

ABSENCE OF JUVENILE EFFECTS CONFIRMED IN STABLE CARBON AND OXYGEN ISOTOPES OF EUROPEAN LARCH TREES

IZOSTANEK JUVENILNEGA EFEKTA POTRJEN V STABILNIH IZOTOPIH OGLJIKA IN KISIKA V BRANIKAH EVROPSKEGA MACESNA

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ABSTRACT

We report carbon and oxygen isotope ratios measured from the pith at breast height (ca. 1.2m) of three European larch (*Larix decidua* Mill.) trees growing in a mixed wood in West Wales, UK. The non-climatic rising trend of carbon isotope ratios reported for other species during early (juvenile) growth is not present and neither isotope shows significant trends as the tree matures. Results from the first ten rings are not significantly different from the next two sets of ten rings. Absence of a juvenile effect in carbon isotopes of European larch has been reported from trees growing in a low density stand in France and attributed to an absence of shading and no use of respired carbon dioxide. This site, in contrast, is densely wooded and the dominant oaks predate the larch trees, suggesting that juvenile effects, when present, may instead be caused by changes in hydraulic conductivity. More research is needed on juvenile effects as the current practice of avoiding juvenile wood is restricting the potential of stable isotope analysis of tree rings for dendroclimatology and plant physiology.

Key words: stable isotopes, *Larix decidua*, Wales, non-climatic trends

IZVLEČEK

Članek obravnava razmerja ogljikovih in kisikovih izotopov v branikah blizu stržena na prsni višini (cca 1,2 m) treh evropskih macesnov (*Larix decidua* Mill.), rastočih v mešanem gozdu s predraslimi hrasti in nasajenimi evropskimi macesni v zahodnem Walesu, Velika Britanija. Ne klimatskega naraščajočega trenda v razmerjih ogljikovih izotopov, ki ga je sicer opaziti pri drugih vrstah v letih juvenilne rasti, ni, in tudi razmerje stabilnih izotopov ne kaže pomembnih trendov med odraščanjem drevesa. Rezultati iz prvih desetih branik ob strženu se bistveno ne razlikujejo od naslednjih dveh nizov desetih branik. O izostanku juvenilnega efekta v ogljikovih izotopih evropskega macesna so že poročali v zvezi z macesni, rastočimi v nesklenjenih sestojih v Franciji in to pripisali nezastrtosti krošenj in posledične neuporabe ogljikovega dioksida, ki ga pri dihanju oddajajo drevesa. Analizirana drevesa v zahodnem Walesu so rasla v nasadu s predraslimi hrasti, ki so bili starejši od podraslih macesnov. Macesni, kot svetloljubne drevesne vrste, so morali tekmovali za prostor in svetlobo, zato domnevamo, da je morebiten pojav juvenilnega efekta pri stabilnih izotopih prej posledica sprememb v hidravlični prevodnosti lesa, kot pa česa drugega. Ker današnja praksa izogibanja juvenilnega lesa omejuje potencial stabilnih izotopov drevesnih branik za dendroklimatološke in fiziološke raziskave, bi bile potrebne dodatne raziskave o učinkih juvenilne rasti na pojav juvenilnega efekta v meritvah stabilnih izotopov.

Ključne besede: stabilni izotopi, *Larix Decidua*, Wales, ne klimatski trend

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1 INTRODUCTION

1 UVOD

European larch (*Larix decidua* Mill.) is a long-lived and widespread deciduous conifer (Gower and Richards, 1990) which has been used for centuries as a building material (Daux et al., 2011). Its natural distribution is fragmented, covering >500,000 Ha, predominantly in central Europe, including Slovenia. In the Alps it is considered one of the most climate-sensitive species (Carrer and Urbinati, 2006). There have been

various studies using tree-ring parameters of European larch trees, investigating phenomena such as snow avalanches (Stoffel et al., 2006), fires and grazing (Genries et al., 2009) and growing season timing and duration (Moser et al., 2009). Long chronologies have been produced using living trees and wood preserved in buildings, peat bogs and lakes (e.g. Büntgen et al., 2005, 2006; Corona et al., 2010, 2011; Esper et al., 2007; Neuenschwander 2009; Nicolussi et al., 2009). Physical properties of larch tree-rings (width and den-

sity) often show a strong relationships with summer temperature (e.g. Buntgen et al., 2005, 2006 and 2008; Carrer and Urbinati, 2004; Frank and Esper, 2005; Vitas and Zeimavicius, 2010). The few studies that have used stable isotopes of larch trees report strong relationships with various climatic factors (e.g. Kress et al., 2009; Johnson et al., 2010; Daux et al., 2011; Hafner et al., 2011, 2014). Bud-moth outbreaks do not seem to affect the isotopic composition of the tree-rings (Kress et al., 2009; Daux et al., 2011). There is, therefore, considerable potential for using long stable isotope chronologies from European larch to reconstruct past climate, including summer sunshine (Hafner et al., 2014).

A significant problem with building long isotope chronologies, particularly those including building timbers, is the perceived need to avoid rings formed when the trees were in their juvenile phase. Freyer (1979a, b) was one of the first to notice a juvenile effect in stable isotopes, finding an increase of between 1.5 and 2‰ in $\delta^{13}\text{C}$ values during the early years of pine growth. An age-related juvenile trend in $\delta^{13}\text{C}$ values has since been found in many trees, including pine (*Pinus*) (Li et al., 2005; Loader et al., 2007; Gagen et al., 2008), beech (*Fagus*) (Duquesnay et al., 1998) oak (*Quercus*) (Raffalli-Delercé et al., 2004; Labuhn et al., 2014) and spruce (*Picea*) (Treydte et al., 2001). The juvenile effect is generally reported as an age-related increase of cellulose $\delta^{13}\text{C}$ of the inner rings lasting for the first few decades (e.g. Freyer, 1979a; Li et al., 2005), although longer age-related trends have been reported (Saurer et al., 2004). The most common way of dealing with the juvenile effect, without resorting to de-trending, which can remove climatic information, is to avoid the juvenile portion of each tree. Hafner et al. (2014), for example, avoided the first 50 rings of the larch trees used to reconstruct summer sunshine in the eastern European Alps using stable carbon isotopes. Procedures for measuring large numbers of stable oxygen isotopes in tree rings, or for measuring carbon and oxygen simultaneously (Young et al., 2011b, Woodley et al., 2011), were developed after the practice of avoiding juvenile wood, so there is less information available for a juvenile effect in oxygen isotopes (Treydte et al., 2006, Labuhn et al., 2014, Young et al., 2011a).

Reasons for the juvenile effect are not well understood. One hypothesis is that young trees, growing close to the canopy floor, use CO_2 which has been respired by the soil and therefore is already depleted in ^{13}C , leading to relatively negative $\delta^{13}\text{C}$ values which increase as trees gain height and use air which has not been recycled (Francey and Farquhar, 1982; Freyer 1979a,b; Schleser and Jayasekera, 1985). Alternatively

the low $\delta^{13}\text{C}$ may reflect low photosynthetic rate due to restricted light availability close to the forest floor (Heaton, 1999). Bonafini et al. (2013), for example, considered a shaded environment to be an important factor that yields low $\delta^{13}\text{C}$ values. Depleted $\delta^{13}\text{C}$ values in the juvenile period have also been attributed to changes in hydraulic conductivity as trees age and grow taller (McDowell et al., 2002). The hydraulic conductance from soil to leaf decreases as branch/trunk length increases leading to a corresponding decrease in stomatal conductance. As the tree grows and stomatal conductance reduces over time, ^{13}C assimilation will increase meaning wood cellulose will become increasingly enriched in ^{13}C . In theory, this increase in $\delta^{13}\text{C}$ should continue until tree height growth ceases, however, there is an offset associated with increased leaf area which enables greater net conductance, meaning $\delta^{13}\text{C}$ does not continually rise after a certain rate of transpiration (Monserud and Marshall, 2001).

Despite a large volume of evidence for a juvenile effect in carbon isotope ratios across a range of different tree types, a study by Daux et al. (2011) found no such juvenile effect in European larch trees from the French Alps. They attribute this unexpected result to the low forest stand density at the site that was sampled, meaning there would be no use of recycled CO_2 or reduction of photosynthesis due to reduced light in the lower levels of the canopy (Heaton, 1999). In this paper we examine the juvenile rings of three individual European larch trees growing under very different conditions to those sampled by Daux et al. (2011), to test whether the unexpected absence of a juvenile effect can be replicated.

2 MATERIAL AND METHODS

2 MATERIAL IN METODE

The sampling site is an 8 ha semi-natural, broad-leaved woodland at Allt Lan-las in the grounds of the National Trust owned Llanerchaeron Estate in Ceredigion, West Wales, UK, where Young et al., (2012) found a very strong relationship between latewood cellulose $\delta^{13}\text{C}$ and summer temperature in oaks. The dominant species is *Quercus petraea* (sessile oak) with *Fraxinus excelsior* (ash) and *Fagus sylvatica* (beech). Mature *Larix decidua* (European larch) are thinly dispersed within the eastern part of the forest. A shrub understorey consists of *Coryllus avellana* (hazel), *Ilex aquifolium* (holly), *Lonicera periclymenum* (honeysuckle) and *Rubus fruticosus* (bramble). In contrast to the study site of Daux et al. (2011), this site has a high stand density and young trees are subject to shading.

Increment cores (12mm) were collected from breast height (*ca.* 1.2m) for five healthy larch trees. Up to three cores were obtained from each tree to identify locally absent rings during cross dating. Samples were air-dried, sanded and the rings measured to an accuracy of 0.01mm using a microscope and a horizontally travelling stage linked to a computer (Tyers 2004). There is no master chronology for British larch, but, once locally absent rings had been identified, the trees cross-dated with each other giving Baillie Pilcher *t*-values >3.5 (Baillie and Pilcher, 1973) and also demonstrated *t*-values >3.5 against an existing oak master chronology for the site (Bale, 2005). Three of the cores reached very close to the pith, which is evident due to extreme curvature of the rings, and these were used to investigate potential juvenile effects. The first rings of trees 1 and 2 were dated as AD 1846 and that of tree 3 as AD1850. The first rings of all three analysed trees are estimated to be between five and ten years from the pith.

Each annual growth ring was cut into slivers using a razorblade and binocular microscope. The whole-ring was used, as suggested by Kress et al. (2009) who found a strong relationship between $\delta^{13}\text{C}$ in earlywood and latewood of larch and concluded that separation of early- and latewood is unnecessary. Prior to isotopic analysis, samples were extracted to α -cellulose

(Loader et al., 1997). Between 0.3 and 0.35mg of sample were then weighed into silver capsules and the isotope ratios analysed by pyrolysis over glassy carbon at *ca.* 1090°C using a Europa ANCA GSL elemental analyser interfaced to a Europa 20/20 isotope ratio mass spectrometer. Analytical precision (σ_{n-1} , n=10) for this on-line approach to dual isotope analysis is typically 0.3‰ ($\delta^{18}\text{O}$) and 0.1‰ ($\delta^{13}\text{C}$) (McCarroll and Loader, 2004). Stable carbon isotope ratios ($\delta^{13}\text{C}$) and oxygen isotope ratios ($\delta^{18}\text{O}$) are reported as per mille (‰) deviations from the Vienna Pee Dee Belemnite (VPDB) and Vienna Standard Mean Ocean Water (VSMOW), respectively (Coplen, 1995). The carbon isotope ratios were corrected for changes in the isotopic ratio of atmospheric carbon dioxide (McCarroll and Loader 2004) but not for potential changes in response to the increase in the amount of atmospheric carbon dioxide (McCarroll et al., 2009).

3 RESULTS

3 REZULTATI

The carbon isotope ratios obtained from the three trees show no evidence of the expected rise in values over the juvenile period (Figure 1). Taking the first 15, 20 or 30 years, none of the trees show a significant rising trend (Table 1). The same is true for the oxygen iso-

Table 1: Results of Pearson's correlation (*r*) and Spearman's rank correlation (*p*) of isotope ratios against ring number for 15, 20 and 30 years

Tree	Years	Carbon / Ogljik			Oxygen / Kisik		
		15	20	30	15	20	30
1	<i>r</i>	-0.05	0.14	-0.02	-0.27	-0.12	0.06
	<i>p</i>	0.01	0.17	-0.04	-0.14	-0.03	-0.04
2	<i>r</i>	-0.16	0.14	0.34	0.11	-0.12	-0.42
	<i>p</i>	-0.09	0.25	0.33	0.03	-0.14	-0.38
3	<i>r</i>	-0.12	0.36	0.21	0.23	0.32	-0.11
	<i>p</i>	-0.09	0.42	0.25	0.09	0.38	-0.09

Preglednica 1: Rezultati Pearsonovega (*r*) in Spearmanovega rang korelacijskega koeficienta (*p*) izotopskih razmerij pri številu branik za 15, 20 in 30 let

Table 2: Results of the Kruskal-Wallis test (*H*) comparing the first, second and third ten years of isotope data, and the Mann-Whitney U-test comparing the first ten years with the next 20 years. Test statistics (*H* and *U*) and two-tail probabilities are tabulated.

Tree	Carbon / Ogljik		Oxygen / Kisik	
	<i>H</i>	<i>U</i>	<i>H</i>	<i>U</i>
1	<i>H</i> = 1.79 <i>p</i> = 0.41	<i>U</i> = 77 <i>p</i> > 0.05	<i>H</i> = 0.11 <i>p</i> = 0.95	<i>U</i> = 93 <i>p</i> > 0.05
2	<i>H</i> = 2.96 <i>p</i> = 0.23	<i>U</i> = 68 <i>p</i> > 0.05	<i>H</i> = 3.4 <i>p</i> = 0.18	<i>U</i> = 65 <i>p</i> > 0.05
3	<i>H</i> = 0.80 <i>p</i> = 0.67	<i>U</i> = 87 <i>p</i> > 0.05	<i>H</i> = 4.7 <i>p</i> = 0.09	<i>U</i> = 92 <i>p</i> > 0.05

Preglednica 2: Rezultati Kruskal-Wallisovega testa (*H*) s primerjavo prvih, drugih in tretjih dekad izotopskih podatkov, in Mann-Whitneyev U-test s primerjavo prvih deset let z naslednjimi 20 leti. Tabelarno so urejene testne statistike (*H* in *U*) in dvostranske verjetnosti.

top results, with no evidence for a juvenile increase or decline. The Kruskal-Wallis H-test, a non-parametric analysis of variance by ranks (McCarroll 2016), demonstrates that the first, second and third sets of ten rings do not significantly differ from one another. For both isotope ratios, the related Mann-Whitney U-test shows that in all cases the first ten years are not significantly different from the next 20 (Table 2). The non-parametric 'change point statistic' (Siegel and Castellan 1988), which assesses the likelihood that a change occurred in a sequence of observations, suggests that in no case was there a change that exceeded the fluctuations that are expected due to chance ($p > 0.05$).

4 DISCUSSION AND CONCLUSIONS

4 DISKUSIJA IN ZAKLJUČKI

The results presented here clearly show that there is no juvenile effect in the carbon or oxygen isotope ratios of European larch trees growing in West Wales, UK. This agrees with the results reported by Daux et al. (2011), who found no juvenile effect in the carbon isotope ratios of European larch trees growing in France. However, Daux et al. (2011) concluded that the absence of a juvenile effect could be explained by the low forest density at their site, meaning that young trees were not using respired carbon dioxide or subject to shading. That explanation cannot be applied to the site in Wales,

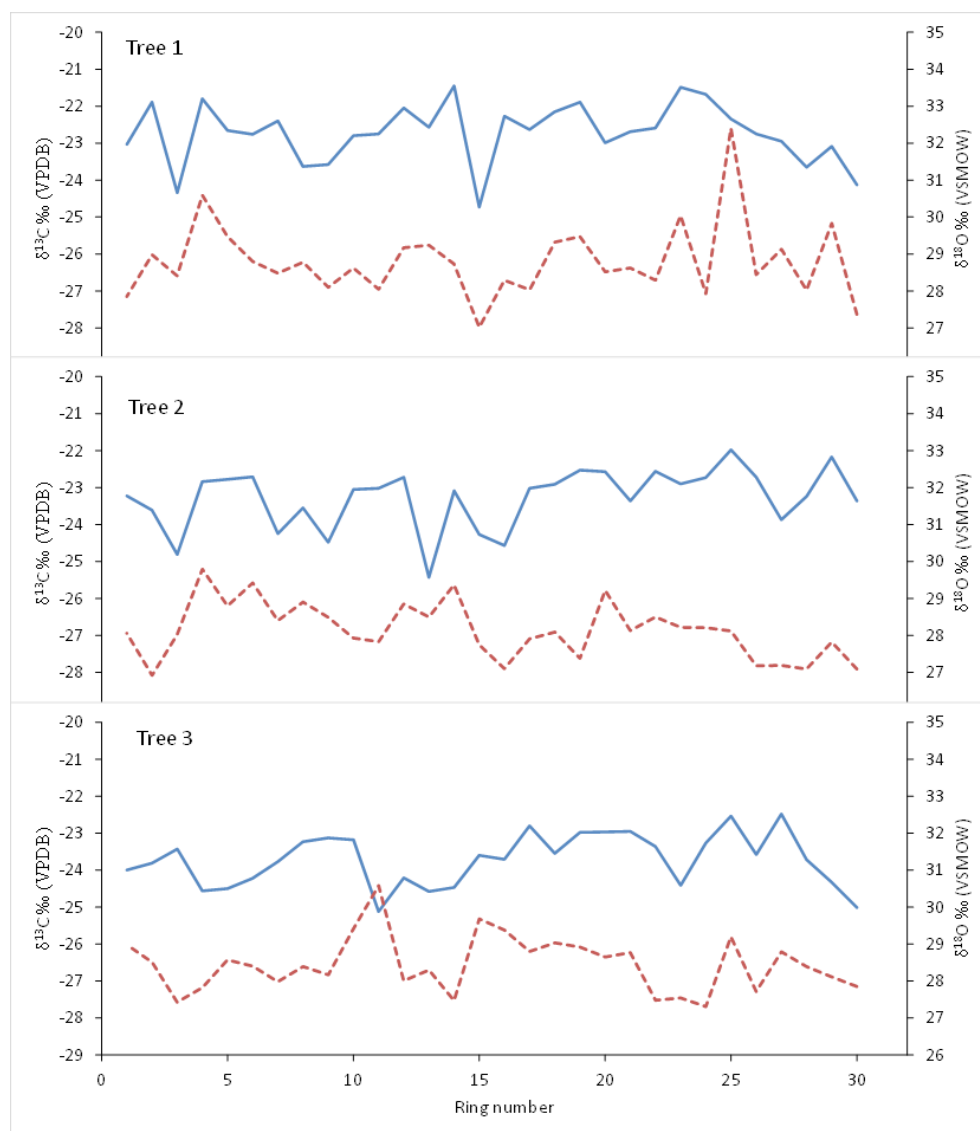


Fig 1: Carbon isotope ratio (solid line) and oxygen isotope ratio (dashed line) of whole ring α -cellulose for the three individual larch trees arranged by ring number. Curvature of the tree rings for each core and knowledge of the forest stand enable estimation of pith age, such that the first measured rings of all trees are within 5-10 rings of the pith.

Slika. 1: Razmerje stabilnega izotopa ogljika (polna črta) in kisika (črtkana črta) α -celuloze iz celotne branike za tri posamezne macesne, urejeno glede na zaporedno številko branike. Število manjkajočih branik do stržena je bilo ocenjeno na podlagi loka drevesnih branik na izvrtku in poznavanju značilnosti priraščanja analiziranega gozdnega sestoja, tako da so prve izmerjene branike vseh dreves znotraj 5-10 branik od stržena.

which is dense mixed broadleaf woodland, dominated by mature oak trees that pre-date the establishment of larch, and contemporary beech and ash. These results raise the possibility that the juvenile effect in carbon isotopes reported for other species is not caused by the use of respired carbon dioxide or by shading, but is a response to changes in hydraulic conductivity, as proposed by McDowell et al. (2002). The juvenile effect reported in the carbon isotope ratios from pine trees from the French Alps (Gagen et al., 2006) support this hypothesis. They were growing in very open stands on a windy hillside, so that respired carbon dioxide and shade are unlikely explanations.

On the basis of the results reported here, and those reported by Daux et al. (2011), we conclude that European larch trees do not necessarily display a juvenile effect in either the carbon or oxygen isotope ratios. The common practice of excluding the inner rings of larch trees from isotope analysis may thus be overly cautious. This means that it may be possible to use longer sequences of rings from living trees to extend palaeoclimate reconstructions and also to make much more efficient use of larch building timbers in producing chronologies that extend beyond the range of most living larch trees.

It is not clear why larch behaves so differently to some other tree species, including pines, which show a clear and strong juvenile effect in carbon isotope ratios. Unfortunately, there is little data available on juvenile effects in other tree species, particularly for oxygen isotopes, because most researchers now routinely exclude juvenile wood. More work is required on the juvenile effect of other tree species, particularly those used as building timbers and thus available to produce very long isotope chronologies.

5 SUMMARY

We report carbon and oxygen isotope ratios of whole ring α -cellulose from the first 30 rings of three European larch (*Larix decidua* Mill.) trees growing in a mixed wood in West Wales, UK. The rising trend of carbon isotope ratios seen in other species, during the early growth years, is not present and neither isotope shows significant trends with ring number over the first 15, 20 or 30 years of growth. Non-parametric analysis of variance (Kruskal-Wallis H-test) shows that the results from the first, second and third sets of ten rings are not significantly different and the Mann-Whitney U-test shows that the values from the first ten rings are not significantly different to the next 20. The non-parametric change point statistic, which assesses the likelihood that a change occurred in a sequence

of observations, suggests that in no case was there a change that exceeded the fluctuations that are expected due to chance.

Absence of a juvenile effect in carbon isotopes of European larch has been reported from trees growing in a low density stand in France and attributed to an absence of shading and no use of respired carbon dioxide. This site in West Wales is, in contrast, densely wooded and the dominant oaks predate the larch trees, suggesting that juvenile effects, when present, may rather be caused by changes in hydraulic conductivity. More research is needed on juvenile effects as the current practice of avoiding juvenile wood is restricting the potential of stable isotope analysis of tree rings for dendroclimatology and plant physiology.

5 POVZETEK

Avtorji prispevka poročajo o razmerjih stabilnega izotopa ogljika in kisika v α -celulozi celotnih prvih 30 branik treh evropskih macesnov (*Larix decidua* Mill.), rastočih v dvoslojnim mešanem gozdu hrasta in macesna v zahodnem Walesu, Velika Britanija. Naraščajočega trenda razmerij ogljikovih izotopov, ki ga je bilo opaziti pri drugih vrstah v letih zgodnje rasti, ni, in tudi razmerje stabilnih izotopov ne kaže pomembnih trendov v branikah v prvih 15, 20 ali 30 letih rasti. Neparametrična analiza variance (Kruskal-Wallisov H-test) kaže, da se rezultati prvega, drugega in tretjega testa desetih branik bistveno ne razlikujejo, pa tudi Mann-Whitneyev U-test kaže, da se vrednosti iz prvih desetih branik bistveno ne razlikujejo od naslednjih 20. Neparametrična statistika analize sprememb, ki ocenjuje verjetnost, da se je sprememba zgodila med zaporedjem opazovanj, nakazuje, da v nobenem primeru ni nastala sprememba, ki bi preseгла naključno pričakovana nihanja.

O izostanku juvenilnega efekta v stabilnih izotopih ogljika v branikah evropskega macesna so poročali v zvezi z drevesi, rastočimi v nesklenjenih sestojih nizke gostote v Franciji in to pripisali nezastrtosti krošenj in posledične neuporabe ogljikovega dioksida, ki ga pri dihanju oddajajo drevesa. Analizirana drevesa v zahodnem Walesu so rasla v nasadu s predraslimi hrasti, ki so bili starejši od podraslih macesnov. Macesni, kot svetloljubne drevesne vrste, so morali tekMOVATI za prostor in svetlobo, zato domnevamo, da je morebiten pojav juvenilnega efekta pri stabilnih izotopih prej posledica sprememb v hidravlični prevodnosti lesa, kot pa česa drugega. Ker današnja praksa izogibanja juvenilnega lesa omejuje potencial stabilnih izotopov drevesnih branik za dendroklimatološke in fiziološke raziskave, bi bile potrebnih dodatne raziskave o

učinkih juvenilne rasti na pojav juvenilnega efekta v meritvah stabilnih izotopov.

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