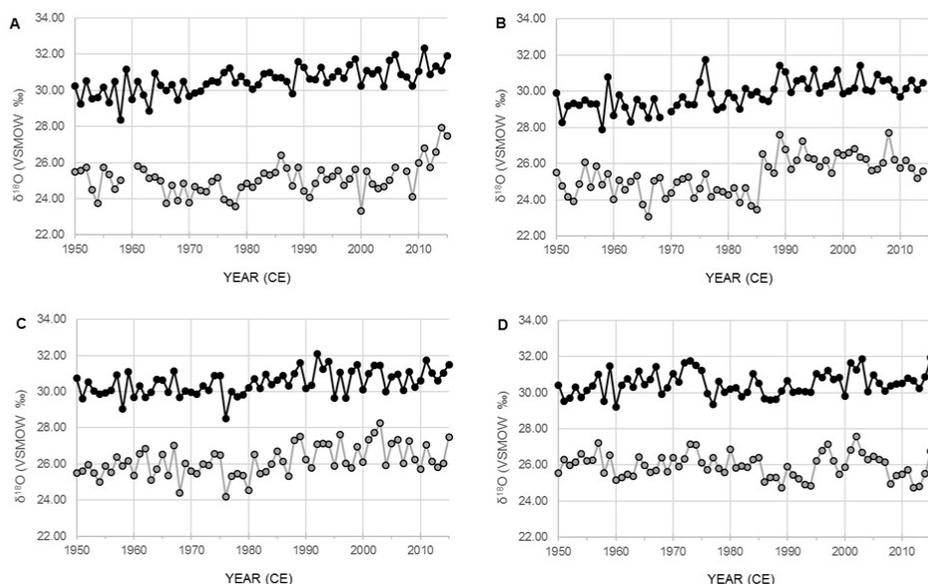


Fig. 3: Stable oxygen isotope time series ($\delta^{18}\text{O}$ VSMOW) 1950 – 2015. (Cellulose: black line filled circles, wholewood: grey line and open circles). Chestnut (A), Oak (B), Beech (C) and Pine (D).

strate the variability in offsets between isotopes and correlations between wood constituents. Although $\delta^{18}\text{O}$ correlations are significant ($p < 0.01$) they are generally lower compared to $\delta^{13}\text{C}$, suggesting the preservation of the different isotopic signals varies between wood constituents. We recommend conducting further investigations into this result if oxygen isotopes in wholewood are to be analysed for dating or climate research using this rudimentary approach, as the correlation (r) values are markedly lower than those produced by previous studies (Barbour et al., 2001; Cullen et al., 2006; Szymczak et al., 2011).

4 CONCLUSION

A study comparing the inter-annual coherence of carbon and oxygen isotopes measured on the wholewood and cellulose of four tree species (*Castanea sativa* Mill., *Fagus sylvatica* L., *Pinus sylvestris* L. and *Quercus robur* L.) was conducted. Significant statistical correlation ($p > 0.01$) were observed between wholewood and cellulose for each of the four European tree species examined. We demonstrated that even using unhomogenised wholewood, significant correlation can be found with cellulose from the same tree rings and that this relationship was preserved across species. This relationship was stronger with $\delta^{13}\text{C}$ than $\delta^{18}\text{O}$. Expected wholewood isotopic depletion (in ^{18}O and ^{13}C), relative

Slika 3: Časovne vrste stabilnega izotopa kisika ($\delta^{18}\text{O}$ VSMOW) za obdobje 1950 – 2015 (celuloza – črna črta, črni krogi, les -siva črta, beli krogi), pravi kostanj (A), dob (B), bukev (C) in rdeči bor (D)

to cellulose, was observed in the oxygen and carbon isotopic series. Our findings therefore support those of a number of similar studies. $\delta^{13}\text{C}$ wholewood does not appear to carry similar enough signal to cellulose to be suitable for correlative dating (Loader et al., in review). For oxygen however, correlation between cellulose and wholewood, using our rudimentary approach for sampling wholewood, were lower than for carbon, suggesting that $\delta^{18}\text{O}$ in our sampled wholewood did not reflect the signal preserved in cellulose as faithfully, or that a more homogeneous wholewood sample is required. The results gathered from this study suggest that for specific applications, $\delta^{13}\text{C}$ measured on wholewood may be sufficient for screening non-resinous species, providing a time efficient and cost effective alternative to cellulose isolation. However for $\delta^{18}\text{O}$, additional comparative studies focussing upon the preparation of homogenous wholewood samples are likely to be required.

4 ZAKLJUČKI

V študiji smo primerjali meritve razmerja stabilnih izotopov ogljika in kisika, merjenih v lesu branike in celulozi, ekstrahirani iz istih branik. Analiza je bila opravljena na lesu štirih drevesnih vrst – pravemu kostanju (*Castanea sativa* Mill.), bukvi (*Fagus sylvatica* L.), rdečem boru (*Pinus sylvestris* L.) in dobu (*Quercus*

robur L.). Korelacijska analiza razmerja stabilnih izotopov v lesu in celulozi iz iste branike je bila statistično značilna za vse štiri drevesne vrste. Uporaba nehomogeniziranih vzorcev lesa, ločeno po drevesnih vrstah, je pokazala, da so bili rezultati do neke mere primerljivi z rezultati meritev stabilnih izotopov v celulozi. Povezava med razmerjem stabilnih izotopov v lesu in celulozi je bila močnejša pri stabilnem izotopu ogljika ($\delta^{13}\text{C}$) kot pri kisiku ($\delta^{18}\text{O}$). Pričakovano osiromašenje (ang. depletion) razmerja stabilnih izotopov ogljika in kisika v lesu v primerjavi s celulozo je bilo opaženo v vseh izotopskih kronologijah ogljika in kisika. Rezultati te študije zato podpirajo rezultate drugih podobnih študij. Zdi se, da razmerje stabilnega izotopa ogljika v lesu ni dovolj podobno signalu v celulozi, da bi bil lahko primeren za primerjalno datiranje (Loader et al., v recenziji).

Za stabilni izotop kisika je bila korelacija med razmerjem stabilnega izotopa kisika v celulozi in nehomogeniziranem lesu nižja kot pri stabilnem izotopu ogljika. To pomeni, da stabilni izotop kisika v lesu nima enakega signala kot celuloza, zato (1) ni možno preskočiti faze ekstrakcije celuloze, ali pa (2) da je treba les za analizo stabilnega izotopa kisika homogenizirati.

Rezultati te pilotne študije nakazujejo, da bi lahko v raziskavah, kjer nas zanimajo meritve stabilnega izotopa ogljika, potencialno uporabili tudi nehomogeniziran les branike, še posebej če gre za drevesne vrste, ki nimajo smole (npr. listavci). Tako bi lahko preskočili zamudno in zahtevno ekstrakcijo celuloze iz lesnih branik. V primerih, ko nas zanima razmerje stabilnega izotopa kisika v branikah, pa je treba uporabiti bodisi homogeniziran les branike bodisi napraviti ekstrakcijo celuloze, kajti signal v nehomogeniziranem lesu branike in v celulozi ni enak.

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