UNIVERZA V LJUBLJANI
BIOTEHNIŠKA FAKULTETA

Polona HAFNER

VPLIV KLIMATSKIH DEJAVNIKOV NA IZOTOPSKO SESTAVO IN ZGRADBO BRANIK EVROPSKEGA MACESNA (*Larix decidua* Mill.) IN DOBA (*Quecus robur* L.)

DOKTORSKA DISERTACIJA

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THE INFLUENCE OF CLIMATIC PARAMETERS ON THE ISOTOPIC COMPOSITION AND STRUCTURE OF EUROPEAN LARCH (*Larix decidua* MIill.) AND PENDUCULATE OAK (*Quecus Robur* L.) TREE-RINGS

DOCTORAL DISSERTATION

Ljubljana, 2015

Na podlagi statuta Univerze v Ljubljani ter po sklepu senata Biotehniške fakultete in sklepa komisije za doktorski študij Univerze v Ljubljani z dne 21. 9. 2011 je bilo potrjeno, da kandidat izpolnjuje pogoje za opravljanje doktorata znanosti na interdisciplinarnem doktorskem študijskem programu Bioznanosti, znanstveno področje Biologija. Za mentorja je bil imenovan prof. dr. Tomislav Levanič.

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AI Na območju jugozahodnih Alp so kronologije izotopskega razmerja ogljika, kisika in vodika v branikah macesna tesno povezane, v najmočnejši korelaciji sta kronologiji vodnih izotopov. Kronologije širin branik vsebujejo informacijo o klimatskih razmerah v obdobju od sredine maja do julija, kronologije izotopskega razmerja pa so v močni korelaciji s temperaturami in trajanjem sončnega obsevanja v juliju in avgustu. Prostorski vzorec korelacije med izmerjenimi temperaturami ter kronologijama izotopskega razmerja ogljika in širine branik se razteza čez širše območje Alp. Pri kronologijah vodnih izotopov vzorec prostorske korelacije pokriva območje južne Italije in zahodnega dela Balkana. Na podlagi kronologije izotopske sestave ogljika je bila izdelana 520 let dolga rekonstrukcija trajanja sončnega obsevanja. Njena primerjava z obstoječimi rekonstrukcijami temperatur razkriva obdobja sinhronega variiranja in razhodov krivulj trajanja sončnega obsevanja in temperatur. V Krakovskem gozdu so dobi s periodično poplavljenega mikrorastišča (W dobi) skozi celotno analizirano obdobje (1970-2008) rasli značilno bolje kot dobi iz bolj suhega mikrorastišča (D dobi). Pri D dobih je potencialna klimatska informacija shranjena v ranem lesu in se nanaša na klimatske razmere v preteklem letu. V kasnem lesu ni razvidnega odziva na okoljske dejavnike oziroma je le-ta zabrisan v odzivu dreves na dolgotrajne stresne razmere. Visoka pozitivna korelacija parametrov kasnega lesa W dobov s poletno količino padavin in poletnim pretokom Krke nakazuje potencial za njihovo uporabo v dendrohidroloških študijah.

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AB Carbon, oxygen and hydrogen isotope chronologies of tree-rings of larch growing in southeastern Alps are strongly correlated, with correlation between water isotopes being the strongest. Chronologies of tree-ring width contain information of climate conditions in the period between May and July, while isotope chronologies are strongly correlated with temperatures and sunshine duration in July and August. Spatial correlation pattern between carbon isotope chronology, tree-ring width chronology and measured temperatures spreads over wider area of Alps, while in case of water isotopes it covers the area of southern Italy and western Balkan. A 520 years long reconstruction of sunshine duration was constructed based on carbon isotope chronology. Its comparison with existent temperature reconstructions reveals period when temperature and sunshine duration covariate and when they diverge. In the Krakovo forest, pedunculated oaks from peridicaly flooded micro-location (W oaks) grew significantly better than oaks from the drier micro-location (D dobi) through the whole analysed period (1979-2008). In D oak, potential climate information is stored in earlywood and is correlated with climate conditions in the previous year. There was no clear response to environmental factors in the latewood and it appears to be blurred by the response of trees to long term stressed conditions. High positive correlation between summer precipitation amount, summer Krka River flow and latewood parameters of W oaks indicates their potential for use in dendrohydrological studies.

KAZALO VSEBINE

KAZALO SLIK

KAZALO PRILOG

1 UVOD

1.1 OPREDELITEV RAZISKOVALNEGA PROBLEMA

Poznavanje variacij klimatskih dejavnikov v preteklosti omogoča uvrščanje aktualnih klimatskih sprememb v širši časovni kontekst naravnega spreminjanja klime. Številni klimatski modeli napovedujejo nadaljnje pospešeno višanje temperature in spremenjene padavinske režime (IPCC, 2007). Za izboljšanje klimatskih modelov, oceno amplitude sedanjih klimatskih sprememb in predviden odziv ekosistemov na spremenjene razmere so potrebni čim daljši nizi informacij o variiranju klimatskih dejavnikov v preteklosti. Nizi instrumentalno izmerjenih meteoroloških spremenljivk so razmeroma kratki, zato se kot dodatne informacije uporabljajo t. i. proxy oziroma nadomestni podatki. Meritve proxy podatkov vključujejo merjenje sprememb kemičnih, fizikalnih in bioloških parametrov, ki odražajo spremembe v okolju v času, ko je nosilec proxy informacije nastajal (Burroughs W. J., 2001).

Drevesa so stalni in najdlje prisotni živ element krajine, ki na istem mestu uspevajo več stoletij in celo tisočletij ter se v tem času bolj ali manj aktivno odzivajo na biotske in abiotske dejavnike, ki delujejo v njihovi okolici, odziv dreves nanje pa se v obliki proxy podatkov shrani v braniki (McCarroll D. in Loader N. J., 2004). Med vsemi poznanimi nosilci proxy podatkov (npr. ledni izvrtki, jezerski in morski sedimenti) so prav branike z vsemi svojimi značilnostmi eden od najdragocenejših in najnatančnejših naravnih arhivov paleoekoloških informacij z ločljivostjo enega leta (Fritts H. C., 1976; McCarroll D. in Loader N. J., 2006) in celo na ravni posameznih mesecev oz. sezone (Helle G. in Schleser G. H., 2004; Rigling A. in sod., 2002). Različni parametri branike (npr. širina branike ter razmerje stabilnih izotopov kasnega in ranega lesa, maksimalna gostota, morfološke značilnosti lesnih celic) vsebujejo različne informacije o odzivu dreves na različne okoljske dejavnike v različnih časovnih obdobjih. Za pridobitev želene klimatske informacije iz posameznih parametrov branike je potrebno dobro poznavanje vpliva klime in okolja na odziv dreves.

1.2 NAMEN RAZISKAV IN DELOVNE HIPOTEZE

Naša raziskava je bila osredotočena na dendroekološko analizo evropskega macesna (*Larix decidua* Mill.) na zgornji gozdni meji, ki predstavlja ekstremno rastišče za rast dreves, ter doba (*Quercus robur* L.) v nižinskih gozdovih, ki sodi med bolj ogrožene gozdne ekosisteme v Sloveniji. S hkratnim proučevanjem različnih parametrov branik smo želeli na v raziskavo zajetih rastiščih rekonstruirati glavne klimatske dejavnike in razložiti njihov vpliv na odziv proučevanih drevesnih vrst.

Za območje Slovenije do sedaj nismo imeli rekonstrukcije okoljskih razmer, ki bi temeljila na hkratnem proučevanju več informacij, ki jih vsebujejo različni parametri branik. Na tem območju, razen širin branik, še ni bila izdelana in analizirana dolga kronologija različnih parametrov branik pri macesnu in hrastu. Opravljenih je bilo zelo malo raziskav, ki bi proučevale okoljski signal v izotopski sestavi branik.

Cilji disertacije so:

- Predstaviti prve dolge kronologije razmerja stabilnih izotopov v braniki ter njihov potencial za rekonstrukcijo klime.
- Vpeljati analizo izotopske sestave branik, ki je na našem območju do sedaj še ni bilo.
- Ugotoviti najpomembnejše klimatske parametre, ki značilno vplivajo na odziv dreves (strukturo branike) ter rekonstruirati izbrano klimatsko spremenljivko.
- S hkratnim proučevanjem različnih informacij, zajetih v parametrih branike želimo dobiti boljši vpogled v odnos med delovanjem okoljskih dejavnikov in fizioloških procesov v drevesu ter oceniti primernost različno vitalnih dreves za rekonstrukcijo klimatskih dejavnikov.

Postavili smo naslednje delovne hipoteze:

- V braniki lahko poleg njene širine analiziramo še številne druge parametre, ki vsebujejo dodatne paleoekološke informacije, ter tako nadgradimo informacijo, pridobljeno iz širin branik. Kombinacija različnih proxy podatkov da boljšo časovno in prostorsko opredeljeno informacijo kot posamezni podatki.

- Z uporabo proxy podatkov, pridobljenih iz parametrov branik, lahko rekonstruiramo izbrane klimatske spremenljivke v obdobju pred instrumentalnimi meritvami.

- Drevesa, ki so v dolgotrajnem fiziološkem stresu, se odzivajo drugače kot vitalna drevesa. V parametrih branik prizadetih dreves je klimatski signal zabrisan zaradi odziva dreves na stres.

2 PREGLED OBJAV

Za proučevanje odziva dreves na klimatske dejavnike so najprimernejša ekstremna rastišča. To pomeni, da je eden od za rast in uspevanje pomembnih dejavnikov na minimumu oziroma omejujoč (Fritts H. C., 1976). Na zgornji gozdni in drevesni meji v Alpah rast dreves praviloma omejuje nizka temperatura in s tem povezana kratka rastna sezona. Za dendroklimatološke raziskave je prav tako pomembno, da so drevesa in gozdni sestoji bolj ali manj prepuščeni naravnemu razvoju. Na drugi strani pa nižinske gozdove v Sloveniji predstavljajo redke še ohranjene dobrave, na katere vpliva mešanica različnih dejavnikov; izpostavljene so močnemu pritisku kmetijstva, poselitve, gospodarjenja z gozdovi in vodami ipd. V takih razmerah nam proučevanje več parametrov branike hkrati pomaga določevanje in proučevanje najbolj vplivnih okoljskih dejavnikov na rast dreves.

2.1 ZGODOVINA RAZISKAV PARAMETROV BRANIK

Za začetnika dendrokronologije štejemo A. E. Douglasa, ki je v začetku 20. stoletja postavil temelje križnega datiranja ter razvil metodo »skeleton plot«. Sistematično je raziskoval razmerje med variiranjem klime in širino branik (tree ring width - TRW) (Fritts H. C., 1976; Speer J. H., 2010). Skozi leta so se uporabljali različni pristopi pri razvijanju kronologij rastišč, v sodobnem času pa se meritve širin branik izvajajo s pomočjo različnih merilnih mizic in povečevalne lupe. Naprednejši sistemi za merjenje širine branik so povezani s kamero in omogočajo analizo slike s pomočjo različne programske opreme. Tudi obdelava podatkov je skozi čas precej napredovala – od stopnje, ko je kronologijo pred objavo »ročno« preveril drug, neodvisni dendrokronolog (Speer J. H., 2010), do danes uporabljanih programskih orodij, ki omogočajo natančno računalniško analizo in sprotno preverjanje rezultatov. Dendrokronološke analize so bile sprva namenjene predvsem datiranju lesenih objektov. Z razvojem tehnologije in uporabe novih metod analize branike so se hkrati razvijale tudi podvede, kot so npr. dendroklimatologija, dendroekologija, dendrohidrologija, … V sedemdesetih letih 20. stoletja se je analizi širine branik pridružila rentgenska densitometrija. Pri njeni aplikaciji v analizi branik med drugim dobimo podatke o maksimalnih gostotah branik (maximum latewood density - MXD) (Polge H., 1970). S pojavom visoko ločljivih skenerjev in programske opreme se je razvila metoda »blue intensity«, ki predstavlja cenejši in lažje dosegljiv nadomestek klasični rentgenski densitometriji (Campbell R. in sod., 2007). TRW in MXD sta tradicionalno najpogosteje uporabljana parametra v dendrokronoloških raziskavah in imata močan signal v okoljih, kjer en okoljski dejavnik, navadno temperatura ali padavine, močno vpliva na rast dreves (Fonti P. in sod., 2010). Korak naprej je prinesla uvedba novih tehnik in možnost analize dodatnih parametrov branike. V primerjavi s tradicionalnima dendrokronološkima metodama kronologije lesnoanatomskih parametrov in izotopske sestave lesa vsebujejo informacije tudi o okoljskih dejavnikih v manj ekstremnih rastnih razmerah in v različnih časovnih oknih (Fonti P.in sod., 2010). Prvi začetki proučevanja variiranja anatomskih značilnosti serij zaporednih branik segajo v šestdeseta in sedemdeseta leta 20. stoletja, ko so meritve mikroskopskih preparatov potekale ročno. Z napredkom in razvojem sistemov analize slike v povezavi z zmogljivimi digitalnimi kamerami, skenerji in programskimi orodji ter novimi metodami priprave vzorcev je danes mogoča natančnejša in učinkovitejša analiza lesnoanatomskih parametrov, kot so traheje, traheide, vlakna in parenhimske celice (Fonti P.in sod., 2010). Prve analize izotopske sestave lesa so bile opravljene že razmeroma zgodaj, v šestdesetih letih preteklega stoletja (Craig H., 1954; Libby L. M. in Pandolfi L. J., 1974), in predstavljajo zametke izotopske dendroklimatologije. Teorija izotopske dendroklimatologije in dendroekologije je podrobneje opisana v sledečih poglavjih.

2.2 IZOTOPSKO RAZMERJE V BRANIKAH DREVES

2.2.1 Definicija izotopov in njihove lastnosti

Atom sestavljajo protoni, elektroni in nevtroni. Za vsak določen element je število protonov vedno enako, medtem ko število nevtronov lahko variira. Število nevtronov v jedru ne vpliva na kemične lastnosti elementa in njegovih spojin, vendar pa razlika v masi povzroča komaj opazne kemične in fizikalne razlike med spojinami, ki vsebujejo elemente z različnim številom nevtronov (Sharp Z., 2007). Atome istega elementa, ki imajo v jedru enako število protonov, med seboj pa se razlikujejo po različnem številu nevtronov in različni atomski masi, imenujemo izotopi. V periodnem sistemu vsi izotopi enega elementa zasedajo isto mesto (Dawson T. E. in Brooks P. D., 2001). Ločimo radioaktivne in stabilne izotope. Za radioaktivne izotope je značilno, da ima vsak svojo hitrost razpadanja atomskega jedra, ki jo imenujemo razpolovni čas. Stabilni izotopi ne razpadajo, vendar zaradi razlike v atomskih masah prihaja do razlik v fizikalnih in kemijskih lastnostih (Clark I. D. in Fritz P., 1997).

2.2.2 Izotopska sestava lahkih elementov

Izotopsko sestavo lahkih elementov (H, C in O) v vzorcu podajamo z δ vrednostjo. Vrednost delta (δ) je relativna vrednost izotopske sestave v vzorcu in je podana kot relativna razlika od mednarodno določenega referenčnega materiala (Dawson T. E. in Brooks P. D., 2001). Vrednost R je razmerje med redkejšim, težjim stabilnim izotopom (^{2}H, ^{13}C, ^{15}N, ^{18}O in ^{34}S) in lažjim, pogostejšim stabilnim izotopom (H, ^{12}C in ^{16}O). Vrednost delta (δ) izračunamo po formuli:

$$\delta(‰) = \left(\frac{R_{vzorec}}{R_{referenčni\ material}} - 1\right) * 1000 \qquad ...(1)$$

in jo podajamo v promilah (‰). Za možnost primerjave relativnih izotopskih meritev so bili za določanje izotopske sestave najpogostejših elementov v naravi sprejeti naslednji referenčni materiali: VSMOW (Vienna Standard Mean Ocean Water) za vodik in kisik ter VPDB (Vienna Pee Dee Belemnite Americana) za ogljik,. Referenčni materiali za posamezen element so izbrani tako, da je izotopsko razmerje referenčnega materiala in povprečnega izotopskega razmerja v naravi čimbolj podobno. Po definiciji je vrednost vseh referenčnih materialov enaka 0 ‰. Pozitivna vrednost δ nakazuje, da vsebuje vzorec v primerjavi s standardom več težjega stabilnega izotopa, medtem ko bolj negativna vrednost pomeni večjo vsebnost lažjega stabilnega izotopa v vzorcu. V laboratorijih se pogosto uporabljajo lastni, »interni delovni standardi« (IWS), ki so za podobne analize v različnih laboratorijih med seboj usklajeni in kalibrirani na referenčne materiale (Dawson T. E. in Brooks P. D., 2001).

2.2.3 Izotopska frakcionacija

Izotopi posameznega elementa imajo različne kemijske in fizikalne lastnostih zaradi razlik v atomski masi. Pri elementih z nizkim masnim številom so razlike v atomski masi posamezniih izotopov dovolj velike, da pri številnih fizikalnih, kemijskih in bioloških procesih prihaja do frakcionacije oziroma spremembe relativnega razmerja izotopov posameznega elementa med reaktanti in produkti (Kendall C. in Caldwell E. A. 1998). Proces, ki vodi do frakcionacije, imenujemo izotopski efekt.

Poznamo dve glavni skupini dejavnikov, ki vodijo do izotopske frakcionacije:
1) Izotopske izmenjalne reakcije, ki se navezujejo na ravnotežno ali termodinamično frakcionacijo. Pri izotopskih izmenjalnih reakcijah poteka izmenjava izotopov posameznega elementa med spojinami, fazami ali molekulami brez kemijske spremembe. S faktorjem izotopske frakcionacije α izražamo z ravnotežnimi reakcijami povezano frakcionacijo med spojino ali fazo A in B:

$$\alpha_{A-B} = \frac{R_A}{R_B} \qquad \text{... (2)}$$

kjer je R razmerje med težjim in lažjim izotopom v spojini A in spojini B.
Frakcionacijski faktor α lahko ob upoštevanju enačbe 2 izrazimo z vrednostjo δ:

$$\alpha_{A-B} = \frac{1000 + \delta_A}{1000 + \delta_B} \qquad \text{... (3)}$$

kjer sta δ_A in δ_B vrednosti izotopske sestave spojine ali faze A in B (Kendall C. in Caldwell E. A. 1998).

2) Kinetične reakcije so odvisne od razmerij izotopskih mas in njihovih vibracijskih energij. Kinetični izotopski efekti so navadno v povezavi s hitrimi in enosmernimi procesi, kot sta npr. evaporacija in difuzija ter večina biokemijskih reakcij. Kinetični izotopski efekt je

posledica različnih reakcijskih hitrosti molekul z različno izotopsko sestavo. Po pravilu se kemijske vezi med lažjimi izotopi lažje cepijo kot vezi med težjimi izotopi. Posledično molekule z lažjo izotopsko sestavo reagirajo hitreje (Kendall C. in Caldwell E. A. 1998; Sharp Z., 2007). Frakcionacijski faktor kinetične reakcije lahko izrazimo z enačbo:

$$\alpha = \frac{R_p}{R_s} \qquad \text{... (4)}$$

kjer sta R_p in R_s razmerji med težjim in lažjim izotopom v produktu (p) in izhodno snovjo (s).

2.2.4 Fiziološko ozadje izotopske sestave lesa oz. izotopska frakcionacija v drevesu

Glavni gradniki lesa so ogljik (50 %), kisik (44 %) in vodik (6 %) in so v naravi zastopani z več kot enim stabilnim izotopom. Izotopska razmerja posameznih elementov v braniki se močno razlikujejo od njihovih razmerij v atmosferskem CO_2, ki je vir v rastlino vgrajenega ogljika, ter razmerij v talni vodi oz. padavinah, ki so vir vodika in kisika v rastlini. Izotopska sestava branike predstavlja občutljiv bioindikator sprememb sestavnih delov vode in CO_2 v drevesu kot odziv na okoljske razmere, v katerih je nastala (McCarroll D. in Loader N. J., 2004).

2.2.4.1 Teorija vrednosti izotopske sestave ogljika v branikah

Encimatski in difuzni procesi frakcionacije vodijo do diskriminacije težjega ogljikovemu izotopu (^{13}C) med fotosintezo. Trenutno razmerje $^{13}C/^{12}C$ v CO_2 v ozračju se odraža v vrednosti $\delta^{13}C$ in znaša približno -8 ‰. Vrednosti v listih ter lesu so posledica frakcionacije in so v primerjavi z vrednostjo v zraku precej nižje, pri C3 rastlinah variirajo med -21 ‰ in -35 ‰ (Badeck F.-W. in sod., 2005). Frakcionacija ogljikovega izotopa se med vgrajevanjem ogljika iz CO_2 zunanjega zraka v listne sladkorje pojavi na dveh točkah (Warren C. R. in sod., 2001). Razločevanje se najprej pojavi pri difuziji CO_2 skozi listne reže, skozi katere le-ta iz okoliškega zraka prehaja do kloroplasta. Lažji $^{12}CO_2$ prehaja na mesto karboksilacije hitreje in zato CO_2 v listu v primerjavi z zunanjim CO_2 vsebuje manj ^{13}C. Proces imenujemo frakcionacija zaradi difuzije CO_2 in ustvarja difuzijski frakcionacijski efekt -4,4 ‰ (Craig H., 1952) in ga označujemo s črko »a«. Druga točka diskriminacije proti ^{13}C se pojavi na ravni asimilacije ogljika in je odvisna od prvega encima, ki pride v stik s CO_2. Pri C3 rastlinah je ta encim RUBISCO (ribuloza bifosfat karboksilaza/oksigenaza), ki diskriminira proti ^{13}C (Taiz L. in Zeiger E., 2006). Frakcionacija temelji na različni hitrosti reakcij s $^{12}CO_2$ in $^{13}CO_2$ in znaša približno -27 ‰, označimo ga s črko »b« (Farquhar G. D. in sod., 1982). Biokemična frakcionacija je neodvisna od temperature (O'Leary M. H., 1981). Na izotopsko sestavo listnih sladkorjev močno vpliva tudi razmerje med intercelularno (c_i) in atmosfersko koncentracijo (c_a) CO_2. Nizka koncentracija CO_2 v listu (c_i) vodi do višje koncentracije ^{13}C in posledično višje (manj negativne) $\delta^{13}C$-vrednosti listnih sladkorjev (McCarroll D. in Loader N. J., 2006). Farquhar in sodelavci (1982) so za predstavitev izotopske zgradbe rastline uporabili model:

$$\delta^{13}C_{(rastlina)} = \delta^{13}C_{(atmosfera)} - a - (b - a) * (c_i \,/\, c_a) \qquad \text{... (5)}$$

kjer »a« predstavlja difuzijski frakcionacijski efekt (-4,4 ‰), »b« frakcionacijo pri karboksilaciji (-27 ‰,), »c_i« intercelularno koncentracijo CO_2 (ppm) ter »c_a« atmosfersko koncentracijo CO_2 (ppm).

2.2.4.2 Učinkovitost izrabe vode in diskriminacija stabilnega ogljikovega izotopa

Medtem ko odprte listne reže omogočajo sprejem CO_2 v rastlino, hkrati dopuščajo tudi izhajanje vodne pare iz listov v okolico. Z omejevanjem izgube vode zapiranje listnih rež omejuje tudi sprejem CO_2. Razmerje med asimiliranim CO_2 in s transpiracijo vode se imenuje fotosintetska učinkovitost izrabe vode (Water use efficeincy – WUE) in predstavlja kvantitativno mero trenutne izmenjave plinov v listu. Kadar je v ospredju zanimanja produkcija suhe snovi celotne vegetacijske sezone, učinkovitost izrabe vode predstavlja razmerje med produkcijo suhe snovi in porabo vode (Larcher W., 2001; Leuenberger M. in sod., 1998). V sušnih razmerah je za rastlino bolj pomembno omejevanje transpiracije (izgube vode) kot sprejem CO_2. Zaradi naštetih lastnosti obeh plinov se morajo listne reže bolj zapreti, da omejijo izhajanje vodnih molekul in s tem postanejo bolj nepropustne za CO_2 molekule (Kozlowski T. T. in Pallardy S. G., 1997b). Večja WUE pomeni več fiksiranega ogljika na enoto izgubljene vode (Farquhar G. D.in sod., 1989).

Glavni okoljski dejavniki, ki nadzorujejo izotopsko sestavo rastlinskega tkiva (v obravnavanem primeru branike), so torej tisti, ki nadzorujejo tudi prevodnost listnih rež ter fotosintezo (McCarroll D. in Loader N. J., 2004). Vrednost $\delta^{13}C$ v rastlini je lahko indikator WUE, saj je izotopska diskriminacija (Δ) funkcija razmerja med intercelularno in atmosfersko koncentracijo CO_2 oz. podaja podatek o izmenjavi izotopov med rastlino in atmosfero. Izotopska diskriminacija ogljika ($\Delta\ ^{13}C$), ki poteka v C3 rastlinah, je rezultat prednosti vezave lažjih ^{12}C pred težjimi ^{13}C atomi med fotosintezo. Definirana je z enačbo (Farquhar G. D.in sod., 1982):

$$\Delta = (\delta^{13}C_{(atmosfera)} - \delta^{13}C_{(rastlina)}) \ / \ (1 + (\delta^{13}C_{(rastlina)}/1000)) \qquad ... (6)$$

Izraz Δ se od $\delta^{13}C$ razlikuje v tem, da vsebuje le informacijo o spremembi izotopske sestave ogljika, ki se zgodi v rastlini in ne upošteva variacij, ki so posledica izotopske sestave CO_2 v atmosferi (Cernusak L. A. in sod., 2013). Izotopska diskriminacija ogljika je močno povezana z razmerjem med intercelularno in atmosfersko koncentracijo CO_2 in je lahko definirana tudi s pomočjo enačbe (Farquhar G. D.in sod., 1982):

$$\Delta = a + (b - a) * (c_i \ / \ c_a) \qquad ... (7)$$

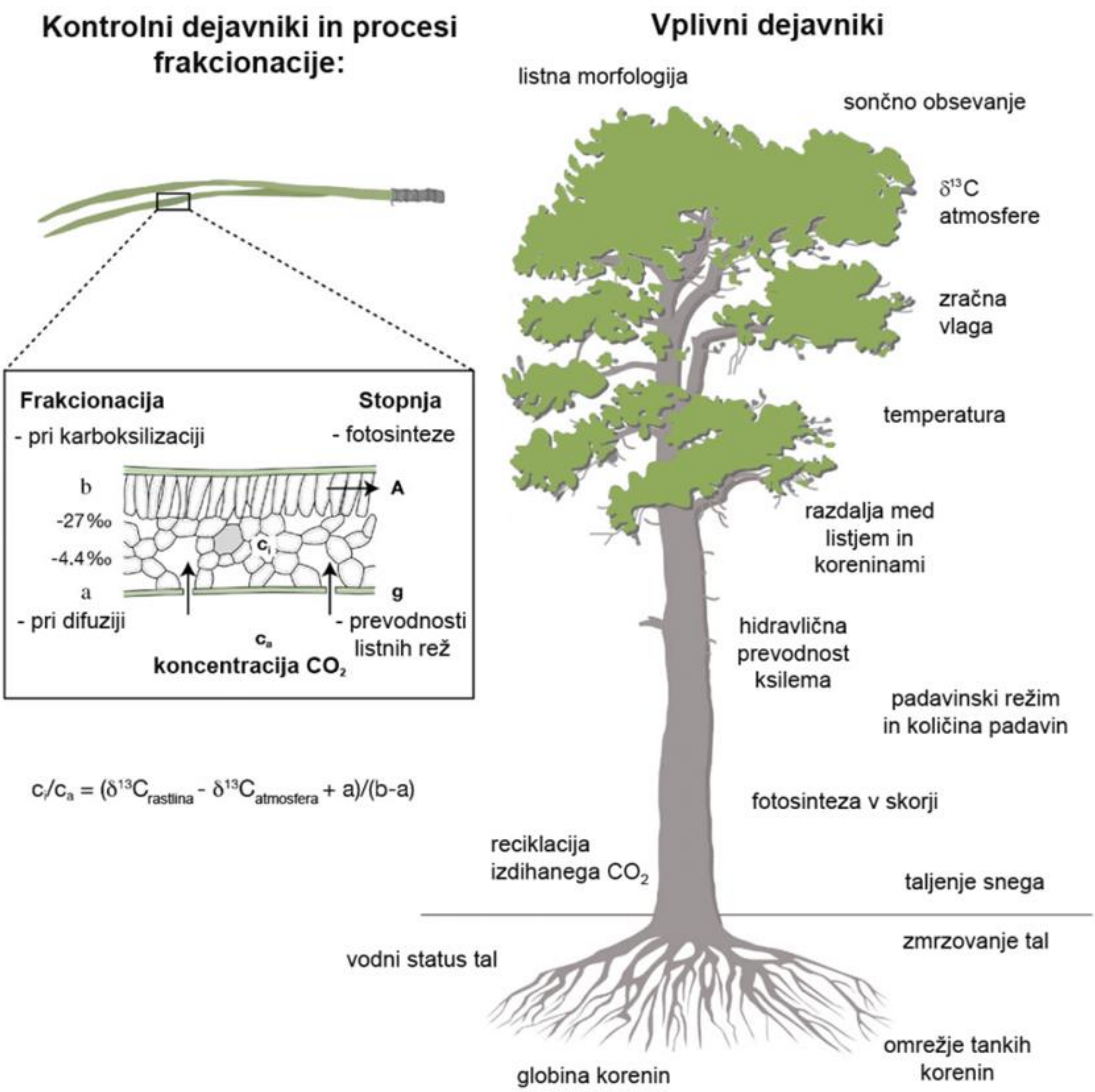
kjer »a« predstavlja difuzijski frakcionacijski efekt (-4,4 ‰), »b« frakcionacijo pri karboksilaciji (-27 ‰,), »c_i« intercelularno koncentracijo CO_2 ter »c_a« atmosfersko koncentracijo CO_2.

Izotopsko razmerje ogljika lahko izrazimo tudi kot WUE, saj je razmerje med fiksiranim kisikom in izgubo vode prav tako kot diskriminacijo ogljikovega izotopa nadzorujeta prevodnost listnih rež in hitrost poteka fotosinteze (Farquhar G. D.in sod., 1982; McCarroll D. in Loader N. J., 2004):

$$iWUE = c_a * (b - \Delta) / (1{,}6 * (b - a)) \quad \text{... (8)}$$

kjer je 1,6 razmerje med difuzivnostjo vodne pare in CO_2 v zraku, »a« predstavlja difuzijski frakcionacijski efekt (-4,4 ‰), »b« frakcionacijo pri karboksilaciji (-27 ‰,) ter »c_a« atmosfersko koncentracijo CO_2.

Ob predpostavki, da je vrednost $\delta^{13}C$ atmosfere konstantna (-8 ‰), je izotopska diskriminacija neposreden odsev sezonske spremembe v braniki, kjer večja vrednost $\delta^{13}C$ odgovarja zmanjšani izotopski diskriminaciji in veliki WUE (Leavitt S. W., 1992).



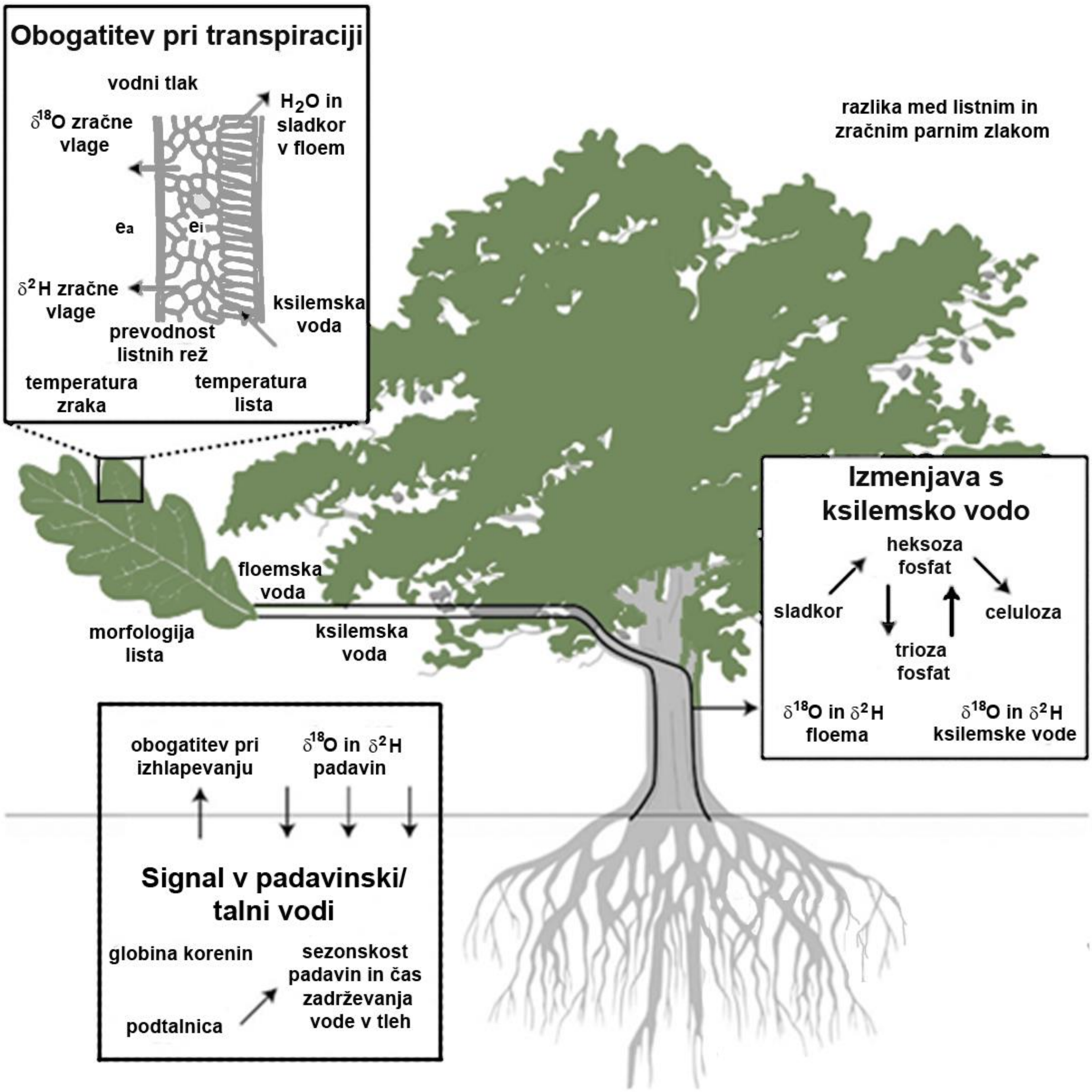
Sl. 1: Shema glavnih dejavnikov, ki vplivajo na frakcionacijo stabilnih ogljikovih izotopov in okoljskih dejavnikov, ki vplivajo nanje (povzeto po: McCarroll D. in Loader N.J., 2004)

Fig. 1: Diagram of the main controls on the fractionation of stable carbon isotopes and the environmental factors influencing them (after: McCarroll D. in Loader N.J., 2004)

2.2.4.3 Teorija vrednosti izotopske sestave stabilnega kisika in vodika v branikah

Izotopsko sestavo kisika in vodika v branikah dreves določajo izotopska sestava vira vode za rastlino (padavin ali podtalnice) (Waterhouse J. S. in sod., 2002), obogatitev listne vode pri transpiraciji (Roden J. S. in sod., 2000) ter biokemičnih frakcionacijskih faktorjev in izmenjave s ksilemsko vodo pri sintezi celuloze (Roden J. S.in sod., 2000; Waterhouse J. S.in sod., 2002).

Številni dejavniki in procesi vplivajo na izotopska razmerja v padavinski vodi. Na nastanek in izotopsko sestavo vlažnih zračnih mas vpliva kondenzacijska temperatura (Dansgaard W., 1964). Ko vodna para kondenzira, se vlažne zračne mase iznad mesta kondenzacije premikajo proti notranjosti topografsko raznolike celine. Prihaja do padavinskih dogodkov, pri katerih se iz vlažne zračne mase najprej odstranijo težji izotopi, ^{18}O in ^{2}H. S pomikanjem zračne mase iznad mesta evaporacije in pojavljanjem padavinskih dogodkov postaja izotopska sestava padavin vedno lažja (Craig H. in Gordon L. I., 1965). Na izotopsko razmerje padavin vplivajo tudi klimatske spremenljivke, kot so količina padavin, zračna vlaga in temperatura (McCarroll D. in Loader N. J., 2004). S spreminjanjem geografska širine od ekvatorja proti poloma ter večanjem nadmorske višine se prav tako spreminja izotopska sestava padavin in postaja obogatena z lažjima izotopoma H in O (Clark I. D. in Fritz P., 1997). Do frakcionacije in mešanja vode z različno izotopsko sestavo prihaja tudi v tleh, kjer se mešajo padavinska voda, podtalnica in talne vode različne starosti (McCarroll D. in Loader N. J., 2004). Korenine med privzemom vode iz tal ne diskriminirajo med lažjimi in težjimi izotopi (Ehleringer J. R. in Dawson T. E., 1992), zato ima ksilemska voda enako izotopsko razmerje kot vodni vir (Roden J. S.in sod., 2000). Do pomembne frakcionacije stabilnih vodnih izotopov prihaja v listih, kjer hitreje izhlapevajo molekule vode z lažjo izotopsko sestavo (Barbour M. M., 2007; McCarroll D. in Loader N. J., 2006; Roden J. S.in sod., 2000). Listni sladkorji so glede na sestavo listne vode obogateni s ^{18}O in osiromašeni s ^{2}H, saj med fotosintezo prihaja do močne diskriminacije prottežjega vodikovega izotopa (med -100 ‰ in -171 ‰) (Yakir D., 1992), medtem ko izmenjava kisika, vezanega na karboksilno skupino organskih molekul z vodo vodi do obogatitve sladkorjev v listu s težjim kisikovim izotopom (McCarroll D. in Loader N. J., 2006). Tretja pomembna točka frakcionacije vodnih izotopov se pojavi pri sintezi celuloze in lignina in je odvisna od stopnje izmenjave v sladkorjih vezanih O in H s ksilemsko vodo (Da S. L. Sternberg L. in sod., 1986).



Sl. 2: Shema glavnih dejavnikov, ki vplivajo na frakcionacijo stabilnih kisikovih in vodikovih izotopov ter okoljskih dejavnikov, ki vplivajo nanje (povzeto po: McCarroll D. in Loader N.J., 2004)

Fig. 2: Diagram of the main controls on the fractionation of stable oxygen and hydrogen isotopes and the environmental factors influencing them (after: McCarroll D. in Loader N.J., 2004)

2.3 METODE PRIPRAVE IN ANALIZE VZORCA

2.3.1 Priprava vzorca

Pri proučevanju izotopske sestave branike lahko analiziramo les ali pa njegove posamezne komponente, navadno α-celulozo in redko lignin. Celuloza je osnovni gradnik celične stene rastlin in je v lesu nemobilna substanca. Poleg tega sta njena konsistentna zgradba in unikatna pot biosinteze dva glavna razloga, da je bila ekstrakcija in analiza α-celuloze sprejeta kot standard v izotopski analizi branik (Verheyden A. in sod., 2005). Vzorec lesa, navadno leseni izvrtek, lahko na posamezne dele ločimo na več načinov. Prva in najpogosteje uporabljena metoda je razrez na posamezne branike na ostružke s pomočjo skalpela ali britvice. Braniko lahko ločimo na rani in kasni les. Če želimo analizirati izotopsko sestavo na več ali točno določenih mestih znotraj branike, lahko uporabimo mikrotom (Helle G. in Schleser G. H., 2004) ali laserske ablacije (Schulze B. in sod., 2004), najnovejše metode pa omogočajo tudi hkratno uporabo obeh omenjenih pristopov (Schollaen K. in sod., 2014).

Pri ekstrakciji α-celuloze se z dodajanjem raztopine natrijevega klorita ($NaClO_2$) odstrani lignin, nato pa se z dodajanjem raztopine natrijevega hidroksida (NaOH) odstrani še smole, maščobe (Green J. W., 1963; Loader N. J. in sod., 1997). Vzorec je pred nadaljnjo analizo potrebno homogenizirati. Najpogosteje uporabljeni metodi sta ultrazvočna homogenizacija ali mletje vzorca. Možna je tudi analiza lignina in/ali celotnega lesa, brez ločevanja na posamezne komponente. Analiza lignina je posebej primerna pri analizi lesa, ki je bil podvržen degradacijskim procesom, ki prej prizadenejo celulozo in hemiceluloze kot lignin (Loader N. J. in sod., 2003). Vse v tej doktorski disertaciji predstavljene analize izotopske sestave branike so osnovane na izotopski sestavi α-celuloze.

2.3.2 Masna spektrometrija

Masna spektrometrija se je po začetnih preizkusih različnih tehnik uveljavila kot edina metoda, s katero lahko dovolj natančno določimo izotopsko sestavo, da lahko iz rezultatov razberemo spremembe, ki so se dogajale v naravi (Pezdič J., 1999). Izotopsko sestavo

oziroma razmerje stabilnih izotopov izmerimo z masnim spektrometrom (IRMS – Isotope Ratio Mass Spectrometry) za analizo stabilnih izotopov lahkih elementov. Vzorec, ki ga želimo analizirati, moramo najprej homogenizirati in pretvoriti v plinsko obliko, kar nam omogočajo različni preparacijski sistemi (elementarni analizator). Plin nato dovajamo v masni spektrometer, kjer so nabiti delci pospeševani z visoko napetostjo električnega toka ter se nato preko cevi transportirajo preko močnega magnetnega polja do ionskih detektorjev. Zaradi različnega razmerja masa : naboj ionov se le-ti na magnetnem polju različno odklonijo in se na podlagi izotopskega razmerja združijo v žarke, ki jih na koncu cevi prestrežejo specifični detektorji (Faradayeve kletke). Ionski trki se v detektorjih pretvorijo v napetost električnega toka, ta pa nato v frekvenco trkov. Razmerje frekvenc trkov za določen vzorec predstavlja vrednost R (razmerje med težjim in lažjim izotopom), ki je eden od glavnih parametrov za izračun končne vrednosti δ (Dawson T. E. in Brooks P. D., 2001).
V nadaljevanju je podan splošen opis poteka analize izotopske sestave snovi, podroben opis v tej doktorski disertaciji uporabljenih metod pa je podan v poglavju Objavljena znanstvena dela (znanstvena članka v podpoglavjih 3.1.1. in 3.1.2).

2.3.2.1 Off-line metoda

Off-line metoda priprave vzorcev CO_2 iz branik dreves temelji na sežigu vzorca v vakuumsko zapečateni nepregorni (Pyrex) epruveti, v kateri je presežek bakrovega oksida, ki priskrbi kisik. Epruveto se 18 ur segreva do temperature 450 °C. Poteče reakcija sežiga celuloze in nastaneta CO_2 in H_2O. Vsako epruveto se nato analizira posamezno ali pa skupaj v serijah. V analizo je vključen tudi vzorec standarda, s čimer se zagotovi zveznost med serijami. Usklajevanje tlaka vzorčnega plina in standarda pred vsako analizo zagotavlja natančnost in zanesljivost rezultatov (približno 0,1 ‰). Glavna omejitev tega postopka je v tem, da je precej dolgotrajen in drag (McCarroll D. in Loader N. J., 2004).

2.3.2.2 On-line metoda

S trajanejem in s stroški raziskav povezane težave so se bistveno zmanjšale z razvojem metode neprekinjenega toka do IRMS. Sistem z elementarnim analizatorjem (EA), vmesnikom ter IRMS (EA-IRMS) omogoča »on-line« pripravo vzorcev, čiščenje in prenos vzorca v masni spektrometer neposredno preko neprekinjenega toka nosilnega plina (McCarroll D. in Loader N. J., 2004).

Za analizo izotopske sestave ogljika zatehtamo 300–350 μg homogenizirane α-celuloze in jo zavijemo v kositrovo kapsulo. V elementarnem analizatorju pri konstantnem toku helija poteče sežig pri 950-1000 °C. Sežig kositra z dodanim impulzom kisika sprosti zadosti toplote, da zagotovi popoln sežig vzorca. Produkti sežiga z nosilnim plinom (He) nato potujejo preko serije pasti. Kemična past odstrani sledi vode, s plinsko kromatografijo pa selektivno polovi mešanico nastalih plinov (CO_2 in N_2). Vzorčni plin (CO_2) nato preko kapilarne cevke potuje v masni spektrometer, kjer poteka nadaljnja analiza. Za analizo enega vzorca je potrebnih 8–12 minut, natančnost analize je ±0,1 ‰ (McCarroll D. in Loader N. J., 2004).

Za analizo izotopske sestave kisika 300–350 μg α-celuloze zavijemo v srebrovo kapsulo. Kapsulo sistem spusti v cev, kjer pri 1100 °C (ali do 1450 °C pri visokotemperaturnih napravah) poteče piroliza. Produkti pirolize (CO, H_2, N_2, CO_2 in H_2O) pri konstantnem toku helija potujejo preko serije pasti, ki odstranijo sledi vode in CO_2. Ker ima N_2 isto maso kot CO, ki ga analiziramo, mora biti v sistemu zagotovljena popolna ločitev teh dveh plinov. Čas analize posameznega vzorca variira med 10 in 20 minutami, natančnost analize je ±0,3 ‰ (McCarroll D. in Loader N. J., 2004). Analiza izotopske sestave vodika poteka podobno kot analiza izotopske sestave kisika. Razlikuje se v tem, da namesto α-celuloze analiziramo celulozni nitrat (Green J. W., 1963; Ramesh R. in sod., 1988) in da je potrebna višja temperatura za pirolizo (1430 °C). Natančnost analize je ±2,5 ‰ (McCarroll D. in Loader N. J., 2006).

Napredek v razvoju sedaj omogoča uporabo α-celuloze pri analizi izotopske sestave vodika (Filot M. S. in sod., 2006). Druge študije so pokazale, da je možno simultano merjenje izotopske sestave kisika in vodika pri pirolizi nastalega ogljikovega monoksida (Woodley E. J. in sod., 2011; Young G. H. F. in sod., 2011). Kot najbolj pomemben napredek, tako z ekonomskega kot znanstvenega vidika, pa se kaže razvoj metode za hkratno merjenje izotopskega razmerja vseh treh v celulozi zastopanih elementov (Loader N. J. in sod., 2014).

2.3.3 Atmosferska in PIN korekcija $\delta^{13}C$ kronologij

Od začetka industrializacije se z vedno večjo porabo fosilnih goriv v ozračje sprošča več lažjega izotopa ogljika (^{12}C) in posledično se je $\delta^{13}C$ atmosfere od leta 1850 znižala za približno 1,5 ‰. Trend se odraža tudi v branikah dreves in ga je pred nadaljnjimi analizami potrebno odstraniti. Korekcija poteka tako, da posamezni vrednosti za vsako posamezno leto prištejemo razliko med $\delta^{13}C$ atmosfere, ocenjene iz vzorcev ledu ali zraka (standardna vrednost) in ocenjeno $\delta^{13}C$-vrednostjo atmosfere pred letom 1850 (-6,4 ‰). Drug način odstranitve naraščajočega trenda vsebnosti ^{12}C v atmosferi je izračun diskriminacije Δ (enačba 6) (McCarroll D. in Loader N. J., 2006).

Kljub korekciji povišane $\delta^{13}C$-vrednosti atmosfere v $\delta^{13}C$ je v braniki še vedno prisoten padajoči trend, za katerega ni klimatskih indicev (Gagen M. in sod., 2007; Treydte K. in sod., 2001; Waterhouse J. S. in sod., 2004). Zato je posebej pri klimatskih rekonstrukcijah potrebno vpeljati še korekcijo, ki upošteva fiziološki odziv dreves na povečane koncentracije CO_2 v atmosferi. Namen te korekcije je prilagoditev $\delta^{13}C$-vrednosti branik tistim vrednostim, ki bi jih dosegli, če bi drevesa rasla v razmerah pred industrijsko revolucijo (McCarroll D. in sod., 2009). Šablonski pristop k tej korekciji predlaga dodajanje standardne, matematično določene $\delta^{13}C$-vrednosti izmerjenim vrednostim in predpostavlja, da je vpliv povišane koncentracije CO_2 na vsa drevesa enak in linearen (Feng X. in Epstein S., 1995; Kürschner W. M., 1996; Treydte K. S. in sod., 2009). McCarroll in sodelavci (2009) pa so predlagali t. i. PIN korekcijo, nelinearno odstranitev trenda za vsako posamezno drevo, ki upošteva teorijo izotopske frakcionacije in fiziološke omejitve, kako naj bi se drevo potencialno odzvalo na povečane koncentracije CO_2. Korekcija temelji na dveh predpostavkah: aktivnem ali pasivnem odzivu rastlin na povišano koncentracijo CO_2 v atmosferi. Korekcija je unikatna za vsako drevo posebej in ne predvideva enakega ter linearnega odziva dreves na povečano koncentracijo CO_2 v atmosferi. Izračun korekcije omogoča skripta »pin« v programu R.

2.3.4 Juvenilno obdobje in rastni trend

Študije navajajo, da je pri proučevanju δ^{13}C-vrednosti v branikah mladih dreves prisoten t. i. učinek juvenilnega obdobja. Uveljavila se je razlaga, da je pojav kombinacija delovanja zmanjšanega potenciala listne vode ter naraščanje razmerja med prevodnim delom lesa in listno površino (McDowell N. in sod., 2002; Schäfer K. V. R. in sod., 2000). Starost kambija vpliva tudi na lesnoanatomske lastnosti, kot so širina branike, dimenzije trahej, delež vlaken, trahej in aksialnega parenhima v kasnem lesu (Gasson P., 1987; Lei H. in sod., 1996). Medtem ko pri proučevanju izotopskega razmerja in anatomskih lastnosti branike juvenilno obdobje navadno izločimo iz raziskave, vpliv drugih dejavnikov (rastni trend in vpliv sestoja) na širino branike, kasnega in ranega lesa odstranimo s standardizacijo (Cook E. R. in Kairiukstis L. A., 1990).

2.4 DENDROKRONOLOŠKE RAZISKAVE NA PODLAGI MACESNOVIH IN DOBOVIH BRANIK

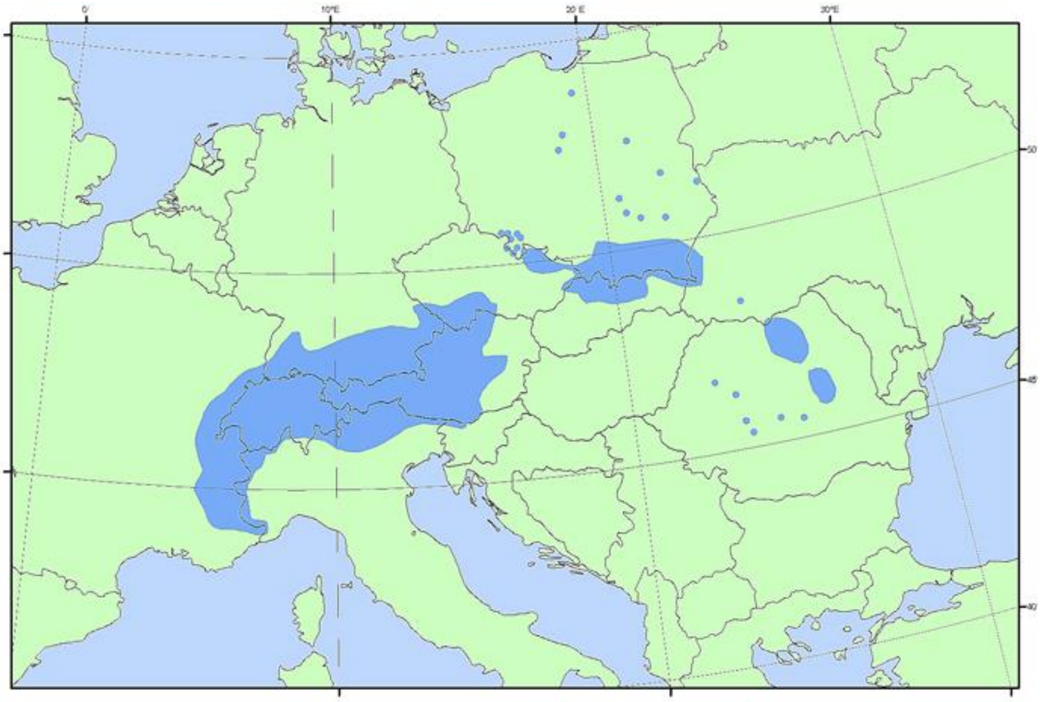
2.4.1 Evropski macesen (*Larix decidua* Mill.)

2.4.1.1 Ekologija in razširjenost evropskega macesna

Evropski macesen je listopadni iglavec, ki sodi v družino borovk (*Pinaceae*). Je dolgoživa drevesna vrsta z močnim srčastim koreninskim sistemom, ki lahko raste v zelo ekstremnih razmerah. Najbolje uspeva na globokih, rahlih, zračnih, svežih ter mineralno bogatih tleh. Bolje kot na silikatu uspeva na apnencu, prenese tudi neutrjene in nestabilne podlage. Poleg talne potrebuje tudi zračno vlago in dobro prevetrene lege. Za uspevanje potrebuje le malo toplote in se zadovolji že s 50 dni dolgo vegetacijsko dobo. Je izrazito svetloljubna vrsta, ki za rast potrebuje celo nekaj svetlobe od strani. Odporen je na zimski mraz (do -50°C), spomladansko in jesensko zmrzal ter požar, dobro prenaša tudi močan veter, obremenitve s snegom in valeče kamenje (Brus R., 2004).

Areal evropskega macesna sestoji iz štirih delov in v vsakem uspeva druga evropska rasa: alpski macesen na območju evropskih Alp, karpatski macesen v Tatrah, poljski macesen na

Poljskem in sudetski macesen na Češkem. Alpski macesen v Sloveniji predstavlja jugovzhodni rob areala te rase (Brus R., 2004; Kotar M. in Brus R., 1999; Maier J., 1992) in je naravno razširjen v Julijskih Alpah, Karavankah in v Kamniško-Savinjskih Alpah, manjša rastišča pa najdemo tudi v predalpskem fitogeografskem območju. Uspeva v višinskem pasu med 520 in 1900 m nadmorske višine, prevladujoč pa je v zgornjem gorskem in subalpinskem pasu med 1600 in 1800 m nadmorske višine (Dakskobler I. in Kutnar L., 2012).



Sl. 3: Naravna razširjenosti evropskega macesna, povzeto po: EUFORGEN (2013)

Fig. 3: Natural distribution of european larch, after: EUFORGEN (2013)

Macesnov les je zelo trajen v vodi, odporen je proti glivam in tudi proti atmosferskim vplivom ter kislinam. Široko je uporaben v tradicionalnem gradbeništvu (pastirski stanovi in kozolci), v splošnem in stavbnem mizarstvu, za zunanje in notranje konstrukcije, pode, vrata, okna, prav tako tudi za vodne konstrukcije, mostove, ladjedelništvo, jambore, drogove itd. (Čufar K., 2006).

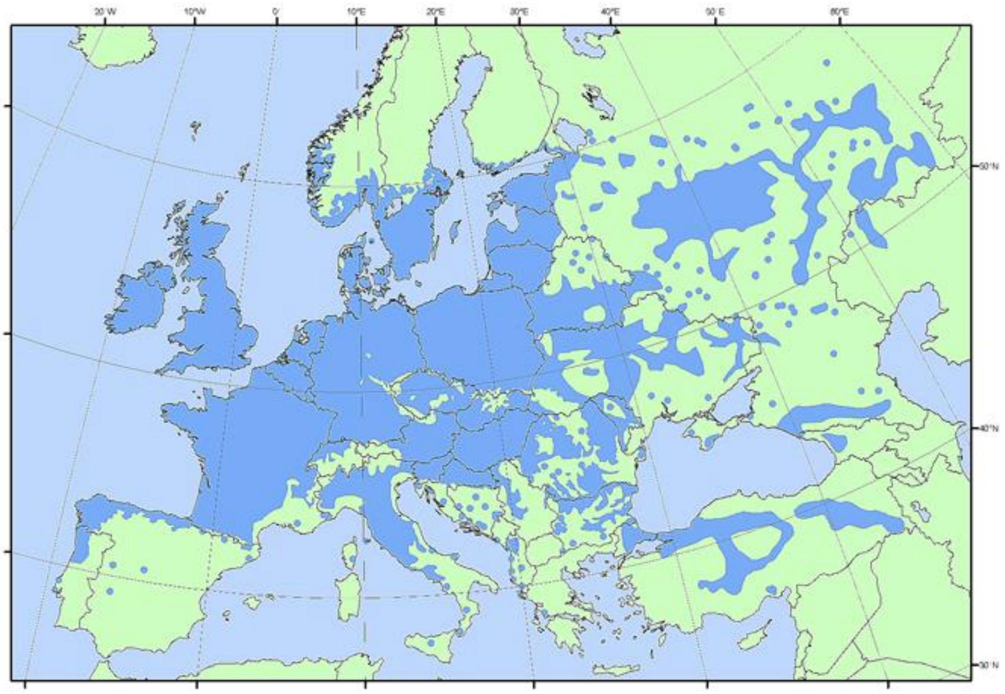
2.4.1.2 Dendrokronološke raziskave macesna

Zaradi dolgoživosti, obstojnega lesa in rasti na izpostavljenih legah je macesen zelo primerna vrsta za dendrokronološke analize, za preučevanje odziva drevesa na klimo in rekonstrukcijo klimatskih spremenljivk v obdobju pred instrumentalnimi meritvami (Levanič T., 2005). Med prvimi objavljenimi dendrokronološkimi raziskavami v Sloveniji je dendrokronološka analiza ostrešja cerkve Sv. Jurija v Piranu, kjer so Levanič in sodelavci (1997) na podlagi analize širin branik datirali pomembne gradbene faze ostrešja in določili izvor uporabljenega lesa. Trebušak (1998) je po planinah okoli Bohinjskega jezera izdelal prvo daljšo slovensko macesnovo kronologijo širin branik, dolgo 518 let. Ta je bila nadgrajena s skupno italijansko-slovensko macesnovo kronologijo (Levanič T. in sod., 2001) in kronologijo macesna za območje jugovzhodnih Alp (Levanič T., 2005). Analiza je pokazala, da se macesni na nadpovprečne junijske in tudi majske in julijske temperature odzivajo s širšo braniko (Levanič T., 2006). Analiza macesnovih branik se je izkazala kot primerno orodje za proučevanje pojavljanja plazov (Stoffel M. in sod., 2006), požarov, paše (Genries A. in sod., 2009), začetka in trajanja rastnega obdobja macesna (Moser L. in sod., 2009), pojavljanja gradacij metulja *Zeiraphera griseana* (Kress A. in sod., 2009), proučevanje sprememb v občutljivosti širin branik na klimatske razmere (Carrer M. in Urbinati C., 2006) in klimatske rekonstrukcije (Büntgen U. in sod., 2006; Corona C. in sod., 2010).

2.4.2 Dob (*Quercus robur* L.)

2.4.2.1 Ekologija in razširjenost doba

Dob je vrsta kolinskega pasu (do 400 m n. v.), najdemo ga v nižinskih legah, na dnu kotlin in na položnejših pobočjih. Je izrazito svetloljubna vrsta. Za njegovo optimalno uspevanje so najprimernejša globoka peščena, ilovnata ali glinena, mineralno bogata in humozna tla, z visokim nivojem podtalnice (0–2 metra) in redno poplavljana tla. Prenese tudi nekoliko bolj suha tla, poletno vročino in nizke zimske temperature. Občutljiv je na pozne spomladanske pozebe, sicer pa so njegove potrebe po toploti razmeroma skromne. Za uspešno rast potrebuje vsaj 4 mesece trajajoče vegetacijsko obdobje (Brus R., 2004). Naravni areal doba obsega skoraj vso Evropo, z izjemo južnega dela Španije, v Skandinaviji pa naseljuje le južno četrtino. Na vzhod se njegov areal razteza do Urala in Kavkaza. Njegova najbolj značilna rastišča so poplavne ravnice evropskih rek (Donava, Ren ...). V Sloveniji so bile po nižinah dobrave nekoč močno razširjene, s časom pa se je mnogo gozdov te avtohtone vrste umaknilo kmetijski rabi tal. Krakovski gozd je največji ohranjeni kompleks nižinskih poplavnih dobrav v Sloveniji, ostanke dobrav pa najdemo tudi po drugih nižinskih in ravninskih predelih Slovenije (Brus R., 2004).



Sl. 4: Naravna razširjenosti doba, povzeto po: EUFORGEN (2013)

Fig. 4: Natural distribution of pedunculate oak, after: EUFORGEN (2013)

Hrastov les je gost in trd. Njegove branike so jasno razločne, rani les z velikimi trahejami se jasno loči od kasnega lesa predhodnega leta, kjer so traheje manjše in včasih komaj razvidne. Velja za skoraj neomejeno trajen les za podvodne konstrukcije (Čufar, 2006). Lesa doba in gradna po zgradbi ni mogoče razlikovati, zato kadar ne poznamo točnega izvora dreves (npr. pri vgrajenem lesu), govorimo o hrastovini in evropskem hrastu (*Quercus sp.*) (Čufar K. in sod., 2014).

2.4.2.2 Dendrokronološke raziskave doba

Začetki dendrokronoloških raziskav hrasta v Sloveniji so potekali na območju Ljubljanskega barja predvsem za potrebe arheologije (Čufar K. in sod., 1999; Čufar K. in Velušček A., 2003; Pavlič T., 1997). Kasneje so se dendrokronološke raziskave hrasta usmerile v proučevanje propadanja hrasta kot posledice človekovega poseganja v prostor, kot so npr. melioracije in gradnje cest ter v proučevanja odziva dreves na klimo (Čater M. in Levanič T., 2004; Levanič T. in sod., 2011). Iz širin branik živih hrastovih dreves in konstrukcijskega lesa je bila zgrajena 548 let dolga kronologija (Čufar K. in sod., 2008b), na podlagi katere so ugotovili, da na širino branik ugodno vplivajo junijske padavine, negativno pa junijske temperature, rekonstruirali so tudi suha in mokra poletja (Čufar K. in sod., 2008a). Številne lokalne hrastove kronologije z različnih predelov Slovenije (Čufar K.in sod., 2008b; Čufar K. in Levanič T., 1999) se med seboj zelo razlikujejo, še posebej dobove kronologije iz nižinskih predelov, kjer na rast pogosto vplivajo mikroklimatske razmere (Čater M., 2003; Čater M. in Batič F., 2006; Čater M. in Levanič T., 2004; Levanič T., 1993; Levanič T.in sod., 2011). Primerjava hrastovih kronologij širin branik iz osrednje in zahodne Evrope je na eni strani pokazala dobro telekonekcijo, na drugi strani pa se kažejo tudi precejšnje razlike v odzivu na klimatske dejavnike (Haneca K. in sod., 2009). Tudi v primerjalni študiji hrastovih kronologij z območij južno in jugovzhodno od Alp z zahodnimi kronologijami se je izkazalo, da na rast hrastov v različnih predelih Evrope verjetno vplivajo različni klimatski dejavniki in njihovi ekstremi (Čufar K. in sod., 2014).

3 ZNANSTVENA DELA

3.1 OBJAVLJENA ZNANSTVENA DELA

3.1.1 Klimatski signal v širinah branik ter izotopski sestavi ogljika, vodika in kisika v branikah *Larix decidua*, rastočega na gozdni meji v severovzhodnih evropskih Alpah

Climate signals in the ring widths and stable carbon, hydrogen and oxygen isotopic composition of *Larix decidua* growing at the forest line in the southeastern European Alps

Polona Hafner, Iain Robertson, Danny McCarroll, Neil J. Loader, Mary Gagen, Roderick J. Bale, Högne Junger, Eloni Sonninen, Emmi Hilasvuori, Tom Levanič
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Območje severovzhodnih evropskih Alp je slabo pokrito s proxy podatki visoke resolucije. Poleg tega je za to območje značilen drugačen klimatski režim kot za severne in zahodne predele Alp. Predstavljamo serijo novih proxy podatkov za obdobje 1907–2006, pridobljenih na podlagi analize širin branik ter izotopskega razmerja stabilnih ogljikovih ($\delta^{13}C$), neizmenljivih vodikovih ($\delta^{2}H$) in kisikovih ($\delta^{18}O$) izotopov v celulozi, izolirani iz branik evropskega macesna, rastočega na gozdni meji v jugovzhodnih Alpah (Slovenija). $\delta^{13}C$, $\delta^{2}H$ in $\delta^{18}O$ so značilno ($p < 0{,}001$) in pozitivno povezani med seboj. Junijska temperatura ima najmočnejši vpliv na širino branike (TRW), medtem ko razmere v juliju in avgustu vplivajo na izotopsko zgradbo branike. Vsi štirje seti proxy podatkov so značilno ($r > 0{,}4$; $p < 0{,}001$) povezani s poletno temperaturo in tudi s številom sončnih ur, medtem ko imajo padavine manjši vpliv. Kombiniranje podatkov TRW in $\delta^{13}C$ vsebuje največji potencial za rekonstrukcijo poletnih (junij–avgust) temperatur v preteklosti, saj je v značilni ($p < 0{,}001$) korelaciji s povprečnimi temperaturami na širšem območju južne in zahodne Evrope zahodno od Karpatov. V vodnih izotopih (kisik in vodik) je shranjena informacija o razmerah na območju Jadrana/Mediterana, kjer je izvorno območje vlažnih zračnih mas, ki prinašajo padavine na proučevano območje. To se kaže v visokih korelacijah s temperaturo v južni Italiji in na zahodnem delu Balkanskega polotoka. Kombinacija proxy podatkov z različnimi prostorskimi in časovnimi signali omogoča pridobitev boljše in močnejše klimatske informacije. Ti rezultati odpirajo nov pogled na klimatske rekonstrukcije na območju jugovzhodnih Alp in zahodnega Balkana.

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ORIGINAL PAPER

Climate signals in the ring widths and stable carbon, hydrogen and oxygen isotopic composition of *Larix decidua* growing at the forest limit in the southeastern European Alps

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Abstract The southeastern border of the European Alps is not well resourced with high-resolution climate proxies and experiences a distinct climatic regime from the northern and western Alpine zones. Here, we present new high-resolution climatic proxies (AD 1907–2006) from ring widths and stable carbon (δ^{13}C), non-exchangeable hydrogen (δ^{2}H) and oxygen (δ^{18}O) isotope ratios of cellulose extracted from *Larix decidua* tree rings, growing at the forest limit in the southeastern European Alps (Slovenia). δ^{13}C, δ^{2}H and δ^{18}O are strongly ($p < 0.001$) and positively correlated with each other. June temperature has the strongest control on tree ring width (TRW), while later summer conditions (July–August) influence the stable isotope composition. All four proxies are strongly correlated ($r > 0.4$; $p < 0.001$) with summer temperature and also sunshine hours, while precipitation has less impact. A combination of TRW and δ^{13}C provides the greatest potential for reconstructing past temperatures (June–August) with significant ($p < 0.001$) correlations with gridded temperatures extending across a very large part of southern and western Europe west of the Carpathian Mountains. The water isotopes (oxygen and hydrogen) record conditions in the Adriatic and Mediterranean, which are the source area for the air masses that bring precipitation to this region giving strong correlations with temperatures in southern Italy and the western part of the Balkan Peninsula. Combining proxies with different spatial and temporal signals allows the strength and spatial footprint of climate signals to be enhanced. These findings open new perspectives for climate reconstruction in the southeastern European Alps and Western Balkans.

Keywords Dendroclimatology · Dendrochronology · European larch · Slovenia · Tree ring · Stable isotopes

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Introduction

European larch (*Larix decidua* Mill.) is a long-lived and temperature-sensitive (Rolland et al. 1998, Carrer and Urbinati 2006) species distributed over the European Alps, Tatra Mountains, Poland and the Sudetan region (Maier 1992). The mountains of Slovenia, in the southeastern corner of the European Alps, represent the southeastern border of the Alpine *Larix decidua* population (EUFORGEN 2009). Since any changes in tree growth and ecological equilibrium are likely to occur first in stands at the boundaries of the ecological range of a species (Tessier et al. 1997), this area offers potential for reconstructing past changes in climate and environment and for predicting the impact of environmental change on the forest ecosystem. One of the concerns about using larch as a palaeoclimatic archive is the regular occurrence of the larch bud-moth (*Zeiraphera diniana* Gn.)

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causing non-climatic variations in the resulting time series. However, bud-moth outbreaks do not appear to affect the isotopic composition of tree rings (Kress et al. 2009) and corrections can be applied for ring-width to be able to extract a climate signal (Büntgen et al. 2005). Furthermore, forests in Slovenia appear to be located beyond the documented range of the larch bud-moth (Bjørnstad et al. 2002) or its projected future distribution (Johnson et al. 2010).

Tree rings of European larch have been so far used to investigate debris flow occurrence, snow avalanches (Stoffel et al. 2006), fires and grazing (Genries et al. 2009); to assess the timing and duration of larch growth season (Moser et al. 2009); to reconstruct bud-moth outbreaks and to assess their influence on climate reconstruction (Büntgen et al. 2009; Kress et al. 2009; Weidner et al. 2010); to study the influence of climate on radial tree growth (Levanič 2006); to detect changes in the sensitivity of tree ring growth to climate (Carrer and Urbinati 2006) and to reconstruct climate in the past. Although Alpine summer temperatures have been reconstructed based on larch tree ring maximum latewood density (MXD) and/or tree ring width (TRW) (Büntgen et al. 2006; Corona et al. 2010), there has been limited dendroclimatological research in the southeastern Alps and western Balkans. The results showed that tree ring growth is closely correlated with temperature in June (Carrer and Urbinati 2006; Levanič 2006). Carrer and Urbinati (2006) exposed the differences in chronology statistics in easternmost sites and attributed them to the lower mass effect of the mountains and the closeness of the Adriatic Sea on more pronounced oceanic climatic regime. In the recent years, the stable isotopic composition of tree rings has been increasingly used both to reconstruct past climate (Young et al. 2010; Etien et al. 2008; Kress et al. 2010; Loader et al. 2008; Treydte et al. 2006) and to monitor changes in tree physiological response to increasing levels of atmospheric CO_2 (Feng 1998; Saurer et al. 2003; Waterhouse et al. 2004). Stable carbon isotope composition (δ^{13}C) of tree rings depends on the environmental variables which influence the uptake of CO_2 from the atmosphere regulated by stomatal conductance and rate of carboxylation during photosynthesis (Farquhar et al. 1989). The dominant control of stable carbon isotopic composition in plant tissue at dry sites is likely to be stomatal conductance, which responds to moisture stress and humidity. At well-watered sites, photosynthetic rate is likely to dominate stable carbon isotope composition and the strongest correlations tend to be seen between δ^{13}C and sunshine and temperature (McCarroll and Loader 2004, 2005). Stable oxygen and non-exchangeable hydrogen isotope composition (δ^{18}O and δ^{2}H, respectively) of tree rings is determined by the isotopic composition of source water (Waterhouse et al. 2002), enrichment of leaf water due to transpiration (Roden et al. 2000), biochemical fractionation factors and exchange with xylem water during cellulose synthesis (Roden et al. 2000; Waterhouse et al. 2002). Therefore, the stable isotope ratios of water isotopes in plant tissues have a complex association with climate.

Very few studies have yet reported stable isotope composition of European larch tree rings (Johnson et al. 2010; Kress et al. 2009, 2010). To date, only one paper has investigated the stable carbon isotope composition of wood (*Picea abies*) in the southeastern Alps and it shows a strong response to July–August temperatures (Levanič et al. 2008). There have been no reported measurements of tree ring δ^{18}O and δ^{2}H in the southeastern Alps and there are a very few long chronologies of their concurrent measurement in tree rings elsewhere (Boettger et al. 2003; Hilasvuori and Berninger 2010; Loader et al. 2008; Szczepanek et al. 2006). Since the stable isotope ratios of carbon, non-exchangeable hydrogen and oxygen are not controlled by the same environmental factors, their combination could potentially offer a better understanding of the environmental physiology of larch trees as well as increasing the potential for palaeoclimatic reconstruction.

In this study, we present the first stable carbon, non-exchangeable hydrogen and oxygen isotope chronologies of *Larix decidua* tree rings from the southeastern Alps, spanning the period AD 1907–2006. We quantified the climate signal in stable isotope composition and tree ring widths to assess the most important periods of the annual cycle and the most significant climate parameters influencing tree ring proxies. The results are used to demonstrate the potential for climate reconstruction in the southeastern Alps and wider surrounding area using both single proxies and multi-proxy combinations.

Materials and methods

Site and sampling

Trees were sampled from two forest line sites, Dleskovška plateau and Vršič, at 1,700 m a.s.l. in the mountains of northern Slovenia, the southeastern part of the European Alps (Fig. 1). Dominant soil types are lithosol and rendzina on limestone or dolomite (Dakskobler et al. 2010). The open forests are classified as "Rhodothamno-Laricetum" and composed of European larch (*Larix decidua* Mill.) in combination with *Picea abies, Pinus mugo*, *Fagus sylvatica*, *Sorbus aucuparia* and *Abies alba*. Mean annual temperature at the nearest relevant weather station at Krvavec (1,740 m a.s.l.; 15 km from Dleskovška plateau and 60 km from Vršič) is 3.3°C with an average summer temperature (June, July and August) of 10.9°C. Mean precipitation amount at sampling sites in the growing season (April–September) is estimated to be between 1,000 and 1,200 mm

Fig. 1 Map of SE Alps with marked location of the study sites (Vršič and Dleskovška plateau) and the nearby meteorological stations (VillacherAlpe and Eisenkappel)

(Ceglar and Kajfež-Bogataj 2008). Tree diameter ranged from 45 to 85 cm and the age of collected samples was between 96 and 562 years. In this study, data are presented for the recent period covering AD 1907–2006.

Tree ring width (TRW) chronology

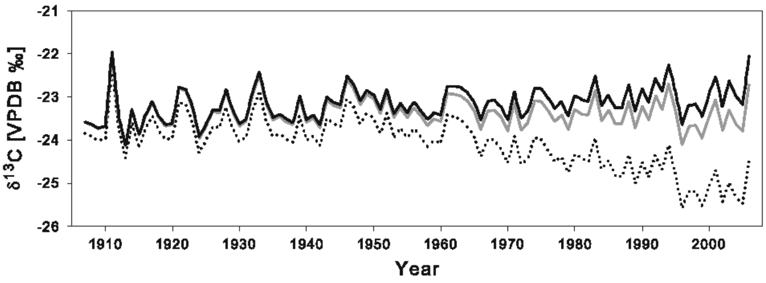
One or two 5 mm diameter increment cores were taken at breast height (1.3 m) from each of 29 dominant trees. The cores were air-dried and glued onto wooden holders, sanded and polished with progressively smoother abrasive paper. Samples were scanned using the ATRICS system (Levanič 2007), ring widths measured using WinDENDRO software (www.regentinstruments.com) and absolute dating achieved using PAST-4 software. Individual TRW series were standardised to remove long-term trends (Cook 1985) using a 67% cubic smoothing spline with a 50% frequency cut-off in ARSTAN (Cook and Holmes 1986). Each year's ring width was divided by that year's fitted value to give a dimensionless index with a mean of 1. This procedure removed non-climatic trends due to tree age, size and the effects of stand dynamics (Cook and Kairiukstis 1989). Index values were pre-whitened using an autoregressive model selected on the basis of the minimum Akaike Criterion and combined across all series using bi-weight robust estimation of the mean to exclude the influence of the outliers. ARSTAN produces four chronologies. For the purpose of this research, we used the standardised (STD) chronology, which represents a robust estimate of the arithmetic mean and contains autoregression (Cook 1985).

Stable isotope ratio chronologies

Samples for isotopic analysis were obtained using a 12-mm borer, air-dried and absolutely dated. Then, they were divided into annual tree rings using a scalpel. The whole ring was used for isotopic analysis as the rings were often too narrow to yield sufficient α-cellulose from separate earlywood and latewood fractions. High correlations have been reported between early and late wood carbon isotope values (Robertson et al. 1997) and Kress et al. (2009) recently concluded that the whole ring of conifers growing at marginal sites is suitable for isotopic analysis. To minimise potential age-related trends in stable isotope chronologies, the first (youngest) 50 tree rings were not used in the analysis (Loader et al. 2007; Gagen et al. 2007). The stable isotopes of carbon ($\delta^{13}C$), oxygen ($\delta^{18}O$) and non-exchangeable hydrogen ($\delta^{2}H$) were analysed for each tree individually and at annual resolution.

α-cellulose was isolated from each sample using the standard techniques (Loader et al. 1997; Rinne et al. 2005) and homogenized using a Hielscher ultrasonic probe to yield a homogenised fibrous sample that was used for carbon and oxygen isotope analysis. The samples were freeze-dried for at least 48 h. For hydrogen isotopic analysis, the exchangeable hydroxyl groups were replaced by nitration using a mixture of nitric and phosphoric acids (Green 1963). The dry cellulose nitrate was dried and dissolved in the minimum volume of anhydrous acetone and poured into an excess of cold deionised water to form a white flocculant product (Ramesh et al. 1988). The samples were dried again in an oven at 50°C for at least 48 h. For

Fig. 2 Effect of corrections on the δ^{13}C chronology. *Dotted line* represents raw δ^{13}C values. *Grey line* represents δ^{13}C chronology after correction for the "anthropogenic" effect. The *black line* shows the "pin" corrected δ^{13}C chronology (McCarroll et al. 2009)



carbon isotope analysis, 0.30–0.35 mg of α-cellulose was weighed into tin capsules and combusted over chromium(III) oxide and copper(II) oxide at 1,000°C (Swansea). For oxygen isotope analysis, 0.30–0.35 mg of α-cellulose was weighed into silver capsules and pyrolysed over glassy carbon at 1,090°C (Swansea) or 1,330°C (Helsinki). Owing to the number of analyses conducted, it was necessary in this study to perform the measurements for oxygen isotopes in two laboratories. Before commencing analysis, both laboratories participated in an intercomparison exercise to evaluate any differences between the methods that may result from the different equipment/pyrolysis temperatures (Boettger et al. 2007). Shared analysis of standard materials and assignment of specific trees to each laboratory further reduced potential analytical/methodological uncertainties and enable the series to be combined. Combustion and pyrolysis were conducted using a PDZ Europa ANCA GSL elemental analyser interfaced to a PDZ Europa 20–20 stable isotope ratio mass spectrometer (Swansea). Pyrolysis was conducted on a Finnigan TC/EA high temperature elemental analyser interfaced (by ConFloIII) to a Thermofinnigan Delta^{Plus} Advantage isotope ratio mass spectrometer (Helsinki). For hydrogen isotope analysis, 0.3–0.4 mg of dry cellulose nitrate was weighed into a silver capsule, dried in open capsules in a vacuum oven, closed, and kept in the vacuum oven until pyrolysed at 1,430°C using a TC/EA high temperature elemental analyser interfaced with a Thermo-Finnigan Delta^{Plus} XL mass spectrometer (Helsinki). Isotope ratios are expressed as per mille deviations using the standard delta notation (δ) relative to VPDB (carbon) and VSMOW (hydrogen and oxygen) standards (Coplen 1995). Analytical precision was typically ±0.1‰ for carbon, ±0.3‰ for oxygen and ±2‰ for hydrogen isotope analysis.

Corrections applied to the δ^{13}C series

Two corrections were applied to the δ^{13}C series. After approximately AD 1850, δ^{13}C values of atmospheric CO_2 have decreased, primarily due to the burning of fossil fuels that are depleted in ^{13}C. This "anthropogenic decline" is directly reflected in tree ring δ^{13}C values and is corrected simply by adding to each year the difference between the atmospheric δ^{13}C value estimated from ice cores or air samples and the estimated value in AD 1850 (−6.4‰). The procedure is described by McCarroll and Loader (2005) and performs a simple mathematical correction to express each δ^{13}C value relative to the pre-industrial atmsopheric δ^{13}C level. This way $\delta^{13}C_{atm}$ chronology is obtained. The second correction takes into account the physiological response of trees to changes in the atmospheric concentration of CO_2, the intention being to adjust tree ring δ^{13}C values to those that would have been obtained under pre-industrial conditions (McCarroll et al. 2009). It is essentially a non-linear de-trending of individual tree δ^{13}C series, based on the stable isotope fraction theory and utilises logical constraints on the way that trees could potentially respond to rising CO_2. The correction is unique to each tree individually and does not assume a uniform and linear response of trees to the increased concentration of CO_2 in the atmosphere. This pre-industrial or "pin" correction is more contentious than the purely isotopic correction, but has been used successfully elsewhere (Gagen et al. 2007, 2008; Loader et al. 2008, 2010; McCarroll et al. 2011; Young et al. 2010). So far no reported research has demonstrated "pin" corrected δ^{13}C chronologies at the Alpine sites as there is a lack of long tree ring δ^{13}C chronologies. $\delta^{13}C_{atm}$ chronology was used in climate correlation analysis for the comparison of the results with $\delta^{13}C_{pin}$ chronology. $\delta^{13}C_{pin}$ chronology was used for all other analysis. All three δ^{13}C chronologies are presented in Fig. 2.

Climate data

The southeastern part of the Alps lacks long-term meteorological stations at high altitude. Data series from the two local stations, closest to the field sites (Zgornje Jezersko at 894 m a.s.l. and Krvavec at 1,740 m a.s.l.) are too short (22 and 32 years, respectively) to be used for the calibration and verification. Meteorological data were, therefore, obtained from two HISTALP (Auer et al. 2007) meteorological stations close to the Slovenian border but in Austria: Villacher Alpe (2,160 m a.s.l.) and Eisenkappel

(623 m a.s.l.). For Villacher Alpe, the temperature record extends back to AD 1851 and the sunshine back to AD 1884. For Eisenkappel, the precipitation dataset extends back to AD 1886. Regression analysis showed a strong correlation between temperatures measured at Villa-cherAlpe and local meteorological stations ($r^2 = 0.98$). As the same trend was observed in both series, so the Villacher Alpe temperature and sunshine duration dataset was used for further analysis. For precipitation, the fit between Eisenkapel and Slovenian stations for June/July/August precipitation was $r^2 = 0.84$. For each month, separately a regression model was established between the short Zgornje Jezersko and long Eisenkappel precipitation data-set and used to extend the Jezersko dataset, which was used in the further analysis.

Statistical analysis

The Expressed Population Signal (EPS) was calculated to assess the common forcing in stable isotope and tree ring width chronologies. An EPS ≥0.85 is generally accepted to be high enough to show that analysed tree ring series represent the common forcing mechanisms (Wigley et al. 1984, Robertson et al. 1997). To check the regularity of combining data from two sites into one chronology, we tested the equality of tree ring series from both sites with ANOVA and *t* test using SigmaStat module of SigmaPlot (version 11). Tree ring proxies were compared to average monthly temperatures, amount of sunshine and precipitation using Pearson's correlation coefficient (*r*). The spatial patterns of correlation between tree ring proxies and summer temperature were investigated using the monthly gridded (0.5° × 0.5° grid) CRU TS 3 temperature dataset (Mitchell and Jones 2005), accessed via the KNMI Climate Explorer website (Oldenborgh et al. 2004).

The potential for using the tree ring proxies for the climate reconstruction was investigated using both single proxies and multi-proxy combinations. The likely skill of regression-based reconstructions was tested using split-period calibration/verification and the procedures recommended by the National Research Council (2006), including reduction of error (RE), coefficient of efficiency (CE), root mean squared error (RMSE) and the squared correlation coefficient (r^2).

Results

Presentation of the chronologies

The validity of combining data from the two field sites was confirmed by a strong significance between-site correlations (*r*) for all isotope proxies, high Gleichläufigkeit score

Table 1 Pearson's correlation coefficient between trees from two sites (r; $p < 0.001$), Gleichläufigkeit score (Glk), modified Student's t value (t_{BP}) and Expressed Population Signal (EPS) for $\delta^{13}C$, $\delta^{18}O$, $\delta^{2}H$ and TRW

Proxy	$\delta^{13}C$	$\delta^{18}O$	$\delta^{2}H$	TRW
n	9	13	7	29
r	0.42	0.41	0.56	0.96
Glk%	–	–	–	68.90
t_{BP}	–	–	–	10.40
EPS	0.86	0.92	0.90	0.92

Table 2 Descriptive statistics for stable carbon ($\delta^{13}C$), oxygen ($\delta^{18}O$), stable hydrogen ($\delta^{2}H$) isotopes and tree-ring width (TRW) chronologies over the period AD 1907–2006

	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)	$\delta^{2}H$ (‰)	TRW (mm)
Average	−23.16	27.56	−118.82	0.74
Standard deviation	±0.72	±1.28	±9.43	±0.47
Minimum	−25.59	21.81	−145.15	0.04
Maximum	−20.27	32.06	−85.12	4.88
Range	5.32	10.25	60.03	4.84

(Glk%) (Eckstein and Bauch 1969) and modified Student's *t* value (t_{BP}) (Baille and Pilcher 1973) for TRW, by EPS values which exceed the threshold of 0.85 (Table 1) and by no significant difference in the variance of the proxies between sites. We therefore assume that, for the purpose of regional-scale climate reconstruction, the trees from both sites exhibit a common signal which is strong enough to consider that they belong to the same population and so the average chronologies were used for further analysis. Descriptive statistics for the larch tree ring width, stable carbon, oxygen and non-exchangeable hydrogen (hereafter referred to as hydrogen) isotope chronologies, covering the period AD 1907–2006, are presented in Table 2. Full chronologies with confidence limits around the mean are shown in Fig. 3.

The $\delta^{13}C$ chronology exhibits a high variation between years and low deviation in the first four decades of the 20th century (Fig. 3), but in the following period (AD 1946–1980) the trees exhibit more variability, with fewer pointer years and high standard deviation from the mean. The individual tree chronologies become more synchronous again in the recent period (AD 1981–2006). A slight increasing trend in the $\delta^{13}C$ chronology is observed over the full period AD 1907–2006, which is also present in the summer temperature data. In contrast, $\delta^{18}O$, $\delta^{2}H$ and TRW do not show any apparent trend in the first half of the analysed period, but increasing values in the last 30 years, most strongly exhibited for $\delta^{18}O$. $\delta^{18}O$ and $\delta^{2}H$ values

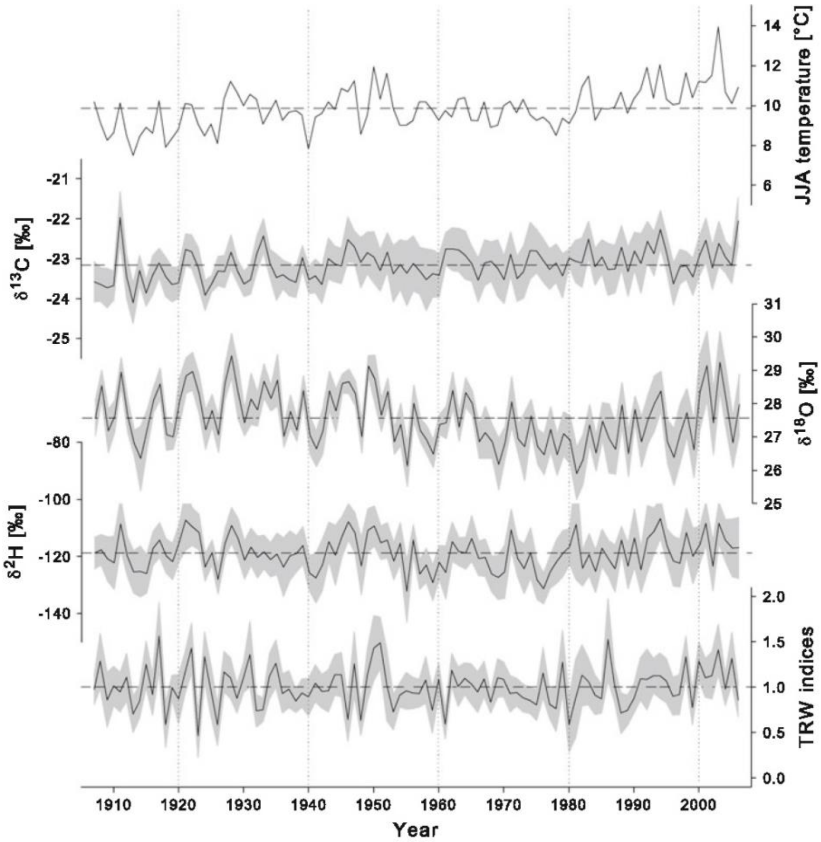


Fig. 3 Mean June–August temperature (JJA temperature) and the average chronology (*black line*) with confidence limits ($p < 0.05$) (McCarroll and Loader 2005) (*grey area*) of the stable isotope ratios of carbon (δ^{13}C), oxygen (δ^{18}O), hydrogen (δ^{2}H) and tree ring widths (TRW). The *horizontal dashed line* represents the mean value of temperature and each proxy

exhibit low standard deviation throughout the 20th century. The TRW chronology is characterised by a strong common signal in the early period followed by alternation of more (e.g. AD 1946–1960, AD 1997–2006) and less (AD 1935–1945, AD 1961–1975) synchronous periods.

Relationship between δ^{13}C, δ^{18}O, δ^{2}H and TRW chronologies

The three stable isotope chronologies are significantly ($p < 0.001$) and positively correlated with each other, the highest correlation being between δ^{18}O and δ^{2}H ($r = 0.76$). The water isotopes (δ^{18}O and δ^{2}H) are also significantly, though less strongly, correlated with TRW ($r = 0.38$ and $r = 0.32$, respectively), but there is no significant correlation between δ^{13}C and TRW ($r = 0.03$). When the isotope chronologies are standardised (z scores) and plotted together, a clear common signal is observed (Fig. 4a). The three isotope chronologies have many common pointer years, with slight offset in the values due to the steadily increasing

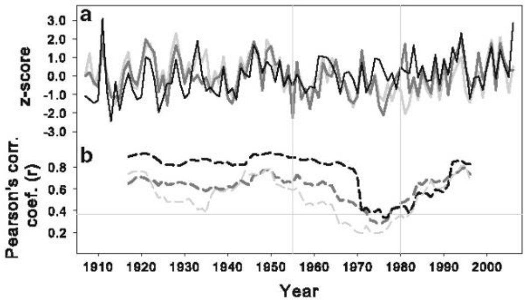


Fig. 4 **a** Z-scored stable isotope chronologies. δ^{13}C, *solid black line*; δ^{18}O, *solid dark grey line*; δ^{2}H, *solid light grey line*. **b** 21-year running Pearson's correlation coefficients (*r*) between stable isotope chronologies. *r*(δ^{18}O, δ^{2}H), *black dashed line*; *r*(δ^{13}C, δ^{18}O), *dark grey dashed line*; *r*(δ^{13}C, δ^{2}H), *light grey dashed line*. *Vertical grey lines* denote period of lower correlation between series. *Horizontal grey line* indicates correlation significance level of 21-year moving window at $p < 0.1$

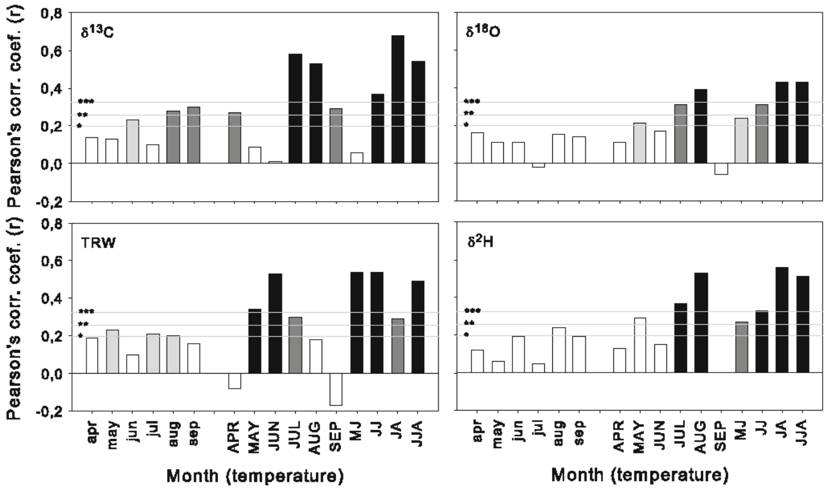


Fig. 5 Pearson's correlation coefficient between average monthly temperature and four tree ring parameters (stable carbon isotope ratios, δ^{13}; stable oxygen isotope ratios, $\delta^{18}O$; stable hydrogen isotope ratios, $\delta^{2}H$; tree ring widths, TRW). *Black bars* and *light grey line* denoted with *** represent significant correlation at $p < 0.001$, *dark grey bars* and *light grey line* denoted with ** at $p < 0.01$, *light grey bars* and *light grey line* denoted with * at $p < 0.05$. Months of previous year are written in *lowercase* and months of current year in *uppercase*. Combinations of months are denoted as: *MJ* May, June; *JJ* June, July; *JA* July, August; *JJA* June, July, August

trend in $\delta^{13}C$. Oxygen and hydrogen stable isotope values are strongly correlated throughout the 20th century. In the period AD 1964–1983, the strength of the common signal drops (Fig. 4b), but correlation between $\delta^{18}O$ and $\delta^{2}H$ is still significant at $p < 0.001$. In the recent period, the correspondence between all three stable isotopes is high.

Relationship between climate and tree ring parameters

The Pearson's correlation coefficient was calculated between proxies and monthly climate variables, from April to September, for the current and previous year. Statistically significant positive r values were obtained for the temperature (Fig. 5) and sunshine duration (Fig. 6), while for precipitation (Fig. 7) negative correlations were found. For all three isotopes, the highest r values were obtained with July and August climate variables, but climate conditions in June provide the highest correlation with TRW. The climate signal in the TRW is enhanced when June and July are averaged and the same is true for the isotope series using the mean of July and August. Correlation analysis also showed that $\delta^{13}C_{pin}$ chronology comprises stronger temperature signal than $\delta^{13}C_{atm}$ ($r_{JA} = 0.52$ and $r_{JA} = 0.68$ for $\delta^{13}C_{atm}$ and $\delta^{13}C_{pin}$, respectively), while the difference in correlation between both $\delta^{13}C$ chronologies and sunshine duration and precipitation is minor. All four proxies are strongly correlated with climate parameters averaged over June–August.

In the meteorological data used in this research, the correlation between temperature and sunshine duration is high ($r_{JJA} = 0.71$), but these parameters are only weakly correlated with precipitation ($r_{JJA} = -0.39$ and $r_{JA} = -0.40$, respectively). All four proxies exhibit strong and positive correlation with summer temperatures, $\delta^{13}C$ values showing the strongest correlation with mean July–August temperature ($r = 0.68$, $p < 0.001$). Oxygen and hydrogen stable isotopes correlate most strongly with sunshine duration in June–August ($r_{JJA} = 0.64$ and $r_{JJA} = 0.58$, respectively). In the case of TRW, the correlation with temperature and sunshine duration is similar. The correlation with precipitation is slightly lower for all proxies, the strongest value being obtained for $\delta^{13}C$ ($r_{JA} = -0.47, p < 0.001$). TRW and $\delta^{13}C$ are significantly ($p < 0.05$) correlated with late summer temperatures of the previous year ($\delta^{13}C$: $r_{Aug} = 0.28$, $r_{Sep} = 0.30$; TRW: $r_{Jul} = 0.21$, $r_{Aug} = 0.22$), but $\delta^{18}O$ and $\delta^{2}H$ are not significantly correlated with any analysed climate variable of the previous year.

The summer (JA and JJ) temperature series from Villacher Alpe correlate significantly ($p < 0.001$) with the CRU TS 3 gridded summer temperature data across a very wide area, covering the greater Alpine region and extending over the British Isles, Iberian Peninsula and across Italy to North Africa (Fig. 8). A near identical pattern is obtained for the tree ring $\delta^{13}C$ data. Since TRW is less strongly correlated with local summer temperature (JJ), the

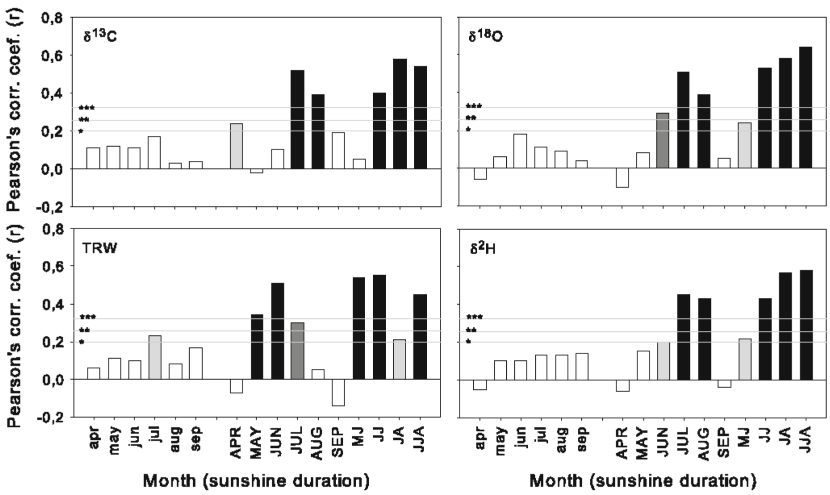


Fig. 6 Pearson's correlation coefficient between average monthly amount of sunshine duration and four tree ring parameters (panels as in Fig. 5)

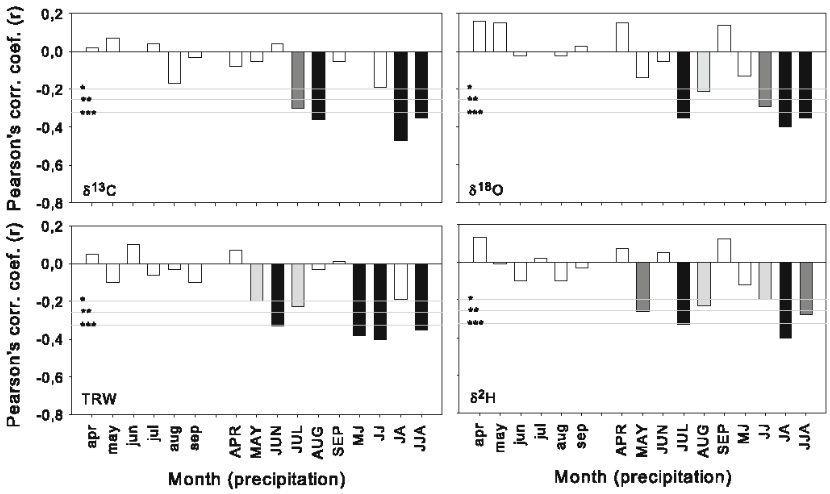


Fig. 7 Pearson's correlation coefficient between average monthly amount of precipitation and four tree ring parameters (panels as in Fig. 5)

significant correlations are less extensive, but the pattern is very similar to that obtained for Villacher Alpe. The water isotopes, in contrast, show spatial fields that are quite different to those obtained using the meteorological data. They correlate strongly with the temperature (JA) to the south and southeast of the field sites, extending across Italy and the Balkans, but the pattern does not extend over the Alps or far to the north and west.

Potential for the reconstruction of climate

The strong correlation between tree ring δ^{13}C and mean July–August temperature, and the similarity in the spatial fields produced by both the instrumental and proxy data suggest that stable carbon isotope ratios may provide a valuable proxy for summer temperature evolution in the southeastern Alpine region. To test this, however, the

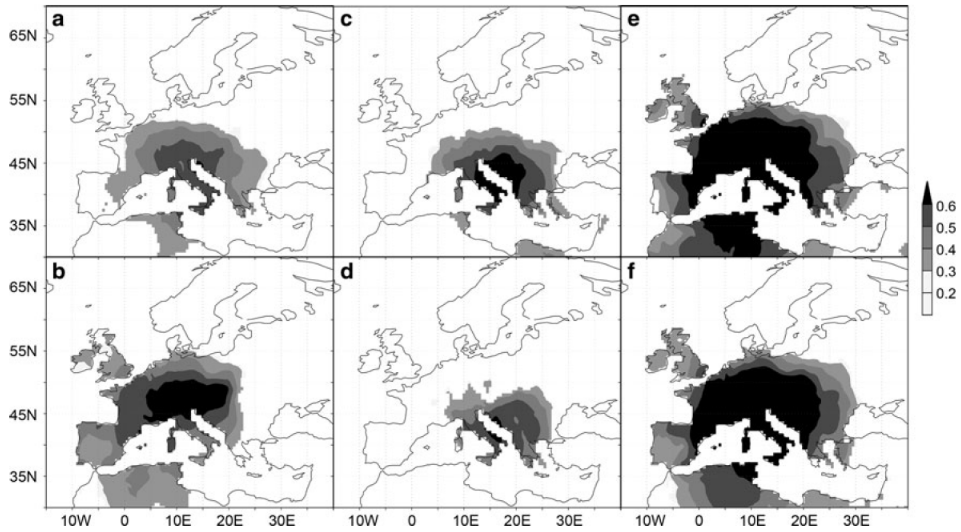


Fig. 8 Spatial field correlation ($p < 0.001$) between summer temperature and tree ring proxies. TRW (**a**) is correlated with mean June–July temperature, $\delta^{13}C$ (**b**), $\delta^{2}H$ (**c**) and $\delta^{18}O$ (**d**) are correlated with mean July–August temperature. **e**, **f** Present spatial field correlation with June–July and July–August temperature, respectively, measured at the Villacher Alpe meteorological station

Table 3 Summary statistics for the temperature and sunshine duration reconstructions

Model	$\delta^{13}C$		$\delta^{13}C$ and TRW		$\delta^{13}C$, $\delta^{18}O$ and TRW	
Target	JA temperature		JJA temperature		JJA sunshine	
Calibration	1907–1956	1957–2006	1907–1956	1957–2006	1907–1956	1957–2006
Verification	1957–2006	1907–1956	1957–2006	1907–1956	1957–2006	1907–1956
r^2	0.52	0.32	0.52	0.49	0.54	0.60
RMSE	±0.94	±0.88	±0.78	±0.77	±16	±18
RE	0.47	0.59	0.54	0.57	0.58	0.52
CE	0.30	0.48	0.34	0.39	0.58	0.52
Var.exp.	0.46		0.52		0.55	

r^2 squared correlation, *RMSE* root mean squared error, *RE* reduction of error statistics, *CE* coefficient of efficiency, *Var.exp.* explained variance

stability of the relationship was explored using separate 50-year calibration and verification periods and the range of verification statistics recommended by the National Research Council (2006). Irrespective of whether the early or later period is used for the calibration, the RE and CE statistics are strongly positive (Table 3) and over the full 100-year period summer temperature accounts for 46% of the variance in $\delta^{13}C$. If $\delta^{13}C$ and TRW are combined, in a multi-proxy approach (McCarroll and Loader 2004; McCarroll et al. 2011), in this case using multiple linear regression, the target covers a larger part of the summer (JJA) and the verification statistics are again strongly positive, with 52% of the variance explained by summer temperatures. The water isotopes correlate most strongly with summer (JJA) sunshine duration, rather than temperature, with $\delta^{18}O$ providing the strongest potential proxy ($r = 0.64$). However, the two water isotope series are so similar that combining them does not strengthen the climate signal. If, however, $\delta^{18}O$, $\delta^{13}C$ and TRW are combined, using multiple regression, to reconstruct summer (JJA) sunshine then the verification statistics are strongly positive and 55% of the variance is explained (Table 3).

Discussion and conclusions

In an extreme environment, such as the altitudinal forest and tree line, a single climatic factor often limits the tree growth, providing a clear (climate) signal in tree ring chronologies (Fritts 1976). Temperature is the most important factor, which exerts control on tree growth at high altitudes in the Alps (Rossi et al. 2007). For larch trees growing at the forest line in the southeastern Alpine region, the strongest control on tree ring width is the temperature of early to mid-summer (average of May–June and June–July) of the current year, with summer temperatures of the previous year also exerting some control. The strong link between ring width and early summer temperature reflects the direct effect of temperature on the onset of cambial activity (Gričar et al. 2007; Rossi et al. 2008), which culminates around summer solstice (Rossi et al. 2006). The strongest signal of June temperatures in larch TRW was also found in other researches (Carrer and Urbinati 2004; Serre 1978), while some analyses identified July and August temperatures to be the most influencing factor for TRW (Büntgen et al. 2005).

The fractionation of carbon within the measured trees is correlated most strongly also with temperature, but that of July and August, with no apparent relationship with the temperature of June (Fig. 5). There are two possible explanations why early summer (June) temperature influences ring width but not δ^{13}C: (1) early wood formation in deciduous trees only partly relies on current photoassimilate and more strongly on carbohydrate stored from the previous year (Helle and Schleser 2004; Kozlowski and Pallardy 1997). Thus, early wood carries information derived from starch δ^{13}C formed in the previous year (Felten et al. 2007; Kagawa et al. 2006), and (2) the amount of cellulose incorporated in early wood cell walls is significantly lower than in late wood (Rossi et al. 2007) and thus much less significant in homogenized δ^{13}C samples. Correlation between δ^{13}C and sunshine duration is slightly weaker ($r_{JA} = 0.58$) (Fig. 6) and with precipitation amount further reduced ($r_{JA} = -0.47$) (Fig. 7).

After correcting for the effects of changing amount and isotopic ratio of atmospheric carbon dioxide, as in this study, variations in tree ring δ^{13}C reflect the amount of CO_2 within the leaf, which is controlled by the balance between stomatal conductance and photosynthetic rate. Neither of these two rates is strongly and directly controlled by temperature; the dominant controls being vapour pressure deficit (VPD) and sunlight (photosynthetically active radiation). The link to temperature is therefore indirect, and δ^{13}C will only provide a reliable palaeotemperature proxy where the series of links (e.g. intercorrelation between VPD, sunshine and temperature) that lead to the strong correlation remain constant (McCarroll et al. 2011). Strong correlations between tree ring δ^{13}C and summer temperature have been reported elsewhere (Hilasvuori et al. 2009; Kress et al. 2010; Sidorova et al. 2010), but caution is required in interpreting this relationship and using it to reconstruct the climate of the past. It has recently been argued that tree ring δ^{13}C records from Northern Fennoscandia, although very strongly correlated with summer temperature, are better regarded as a record of past changes in sunshine or cloud cover (Young et al. 2010, Gagen et al. 2011). Stable carbon isotope ratios from larch trees in Switzerland have also been interpreted as indirectly related to temperature, with equally high correlations obtained for temperature and precipitation (Kress et al. 2010), while tree ring δ^{13}C from the French Alps correlated more strongly with precipitation than with temperature (Gagen et al. 2004). The correlation between summer temperature and tree ring δ^{13}C observed in this study is unusually strong and consistent, and the spatial field represented by the temperature of this region covers a very large part of southern and Central Europe west of the Carpathian Mountains. If it can be confirmed that long-term changes in larch tree ring δ^{13}C faithfully record changes in summer temperature, then this would be a very important archive. By combining δ^{13}C and TRW, the average temperature of a larger part of the summer could be reconstructed (June–August), allowing direct comparison with other reconstructions that use the same target (Büntgen et al. 2005; Corona et al. 2010).

While δ^{13}C and TRW chronologies reflect a strong temperature signal centred over the study area (southeastern Alps), the spatial correlation between stable water isotopes in tree rings and temperature is biased towards southern Italy and the western part of the Balkan Peninsula (Fig. 8). This pattern can be explained by large-scale atmospheric air pressure patterns, which govern the movement of humid air masses and influence the isotopic composition of the precipitation. Field (2010) showed that North Atlantic Oscillation (NAO) controls δ^{18}O of precipitation in the winter, however there is the absence of NAO influence at the alpine locations in the summer time (JJA) when control of local (orographic) mechanisms prevail. Southwest winds bring humid air masses from the Mediterranean Sea, which leave a temperature fingerprint in the water isotopes. When they reach the southeastern Alpine ridge, precipitation is released, yielding up to 2,000 mm per year (Petkovšek and Trontelj 1987). Larches growing in the southeastern Alps at sites with a combination of dry karstic soil and high amount of precipitation have great potential for recording information about the source water (Roden et al. 2000; White et al. 1985) and therefore the characteristics of the air masses from which their precipitation is sourced.

δ^{18}O and δ^{2}H of source water co-vary with a relationship described by a global meteoric water line (GMWL) (Craig 1961). The co-variation is observed also in the isotopic composition of tree ring cellulose in our study confirmed by the high EPS for each isotope and the strong correlation between both water isotopes ($r = 0.76$). One may therefore expect such a strong correlation to be retained in the isotopic composition of all tree ring cellulose series, however this is not always the case. Several long-term studies of water isotopes in tree ring cellulose (Hilasvuori and Berninger 2010; Loader et al. 2008) have identified that climatically sensitive coherent oxygen and hydrogen isotopes do not always co-vary. In contrast, this study demonstrates a high degree of covariance within the oxygen and hydrogen isotope series, indicating that perhaps, the models currently employed to interpret stable oxygen and hydrogen isotope variability in plant cellulose may require further development. Variability in the strength of the fractionation factors operating during leaf water enrichment and the nature of the biochemical reactions influencing oxygen and hydrogen may contribute to differences in the $\delta^{18}O$ and $\delta^{2}H$ of specific photosynthetic sugars which then enter into the process of cellulose synthesis where they are further exposed to second set of fractionation factors, mainly determined by the rate of exchange with medium water—xylem water in case of tree rings (Yakir 1992; Roden et al. 2000). As the proportion of the carbon-bound hydrogen and oxygen that undergo exchange with xylem water may dampen the leaf water signal thereby enhancing the source water signal in the plant tissue (Barbour 2007; Roden et al. 2000; Yakir and DeNiro 1990), we hypothesise that the larch trees analysed in this study may exhibit a higher degree of exchange with xylem water than that experienced by trees reported in other studies and that this may account, in part, for the higher degree of co-variation between hydrogen and oxygen isotopes observed.

The observed strong correlation between the water isotopes and summer sunshine duration is difficult to explain in terms of a direct control. It seems likely that the link with sunshine is indirect, via the relationship between sunshine and circulation patterns (and thus source water trajectory) and between sunshine and relative humidity (and therefore the gradient between internal and ambient vapour pressures). Kahmen et al. (2011) pointed out that $\delta^{18}O$ of tree ring cellulose in tropic environment is besides relative humidity-dependent also on air temperature and additional information on other biological and environmental data would be needed to distinguish the prevailing influence of one of these two factors. A combination of $\delta^{18}O$, $\delta^{13}C$ and TRW of larch tree rings in our study correlates very strongly with summer sunshine duration and over the last century produces very strong calibration and verification statistics. It is uncertain whether this method would produce reliable results over longer timescales, when there may have been changes in circulation that are not evident over the 20th Century.

Our research has shown that different tree ring proxies developed from larch trees growing in the southeastern Alps record temperature, sunshine duration and circulation patterns of early and late summer. The particular value of investigated proxies is the area they cover; the Alps and the Adriatic, as the existing tree ring isotope (Treydte et al. 2007) and TRW networks (Büntgen et al. 2010) have a notable lack of sites in this region, which represents a linkage between the Central European Alps and Dinaric Alps. The archive offers good palaeoclimatic potential because of the presence of long-lived trees in the forests (up to 560 years of age) and the existence of locally sourced archaeological timbers that provide the potential for extending larch tree ring series in the southeastern Alps back further over the last millennium.

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3.1.2 520-letna kronologija poletnega sončnega obsevanja za območje vzhodnih Alp na podlagi stabilnih ogljikovih izotopov v branikah navadnega macesna

A 520 year record of summer sunshine for the eastern European Alps based on stable carbon isotopes in larch tree rings

Polona Hafner, Danny McCarroll, Iain Robertson, Neil J. Loader, Mary Gagen, Giles HF Young, Roderick J. Bale, Eloni Sonninen, Tom Levanič
Climate Dynamic, 2013, doi: 10.1007/s00382-013-1864-z

Na podlagi analize izotopske sestave celuloze branik evropskega macesna (*Larix decidua*), rastočega na visokih nadmorskih višinah v vzhodnih evropskih Alpah, smo dobili 520 let dolgo kronologijo izotopskega razmerja ogljika. Kronologija izkazuje boljšo korelacijo s temperaturami kot s trajanjem sončnega obsevanja. Poteka krivulj instrumentalnih meritev temperatur in trajanja sončnega obsevanja se po letu 1980 razideta. Kronologija branik ne sledi povečanju poletnih temperatur, temveč trendu trajanja sončnega obsevanja. Rekonstrukcija temperature na podlagi izotopskega razmerja ogljika v branikah ni robustna. Rekonstruirane temperature pred dvajsetim stoletjem so višje kot izmerjene regionalne temperature, prav tako tudi potek temperature ne sovpada z objavljenimi regionalnimi temperaturnimi rekonstrukcijami. Na podlagi teh ugotovitev zaključimo, da je trajanje sončnega obsevanja preko vpliva stopnje fotosinteze na notranji parcialni tlak CO_2 dominantni dejavnik, ki vpliva na frakcionacijo ogljikovih izotopov v proučevanih drevesih. Zato je najbolj primerno rekonstruirati trajanje sončnega obsevanja v visokem poletju (julij–avgust). Predstavljamo prvo rekonstrukcijo trajanja sončnega obsevanja v vzhodnih Alpah in njeno primerjavo z razvojem regionalnih temperatur.

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Abstract A 520-year stable carbon isotope chronology from tree ring cellulose in high altitude larch trees (*Larix decidua* Mill.), from the eastern European Alps, correlates more strongly with summer temperature than with summer sunshine hours. However, when instrumental records of temperature and sunshine diverge after AD1980, the tree ring time series does not follow warming summer temperatures but more closely tracks summer sunshine trends. When the tree ring stable carbon isotope record is used to reconstruct summer temperature the reconstruction is not robust. Reconstructed temperatures prior to the twentieth century are higher than regional instrumental records, and the evolution of temperature conflicts with other regional temperature reconstructions. It is concluded that sunshine is the dominant control on carbon isotope fractionation in these trees, via the influence of photosynthetic rate on the internal partial pressure of CO_2, and that high summer (July–August) sunshine hours is a suitable target for climate reconstruction. We thus present the first reconstruction of summer sunshine for the eastern Alps and compare it with the regional temperature evolution.

Keywords Carbon isotopes · Dendrochronology · Climate change · Cloud cover

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1 Introduction

The ring widths of larch (*Larix decidua* Mill) trees growing high in the eastern European Alps tend to be sensitive to the temperature of June, and to a lesser extent July (Hafner et al. 2011). Stable carbon isotope ratios measured on cellulose from the same tree rings correlate with the temperature of July and August. In a pilot study Hafner et al. (2011) suggested that combining these two proxies might provide a strong record of past changes in temperature for the entire summer season, June to August. However, recent work in northern Fennoscandia has raised concerns about using stable carbon isotopes in tree rings to reconstruct temperature, even when correlation and verification with local temperature data is very strong (Young et al. 2010; Gagen et al. 2011).

The ratio of ^{13}C–^{12}C in wood cellulose, expressed relative to a standard using the delta notation ($\delta^{13}C$), is strongly linked to the concentration of carbon dioxide inside the (leaves or) needles averaged over the growing season (McCarroll and Loader 2004). Air (containing carbon dioxide) enters the needles via the stomata and the carbon dioxide is enzymatically fixed during photosynthesis to produce carbohydrates, which are used to supply all of the needs of the growing tree, including the formation of wood cells. The main controls on the carbon isotope ratio of the resulting photosynthates are the relative rates at which CO_2 enters (stomatal conductance) and is removed

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(photosynthetic rate) from the internal leaf spaces. In dry environments stomatal conductance dominates the $\delta^{13}C$ signal, recording changes in air humidity and soil moisture availability, linked to antecedent precipitation. In cool, high altitude or high latitude settings, where moisture stress is less common, the dominant signal in $\delta^{13}C$ is likely to be the rate of photosynthesis. Photosynthetic rate is linked to temperature, via the rate of production of the photosynthetic enzyme, but more commonly the limiting factor is the supply of energy in the form of photon flux, or sunlight (Beerling 1994) which drives the photosynthetic reation. The $\delta^{13}C$ of tree rings measured at sites that do not experience frequent moisture stress is theoretically controlled more strongly by sunlight than by temperature.

Although sunlight is, in theory, the strongest control on carbon isotope fractionation under cool moist conditions, in practice $\delta^{13}C$, in the recent calibration period, often correlates most strongly with summer temperature. A likely explanation is that temperature and sunshine are very strongly correlated at the inter-annual scale and temperature is measured precisely and accurately, whereas long records of photosynthetically active radiation (PAR) flux are rarely available, forcing reliance on less direct measures such as hours of sunshine or percentage cloud cover.

If summer sunshine and summer temperature remained strongly and uniformly covarying over long timescales it would not matter which one was used for calibration and reconstruction, and this was the assumption in some earlier work (McCarroll and Pawellek 2001; McCarroll et al. 2003). In northern Finland, for example, $\delta^{13}C$ of pine trees correlated more strongly with summer temperature than either tree ring widths or densities and also gave better verification statistics using local meteorological data (McCarroll et al. 2011). However, when this proxy was used to reconstruct summer temperature back to AD1640 (Gagen et al. 2007) it was clear that temperature estimates prior to the calibration period were too high. The offset was demonstrated by comparison with the long early instrumental temperature records from Tornedalen (Gagen et al. 2011). Similar results were obtained in north-west Norway, but in this case there was a period in the instrumental measurements where there was a clear divergence between summer temperature and percentage cloud cover, indicating that temperature and sunshine may not remain tightly coupled at non-interannual timescales (Young et al. 2010). In this case when temperature and cloud cover diverged, the $\delta^{13}C$ values followed the cloud cover (sunshine) rather than temperature (Young et al. 2010). The long $\delta^{13}C$ records from pine trees in northern Fennoscandia have now been robustly reinterpreted as records of past summer sunshine or cloud cover, depending on the availability of local meteorological data for calibration and verification (Gagen et al. 2011; Young et al. 2012; Loader et al. 2013). In this area there are very clear offsets between the tree ring $\delta^{13}C$ records and high quality reconstructions of summer temperature (Melvin et al. 2012; McCarroll et al. 2013), pointing to long periods of divergence between temperature and sunshine, perhaps indicating large scale changes in circulation in the past (Loader et al. 2013). Similar divergence between summer temperature and sunshine, at decadal or multi-decadal timescales, has also been found within a General Circulation Model (Gagen et al. 2011).

In the European Alps, as elsewhere, tree ring $\delta^{13}C$ has generally been interpreted either as an indicator of moisture stress, in drier areas, or of summer temperature in higher, cooler ones. For example, Kress et al. (2010) measured carbon isotope ratios in larch trees from the Lötschental in the Valais; one of driest regions in Switzerland, and found very strong correlations with a drought index based on temperature and precipitation amount. In the same valley Treydte et al. (2001) reported strong correlations between $\delta^{13}C$ in spruce tree rings and temperature, precipitation and relative humidity, concluding that relative humidity is a suitable target for reconstruction. In the French Alps, Daux et al. (2011) also report strong correlations between larch $\delta^{13}C$ and summer relative humidity. Strong correlations with parameters linked to moisture stress have also been reported for beech trees (Saurer et al. 1997) and for pines growing at dry Alpine sites (Gagen et al. 2004, 2006). On the more moist southern side of the Swiss Alps, Reynolds-Henne et al. (2007) report a low but consistent correlation between $\delta^{13}C$ in Scots pine trees and summer temperature. Alpine tree ring $\delta^{13}C$ chronologies have not, thus far, been interpreted in terms of past variations in sunshine.

The aim of this paper is to use a 520 years $\delta^{13}C$ chronology from the eastern Alps to test the hypothesis that, based on first principles, the dominant control on isotopic fractionation is summer sunshine rather than temperature in this region. Given the paucity of sunshine records over this region, this is tested by critically assessing the veracity of temperature reconstructions by comparing them with both long instrumental series and temperature reconstructions, based on other lines of evidence.

2 Sites and data description

Tree ring samples for isotopic analysis were obtained by sub-sampling 12 trees from a *L. decidua* tree ring chronology built using living trees from two alpine (1,700 m a.s.l.) sites; Dleskovška plateau (46°21′N, 14°42′E, close to the border with Austria) and Vršič (46°26′N, 13°43′E, close to the border with Italy) in the Slovenian Alps (see Hafner et al. 2011 for more details of site selection).

The chronology also includes some roof timbers from St. George's church in the coastal region of Slovenia (Piran, 45°31′N, 13°34′E) and two of these were included to extend and strengthen the early part of the isotope chronology. The building timbers originate from 'alpine sites near the border between Slovenia and Italy' (Levanič et al. 1997).

From each tree or beam, 12-mm cores were extracted, air-dried, absolutely dated and then divided into annual tree rings using a scalpel under magnification. The youngest 50 tree rings of each tree were excluded from further analysis to avoid potential "juvenile trends" in the $\delta^{13}C$ chronology (Gagen et al. 2007), although recent work on larch suggests that this might not be necessary (Daux et al. 2011). Tree rings were not separated into early and late-wood components because the rings were often too narrow to provide enough α-cellulose and because it has been shown that for conifers the isotopic ratios of early and late wood are strongly correlated and good results are obtained when using the whole ring (Kress et al. 2009). For each tree ring the α-cellulose was isolated using standard techniques (Loader et al. 1997; Rinne et al. 2005). The purified samples were homogenized using a Hielscher ultrasonic probe (Laumer et al. 2009) and freeze-dried for at least 48 h prior to measurement of $\delta^{13}C$ ratios.

Carbon isotope analysis, using samples of 0.30–0.35 mg of α-cellulose, was performed using a mixture of combustion and pyrolysis techniques. Pyrolysis allows both carbon and oxygen isotope ratios to be measured on the same sample (Young et al. 2011a; Woodley et al. 2012), which is advantageous when dealing with thin rings, as in this study. For combustion the samples were weighed into tin capsules and combusted over chromium(III) oxide and copper(II) oxide at 1,000 °C. For pyrolysis, samples were weighed into silver capsules and pyrolysed over glassy carbon at 1,090 °C. In both cases work was conducted in the Swansea University stable isotope laboratory using a PDZ Europa ANCA GSL elemental analyzer interfaced to a PDZ Europa 20–20 stable isotope ratio mass spectrometer using the methods described by Young et al. (2011a). For comparison, samples from one tree were pyrolysed at higher temperature (1,330 °C) in a Finnigan TC/EA high temperature elemental analyzer interfaced (by ConFloIII) to a ThermoFinnigan DeltaPlus Advantage isotope ratio mass spectrometer at the University of Helsinki. Analytical precision was typically ± 0.1 per mille ($\delta^{13}C$). The $\delta^{13}C$ values obtained by pyrolysis and combustion on the same samples were very similar (mean values −22.98 ‰ for combustion and −23.11 ‰ for pyrolysis, correlation $r = 0.94$, $n = 512$). The small differences in mean and variance were removed by scaling the pyrolysis values to match those of combustion following the procedure described by Young et al. (2011a).

After scaling, the $\delta^{13}C$ values were corrected first to remove the atmospheric decline in the $\delta^{13}C$ values of atmospheric carbon dioxide, by simple addition using an extrapolation of the values provided by McCarroll and Loader (2004), and then for changes in the response to the rising carbon dioxide content of the atmosphere using the pre-industrial (PIN) correction proposed by McCarroll et al. (2009). The effect of the two corrections is shown in Hafner et al. (2011). The PIN correction is based on the physiological constraints on a plastic response to rising carbon dioxide levels and makes no attempt to fit the isotope data to any climatic signal, thus preserving the independence of the meteorological data sets to be used for calibration. The effect of the PIN correction on the reconstruction was checked by including and excluding the period that is affected (discussed later).

Examination of the individual tree $\delta^{13}C$ series suggests that the temporal evolution of the mean isotope curve is not an artifact of changes in sample depth or the mixture of different tree cohorts, and the longer tree series follow the trend of the mean. With only 14 trees, including cohorts of similar age, it is difficult to test conclusively for the presence of age trends. Much larger data sets for Scots pine in Fennoscandia show no evidence for an age trend after a short (<50years) juvenile period (Gagen et al. 2008; Young et al. 2011b) and a similar study would be required for Alpine larch, but there are insufficient published data at present. However, the trend of a linear correlation between $\delta^{13}C$ and ring number provides some indication of the extent of age related trends in these trees, and seven of the trees show a rise over their series length, of which four are statistically significant ($p < 0.05$), whilst the other seven show a decline, of which three are significant. There is certainly no evidence to suggest a consistent age trend in larch $\delta^{13}C$ that might confound any climatic interpretation of the mean series.

Of the five calendar centuries covered by the mean $\delta^{13}C$ record (Fig. 1), the highest century-mean occurs in the 18th (−22.64 ‰) followed by the 17th (−22.78 ‰) and the lowest in the 19th (−22.92 ‰). Mean values of the sixteenth and twentieth centuries are almost the same (−22.88 and −22.89 ‰). Taking half centuries, the mean value for the period 1951–2000 (−22.79 ‰) is the fourth highest, with higher values covering the period AD1651–1750. The lowest is the first half of the twentieth century, followed by the first half of the seventeenth century.

3 Calibration and verification of the climate signal

A pilot study (Hafner et al. 2011) calibrated carbon isotopes from this chronology, together with other potential paleoclimate proxies for the last 100 years, using

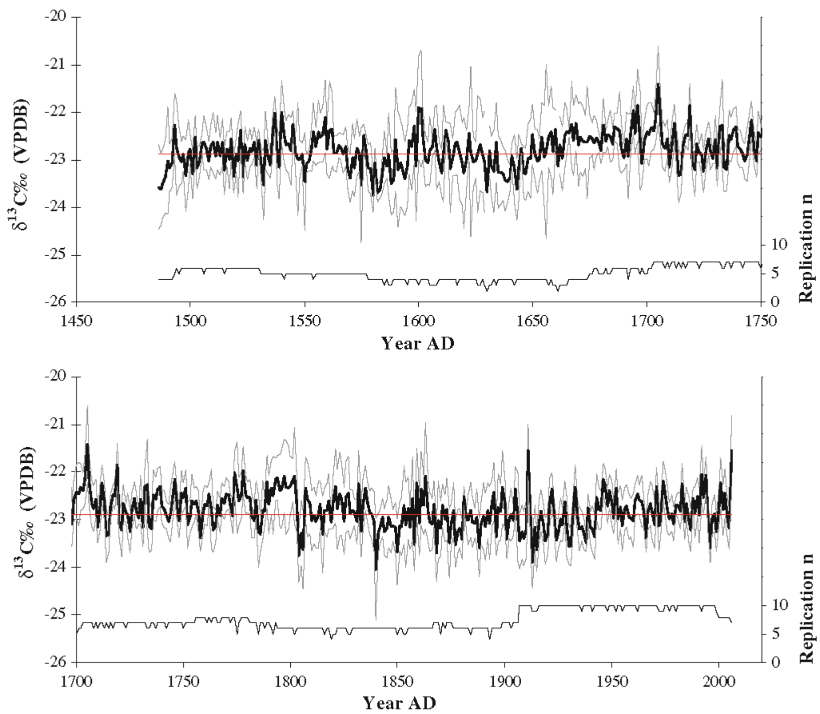

Fig. 1 The mean isotope chronology (*black*) with 95 % confidence limits (*grey*) and replication. The *horizontal* (*red*) *line* is the mean of the twentieth century

meteorological data from Villacher Alps meteorological station in the Austrian Alps, which is part of the HISTALP network (Auer et al. 2007). Strong positive correlations were reported with the mean temperatures of July and August, weaker but significant correlations with September temperatures but not with June. Sunshine hours in July and August also gave strong correlations, with insignificant values for June and September. Significant negative correlations were reported for the precipitation totals of July and August. The data presented here give very similar results, but we are able to extend the correlations back to AD1884, which is the length of the sunshine data (Table 1). In all cases combining the meteorological data from July to August improves the correlation with $\delta^{13}C$.

Table 1 Pearson's correlation coefficients and statistical significance (*p*) of the correlation between climate variables and $\delta^{13}C$ over the common period AD1884-2006

Month(s)	Temperature °C	*p*	Sunshine hours	*p*
June	−0.05	0.57	0.05	0.60
July	0.51	0.00	0.48	0.00
August	0.48	0.00	0.37	0.00
September	0.26	0.00	0.20	0.03
June/July	0.29	0.00	0.36	0.00
July/August	0.62	0.00	0.55	0.00
June to August	0.45	0.00	0.46	0.00

Over the period for which both temperature and sunshine data are available (AD1884 to 2006), $\delta^{13}C$ correlates more strongly with July to August (JA) temperature than with JA sunshine, and these two climatic variables are strongly correlated with each other ($r = 0.73$, $p < 0.001$). Since JA temperature gives the highest correlation, in the absence of other information that would be the logical choice as a target for reconstruction. However, some care is required when comparing sunshine and temperature correlations, because temperature is measured with greater accuracy and precision than sunshine hours and also because temperature changes smoothly across space, whereas sunshine (cloudiness), and precipitation variations are more spatially variable. This means that the inter-annual temperature variations experienced in the sampled forest and measured at a distant meteorological station are likely to be more similar than the same values for sunshine, so if the real influence of temperature and sunshine on a proxy are equally strong, temperature will likely give higher correlation values due to the superior instrumental robustness and spatial homogeneity of the temperature variable.

Split-period verification tests conducted over the common period for which local temperature and sunshine data are available show that temperature may not be the best target for reconstruction (Fig. 2). The squared correlation (R^2) values over the two halves of the instrumental data are very similar for sunshine, but for temperature the correlation over the recent period (AD1946 to 2006) is much lower than that over the earlier half (AD1884 to 1945). Also, although the Reduction of Error (RE) values for temperature are higher than those for sunshine, this is not true for the more challenging Coefficient of Efficiency (CE) statistic. Low CE results indicate an offset in the absolute values of the measured and predicted temperatures. In this case the offset occurs because the temperature and sunshine records diverge after 1983 and the isotope values follow the evolution of the sunshine record, rather than rising with summer temperatures.

There are two reasonable explanations for the offset between summer temperature and stable carbon isotope ratios over the last few decades. One possibility is that the offset reflects the direct influence of increasing carbon dioxide concentrations on fractionation of carbon by these trees. A correction has already been made for this effect, but the PIN correction used (McCarroll et al. 2009) only removes any decline in the isotope values that could be accounted for by rising CO_2. Treydte et al. (2009) have proposed an alternative correction that effectively tunes the stable isotope values to the target climate variable. Applying this procedure would certainly remove the offset but the calibration and verification would be compromised because the isotope and temperature data would no longer be independent. The alternative explanation is that the dominant control on photosynthetic rate, and therefore on stable carbon isotope fractionation, is photon flux rather than temperature and the divergence between $\delta^{13}C$ and summer temperature represents the divergence between temperature and sunshine. If the strong correlation between temperature and $\delta^{13}C$ is indirect, via photon flux, and recent warming is due to increased greenhouse gas concentrations, then a divergence between temperature and sunshine, and between temperature and $\delta^{13}C$, is precisely what would be expected.

Given the available sunshine data, it is not possible to conclude definitively, using correlation analysis, whether the dominant signal in the $\delta^{13}C$ of these larch trees is summer temperature or summer sunshine. Apart from the last few decades, where steeply rising atmospheric CO_2 is a confounding factor, there are no prolonged periods of divergence between temperature and sunshine at Villacher Alps.

The veracity of a JA temperature reconstruction based on the correlation with $\delta^{13}C$ can be investigated to some extent using longer gridded temperature data (Böhm et al. 2010) that is available for the Eastern Alps (43°–49° N and 12°–19°E). Over the common period AD1851 to 2006 the Villacher Alps and eastern Alpine temperatures are very strongly correlated ($r = 0.93$, $p < 0.001$) and over that period they each give the same correlation with the $\delta^{13}C$ results ($r = 0.56$, $p < 0.001$). However, the eastern Alpine series extends back to AD1763, allowing much longer periods for calibration and verification.

Using longer calibration and verification periods (122 years each) should reduce the impact of the recent short period of clear offset, but even so, when $\delta^{13}C$ values calibrated over the recent period, AD1885 to 2006, are used to predict the temperatures for the earlier period, AD1763 to 1884, there is a clear overestimate of summer temperatures, resulting in a CE value close to zero (Fig. 3). If the period of offset after AD1983 is removed, and the $\delta^{13}C$ values are calibrated over AD1885 to 1983, the offset remains and in this case the verification statistics are even worse, because there is then no difference between the average temperatures over the calibration and verification periods, so that RE and CE become equivalent.

Although comparison with the long eastern Alps summer temperature record produces verification statistics that are above zero, it indicates a clear problem with interpreting $\delta^{13}C$ as a record of temperature. Even if the last few decades, when rapidly rising CO_2 causes some uncertainty in the $\delta^{13}C$ values, are ignored, it is clear that $\delta^{13}C$ will tend to overestimate the temperature of the past. The reason is simply that although the mean JA temperature for the

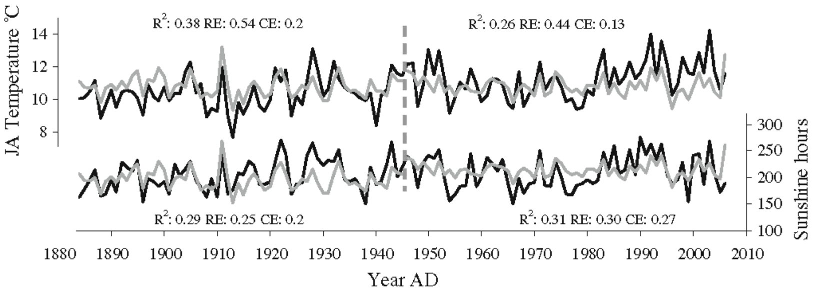

Fig. 2 Measured (*black*) and predicted (*grey*) values for July/August temperature and sunshine hours using split-period calibration and verification. R^2 is the squared correlation between predicted and measured values, RE and CE are Reduction of Error and Coefficient of Efficiency statistics

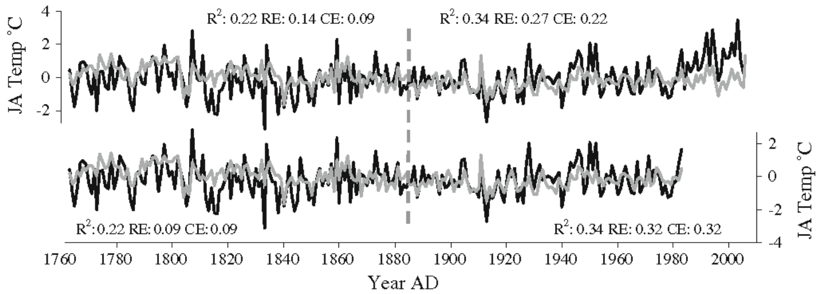

Fig. 3 Measured (*black*) and predicted (*grey*) values for July/August temperature anomalies (relative to AD1901 to 2000) using split-period calibration and verification. R^2 is the squared correlation between predicted and measured values, RE and CE are Reduction of Error and Coefficient of Efficiency statistics. In the lower graph the modern part of the records has been truncated at AD1983 to check the effect of removing the period of clear offset

period AD1760 to 1884 is the same as that for AD1885 to 1983, the mean $\delta^{13}C$ values for the earlier period are higher than those for the recent period. Unfortunately, meteorological records are not sufficiently long to determine whether there is a similar long-term offset between temperature and sunshine or cloud cover.

Comparisons of the $\delta^{13}C$ chronology with other reconstructions of summer temperature for the Alpine region also suggest that temperature is not the appropriate interpretation (Fig. 4). Documentary evidence provides perhaps the most powerful proxy measure of past temperature (Brázdil et al. 2005, 2010) and reconstructions with monthly resolution, based mainly on data from Switzerland, Germany and the Czech lands, have been provided by Dobrovolný et al. (2010). When the $\delta^{13}C$ chronology is scaled to the mean documentary-based JA temperature values there are many clear anomalies and long periods where the two records disagree, despite being forced to the same mean value over the common period AD1500 to AD1854. The isotopes underestimate temperatures between AD1630 and 1660, but generally overestimate between AD1660 and 1840. Very high over-estimates occur in AD1600 and 1601 (3.75 and 3.95 °C) and between AD1692 and 1697 (3.24 °C in 1694). A similar comparison with the longer reconstruction of JJA temperature by Trachsel et al. (2012), although scaled over a different period (AD1486 to 1996) shows a similar pattern of prolonged offsets. In particular, whereas the Trachsel et al. (2012) record shows a long-term increase between about AD1700 and 1950, the isotope record shows a long-term decline. The period between AD1825 and 1996 is almost continuously negative.

An alternative and arguably more reasonable interpretation of the long-term evolution of the $\delta^{13}C$ chronology is that it represents mainly changes in sunshine. If this is true then comparing the $\delta^{13}C$ curve with temperature reconstructions may reveal periods when sunshine and temperature were less coupled than they appear to be over the period for which sunshine records are available. Using both the documentary (Dobrovolný et al. 2010) and multi-proxy based reconstructions (Trachsel et al. 2012) there are several clear periods where temperature and $\delta^{13}C$ (interpreted for sunshine) behave in-phase, but several where they diverge (Figs. 4, 5). The period between about AD1570 and 1590 stands out as particularly cold and cloudy, whereas the years around AD1600 were sunny. Between AD1625 and 1650 it was not cold but very cloudy and as temperatures declined in the second half of the seventeenth century it become increasingly sunny, culminating in the sunniest period in the record in the first decade of the eighteenth century, when it was also warm. Most of the remainder of the eighteenth century is unremarkable, with both temperature and sunshine oscillating close to the values experienced in the latter half of the twentieth century, apart from the AD1790 s, which were sunny. From AD1800 to 1825 it is both cold and cloudy. Between AD1825 and 1875 there is a rise in temperature but a drop in sunshine, so that compared to the last half millennium, the period between AD1825 and 1940 is relatively warm but cloudy. It is notable that when the $\delta^{13}C$ record is scaled over a very long period, to match the Trachsel et al. (2012) reconstruction, and expressed in anomalies relative to the twentieth century, the offset between temperature and sunshine of the last few decades does not look unusual at all.

4 Sunshine reconstruction based on $\delta^{13}C$

Given the long term evolution of the $\delta^{13}C$ chronology, and the reasonably good correlation with measured values, we propose that July/August sunshine is the most appropriate target for climate reconstruction using stable carbon isotope ratios in larch trees growing at high elevation in the southeastern Alps. Climate reconstructions based on

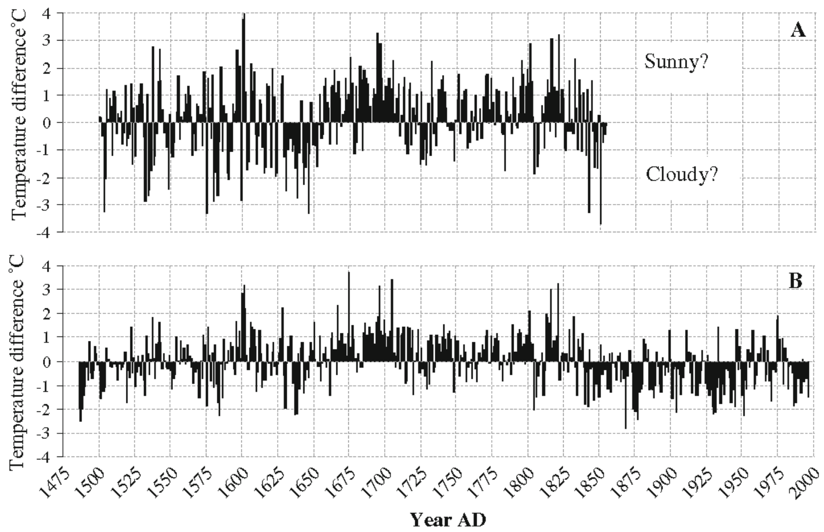

Fig. 4 Difference in temperature reconstructed by scaling the $\delta^{13}C$ chronology to fit the mean and variance of two summer temperature reconstructions for **a** Central Europe (Dobrovolný et al. 2010) and **b** the Greater Alpine Region (Trachsel et al. 2012). The shorter Central European record is based on documentary evidence and represents the mean *JA* temperature relative to AD1961 to 1990. The longer Greater Alpine Region record is a multi-proxy reconstruction of *JJA* temperature relative to the period AD1901 to 2000. Positive values indicate that $\delta^{13}C$ would over-estimate temperature. An alternative explanation is that the *positive* and *negative values* indicate periods of high and low sunshine

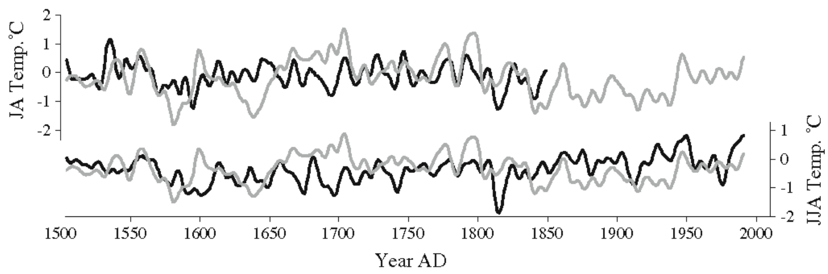

Fig. 5 Temperature reconstructions (*upper*) for Central Europe (July/August: Dobrovolný et al. 2010) and (*lower*) for the Greater Alpine Region (June–August: Trachsel et al. 2012) compared with the $\delta^{13}C$ chronology (in *grey*) scaled to match their mean and variance. *Values* are temperature anomalies relative to AD1961 to 1990 (*upper*) and to AD1901 to 2000 (*lower*) smoothed with a 10 years Gaussian filter

regression (inverse calibration; using the proxy to predict climate) always underestimate the variability of climate in the past, the magnitude of the effect being proportional to the amount of unexplained variance (McCarroll et al. 2013). Given a correlation of r = 0.55 with the total hours of sunshine for July and August combined, a regression-based reconstruction grossly underestimates the variability of sunshine over the period of measurement, so the reconstruction has been scaled to match the mean and variance of the meteorological data (Fig. 6).

It must be stressed that this sunshine reconstruction needs to be interpreted with caution. It is based upon a small sample of trees from only two sites and the uncertainty around the annual values is very large. Ignoring uncertainty in the estimate of the mean isotope values, two standard errors of the prediction gives ± 102 h of sunshine. However, it is the first such reconstruction for the Alpine region and when considered alongside reconstructions of temperature and precipitation may provide a more synoptic view of changes in climate over time.

Three years stand out as having very high isotope and predicted sunshine values: AD2006, AD1911 and AD1705. The autumn of AD2006 was exceptionally warm and dry (Luterbacher et al. 2007). The summer of AD1911 was both hot and sunny and it also had the lowest July/August rainfall in all the available records. The scaled

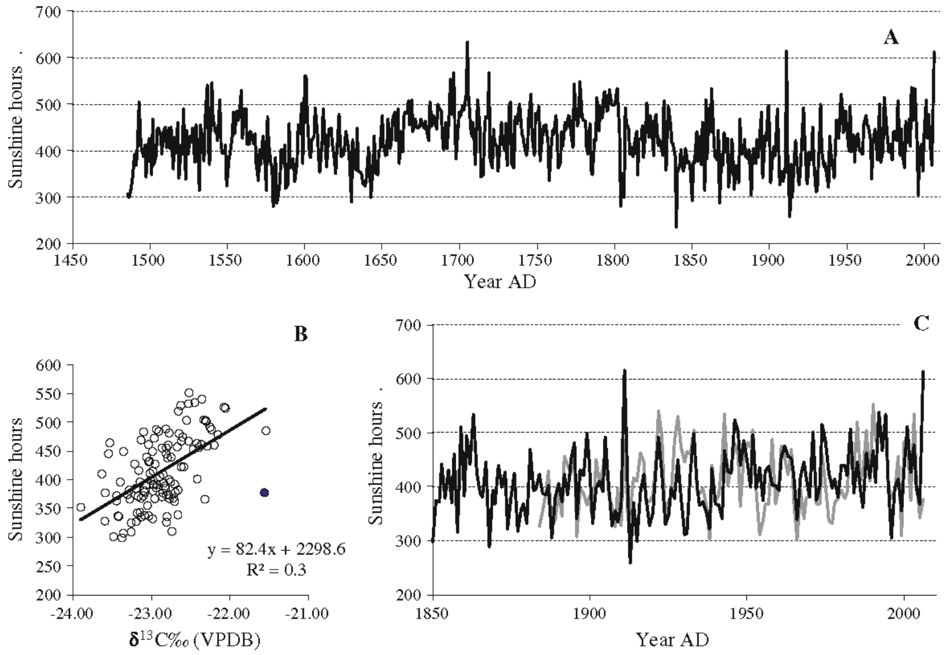

Fig. 6 July and August total sunshine hours reconstruction based on δ^{13}C from high altitude larch trees **a** and a scatter plot **b** and line-graph **c** showing the fit with sunshine hours measured at Villacher Alps in the Austrian Alps (measured series is the shorter *grey line*). Note that AD2006 is a clear outlier (*filled dot* on **b**) possibly reflecting the extreme dry conditions experienced during this year

reconstruction over-estimates sunshine and this probably represents increased stomatal control on fractionation due to very dry conditions. Similar conditions would explain the very high value for AD1705, and in the summer (July–August) temperature reconstruction of Dobrovolný (2010) AD1705 is strongly positive, and is warmer than AD1706. AD1706 in the Casty et al. (2005) reconstructions is listed as one of the warmest and driest summers, but those records also include June. Other very sunny summers include: AD1696, 1719, 1600 and 1601.

Two summers have anomalously low isotope values: AD1840 and 1913. The summer of 1913 was very wet and cold and although sunshine measured at Villacher Alps was not anomalously low, it may have been considerably cloudier at the field site. The summer of AD1840 was cold but is not listed by Casty et al. (2005) as particularly wet regionally. Other summers inferred to be very cloudy include: AD1804, 1580, 1868, 1582, 1630 and 1850.

5 Discussion and conclusions

Although δ^{13}C from high altitude larch trees in the Slovenian Alps correlate most strongly with mid to late summer temperature, we conclude that the dominant control, and most suitable target for reconstruction is actually mid- to late-summer sunshine. This argument is based on four lines of evidence:

1. Mechanistic models of carbon isotopic fractionation by trees suggest that the dominant control can be either stomatal conductance or photosynthetic rate. Photosynthetic rate is controlled more strongly by sunshine (photon flux) than it is by temperature.
2. Since the 1980s there has been an increase in summer temperature but not in hours of summer sunshine. The δ^{13}C results follow the stable sunshine data rather than the rising temperature record.
3. Even if the recent period of divergence between summer temperature and sunshine is ignored, when δ^{13}C is calibrated over the last century it tends to overestimate the measured summer temperatures of the past. This is not the case when sunshine is the reconstruction target.
4. If δ^{13}C is calibrated to temperature it produces a time-series that conflicts with other temperature reconstructions for the Alpine region that are based on strong evidence.

The summer sunshine reconstruction that we provide is the first for the Alpine region, but it needs to be treated with caution. It is calibrated using the best available data, which is total hours of sunshine of July and August, which is not the same as the mechanistic control on fractionation, which we suggest would be PAR. Given a correlation between carbon isotope ratios and hours of summer sunshine of 0.55, only about 30 % of the variance in the isotope chronology is explained by the available sunshine record. We would normally avoid using isotopes for climate reconstruction unless about half of the variance is explained (McCarroll et al. 2003), but since this is a novel interpretation of this proxy in an Alpine setting it is presented to encourage discussion and critique. A more reliable reconstruction would require greater replication and sampling of trees from several different sites, but even then the quality of the calibration might be compromised because of the paucity of suitable meteorological data.

If the $\delta^{13}C$ chronology is interpreted in terms of changes in sunshine rather than temperature, it likely provides a more synoptic view of changing climate over the last 500 years. Perhaps the harshest period of the Little Ice Age in the Alps, between AD1560 and 1600 is the largest negative anomaly in terms of sunshine, and this is followed by another cloudy period in the first half of the seventeenth century. The second half of the seventeenth century sees sunshine increase and the eighteenth century is generally sunnier than the twentieth century. Between AD1800 and 1950 there is a general decline in sunshine followed by a recovery in the last 60 years.

There is no reason to expect sunshine records in northern Fennoscandia to correlate with those in the Alps, but there are some similarities that are worthy of note. In both areas the eighteenth and nineteenth centuries were generally sunnier than the twentieth century and in both areas there are cold intervals that were relatively sunny and warmer intervals that were cloudy, indicating divergence of temperature and sunshine at multi-decadal timescales. However, just as the periods of most extreme warm and cold summers do not coincide between the two regions, so the sunniest and cloudiest periods are also out of phase.

If temperature and sunshine have not remained strongly coupled at multi-decadal timescales in the Alpine region it has implications for understanding and modelling climate change. The influence of changing temperatures on clouds, and therefore on sunshine, is the greatest source of uncertainty in the modeling of climate change (Trenberth and Fasullo 2009; Gagen et al. 2011). Reliable records of the changing relationship between temperature and sunshine over a long period would provide a powerful test of the ability of general circulation models to deal effectively with temperature/cloud feedbacks. The European Alps is an area with unrivalled evidence of changes in past temperature (Dobrovolný et al. 2010; Trachsel et al. 2012) but given that the sunshine reconstruction is based on a single $\delta^{13}C$ chronology, it would be imprudent to use the relationship between temperature and sunshine presented here for model evaluation. Greater replication and addition of different field sites would strengthen the record, but will not deal with the problem of calibration. Perhaps the most powerful test of the hypothesis that tree ring $\delta^{13}C$ chronologies can represent changes in sunshine would be an independent reconstruction of a sunshine parameter based on the wealth of documentary sources that have been collected for this region (Brázdil et al. 2010). A reconstruction of past changes in circulation, using stable oxygen isotopes in tree rings, might also help to explain periods of divergence between sunshine and temperature and also account for the temporal offsets in climate extremes in different regions.

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3.1.3 Anatomske značilnosti in hidrološki signal v branikah hrasta (*Quercus robur* L.)

Anatomical characteristics and hydrologic signals in tree-rings of oaks (*Quercus robur* L.)

Jožica Gričar, Martin de Luis, Polona Hafner, Tom Levanič
Trees – Structure and function, 2013, 27:1669–1680

Propadanje vrste *Quercus robur* v evropskih poplavnih gozdovih v zadnjih letih je močno povezano s spremenjenim hidrološkim režimom. Da bi določili vpliv mikrolokacijskih razmer na hidrološki signal v lesnoanatomskih značilnostih, smo proučili vpliv pretoka reke Krke na strukturo branik *Q. robur* v poplavnem Krakovskem gozdu (Slovenija). Na dveh bližnjih lokacijah smo izbrali dve skupini dreves *Q. robur*. Lokaciji se med seboj razlikujeta po hidroloških razmerah, in sicer je bolj vlažno rastišče (W-wet hrasti) spomladi in jeseni pogosto poplavljeno, medtem ko na bolj sušnem rastišču (D-dry hrasti) poplav ni opaziti. Odkrili smo razlike v anatomski zgradbi branik med obema skupinama hrastov, pri čemer se je širina branike pokazala kot glavni parameter, ki določa anatomsko zgradbo hrastovine. D in W hrasti so se različno odzvali na pretok reke Krke v proučevanem obdobju. Na debelinsko rast D hrastov je negativno vplival spomladanski pretok, pozitivno pa minimalni poletni pretok, pri W hrastih je širina branike pozitivno povezana s srednjim poletnim pretokom. Okoljska informacija, shranjena v lesnoanatomskih značilnostih, lahko variira celo znotraj enega sestoja, in je v veliki meri odvisna od mikrolokacije. Zmanjšan prirastek D hrastov nakazuje, da so rastne razmere zanje manj ugodne in iz tega sledi povezava med zdravstvenim stanjem hrastov nižinskih gozdov in hidrološkimi razmerami. Drevesa za hidrološke rekonstrukcije morajo biti pazljivo izbrana, s čimer se izognemo izgubi informacij in napačnim interpretacijam rezultatov. Anatomske značilnosti in hidrološki signali v branikah doba na redno poplavljenih območjih lahko variirajo, celo znotraj enega gozdnega sestoja, in so v veliki meri odvisni od mikrolokacijskih razmer.

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ORIGINAL PAPER

Anatomical characteristics and hydrologic signals in tree-rings of oaks (*Quercus robur* L.)

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Abstract

Key message **Anatomical characteristics and hydrologic signals in tree-rings of oaks from areas with regular flooding may vary, even within the same forest stand, and largely depend on the micro-environmental conditions.**

Abstract *Q. robur* decline in European floodplain forests in recent years seems to be strongly associated with the deteriorating hydrological regime. We investigated the influence of the Krka River flow on tree-ring patterns of *Q. robur* from the Krakovo floodplain forests (Slovenia) to assess the effect of micro-location conditions on hydrological signals in wood-anatomical characteristics. We selected two groups of *Q. robur* trees growing at nearby locations with different hydrological conditions, resulting in frequent autumn and spring flooding at the wetter site (=W oaks) but no flooding at the other, drier site (=D oaks). We found differences between the two groups in the anatomical structure of tree-rings; however, ring width proved to be the main variable determining the anatomical structure of oak wood. D and W oaks responded differently to the Krka River flow in the studied period. Radial growth of D oaks was negatively influenced by spring flow, but positively influenced by minimum summer flow. In W oaks, ring width was positively correlated with mean summer flow. Thus, environmental information stored in wood-anatomical features may vary, even within the same forest stand, and largely depends on the micro-environment. Reduced wood increments of D oaks suggest that growth conditions are less favourable, implying a link between the health state of oaks from lowland forest and hydrological conditions. Trees intended for hydrological reconstruction must therefore be carefully selected to avoid the possibility of error and potential loss of information. Anatomical characteristics and hydrological signals in tree-rings of oaks from areas with regular flooding may vary, even within the same forest stand, and largely depends on the micro-environmental conditions.

Keywords Hydrological sensitivity · European oak · Tree-rings · Vessels · Wood structure · River flow · Micro-environment

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Introduction

In Slovenia, oaks (*Quercus robur* L. and *Quercus sessiliflora* Salisb.) are economically very important wood species, representing about 7 % of the entire wood stock (Gozdnogospodarski etc. 2006). In relation to *Q. robur*, the lowland forest area has been shrinking, due to human settlement in the past, intensive and unplanned silvicultural and agricultural exploitation of the land and conflicts of interest, so only a few lowland oak forest stands have managed to survive (Kadunc 2010). In addition, as in many European countries (e.g., Klimo and Hager 2001), a trend of decreasing vitality of *Q. robur* has been observed in most sites in recent decades (Čater et al. 2001). One of the main reasons for this situation in Slovenia is ascribed to decreasing ground water levels due to changes in climatic conditions and unsuitable artificial melioration of land for agricultural purposes, for which numerous drainage ditches were excavated in the 19th century (Čater et al. 2001). The most obvious response of *Q. robur* to the changing environmental (hydrological) conditions is seen in its decreased vitality (e.g., Hager and Buchleitner 2001; Vukelić and Rauš 2001), resulting in reduced wood increment (Levanič et al. 2011), which is closely related to the structure of wood and its quality (Rao et al. 1997).

In addition to major economic consequences in these areas, ecological issues associated with decreasing vitality of *Q. robur* stands cannot be neglected. The many local oak tree-ring chronologies from various sites of Slovenia (Čufar and Levanič 1999a, b; Čufar et al. 2008) differ greatly among each other, particularly in the case of *Q. robur* from lowland sites, where tree growth is often influenced by micro-site hydrological conditions (Čater 2003; Čater and Batič 2006; Čater and Levanič 2004; Levanič 1993; Levanič et al. 2011). However, tree-ring width (TRW) is only one environmental proxy, whereas others still remain to be explored. In this respect, the use of other proxies, such as wood-anatomical variables, have proved to be particularly promising (e.g., Fonti et al. 2010).

Q. robur is a regular constituent of floodplain forests and is generally considered to be one of the most flood-tolerant *Quercus* species with respect to growth and survival (Prpić 2003; Schmull and Thomas 2000). However, knowledge of its growth response in such environments is scarce (e.g., Leuschner et al. 2002; Sass-Klaassen and Hanraets 2006). Due to changes in the hydrological regime, the groundwater level and water supply might differ even in the same forest stand, which raises the question of the extent to which micro-location conditions affect tree-ring patterns of *Q. robur*. This could be an important factor for assessing the environmental sensitivity of *Q. robur* from floodplain forests in Slovenia.

To test this hypothesis, we selected two groups of adult *Q. robur* trees, growing at nearby locations with different hydrological conditions. The wetter site (=W oaks) is characterised by frequent autumn and spring flooding, whereas no flooding occurs at the drier site (=D oaks). To reduce any geographical differences in climate affecting the trees under study to a minimum, the sites were chosen in the same forest stand in Krakovo forest, which is in some parts often flooded by the Krka River. In particular, aims of the study were to: (1) evaluate if TRW and their anatomical characteristics (including latewood) of *Q. robur* differ in flooded and unflooded areas. For this purpose, we used 10 different wood-anatomical variables for the period 1970–2008; (2) to assess if selected 10 anatomical variables show the same pattern of temporal variation and if they contain complementary or redundant information; (3) to determine if morphological characteristics of the selected variables are related to the Krka River flow.

Materials and methods

Study site characteristics

The research was carried out in Krakovo *Querco robori–Carpinetum* mixed forest, Slovenia (45°54′N, 15°25′E, elevation 150 m), which is the largest lowland oak forest complex in Slovenia and is mainly composed of *Quercus robur*, *Carpinus betulus* and *Alnus glutinosa* in combination with *Tilia sp., Prunus avium*, *Acer campestris*, *Fraxinus angustifolia* and *Ulmus campestris* tree species.

The appearance of the specific forest association is mostly determined by microtopography and soil properties, which, to a large degree, influence runoff, distribution and water movement into the soil. Hydromorphic soils, such as pseudogley and gleysoils (amphygley), on pleistocene clays and loams with low infiltration capacity prevail. The occurrence of different forms of deposits is responsible for the difference in permeability of the soil surface, which in turn affects groundwater level. In rainy periods, water can temporarily stagnate on the surface and slowly evaporates or absorbs into the soil. The area may remain flooded for weeks. However, due to low infiltration capacity of this soil type, most of the rainwater on slightly inclined slope or a bit higher elevation can runoff the surface before the soil absorbs it. This could negatively affect the hydrological conditions at such micro-locations (Čater et al. 2001). Differences in the depth of the groundwater table at the same surface level can be up to 90 cm within a horizontal distance of 2 m. The level of water table varies during the year, being very high in the fall, winter and early spring (Žibert 2006).

In addition to the groundwater level and rainwater, which may stagnate on the low permeable soil surface, frequent autumn and spring flooding of the Krka River, the main river in this area, significantly affect hydrological conditions in the Krakovo forest. The Krka River belongs to the Karst Rivers. It has a rain-snow regime, with runoff peaks in April and November, and minima in August and January. The Krka River has numerous small tributaries and regularly floods, especially in spring and autumn. Data for minimal (Q_{np}), average (Q_s), maximal (Q_{vp}) monthly rate (Fig. 1) and annual (Fig. 2) rate of flow for the Krka River for the period 1970–2008 were obtained from the Environmental Agency of the Republic of Slovenia within the Ministry of the Environment and Spatial Planning.

The study area is characterised by sub-pannonian continental climate. About 70 % of all precipitation thus falls during the growing season (March–October) and a very small amount in winter. The mean annual temperature is 10.1 °C (range 8.6–12.0 °C, $T_{Jan} = -0.1$ °C, $-T_{Jul} = 20.1$ °C) and total annual precipitation 1,149 mm (range 827–1405 mm, $P_{Jan} = 51.3$ mm, $P_{Aug} = 126{,}9$ mm), as calculated from the

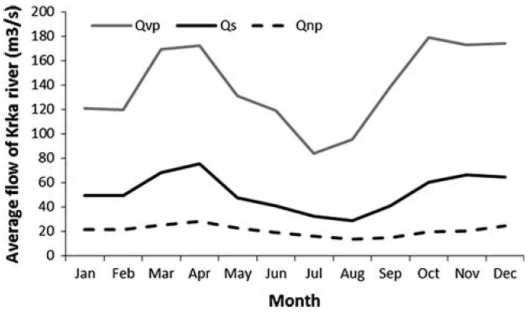


Fig. 1 Average monthly flow of the Krka River (Q_s, Q_{vp}, Q_{np}). *Qnp* minimal monthly rate of flow (daily average) [m3/s], Q_s average monthly rate of flow [m3/s], Q_{vp} maximal monthly rate of flow (daily average) [m3/s]

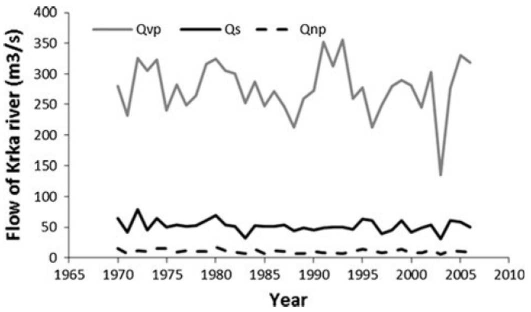


Fig. 2 Krka River flow variables (Q_s, Q_{vp}, Q_{np}) calculated at annual scales. Q_{np} minimal monthly rate of flow (daily average) [m3/s], Q_s average monthly rate of flow [m3/s], Q_{vp} maximal monthly rate of flow (daily average) [m3/s]

1970 to 2008 climate dataset from the nearby Novo mesto climate station of the Environmental Agency of the Republic of Slovenia. The station is located approximately 20 km from the forest site (45°48′N, 15°11′E; altitude 220 m).

Tree selection, sampling and anatomical observations

Pedunculate oak (*Quercus ruber* L.) trees were sampled at two research plots in a Krakovo forest stand. To reduce any geographical differences in climate affecting the trees under study to a minimum, the research plots were only about 600 m apart and belong to the same forest association, but differ in the hydrological conditions. Oaks growing on the first plot were exposed to occasional flooding (=W oaks), especially in autumn and spring periods, whereas in the second plot, flooding does not occur (=D oaks). The reason could be in a slightly inclined surface and a bit higher elevation, which prevent the retention of the surface water.

Six dominant or codominant trees were selected on each plot; a total of 12 trees were thus analysed. Selected trees were 80–100 years old, with DBH 30–60 cm, without any visible mechanical injures of stems or roots. During winter 2008–2009, we took 1 cm wide cores about 1.3 m above the ground from each of the trees to prepare microscopic slides. The material was fixed in formalin-ethanol-acetic acid solution (FEA) and dehydrated in a graded series of ethanol (30, 50 and 70 %) after 1 week. Each core was cut exactly at the growth ring boundary into pieces about 5–6 cm long, so that they could be placed on microscope slides. Permanent transverse sections of 25 µm in thickness were prepared on a "G.S.L. 1" Sledge microtome (©Gärtner and Schweingruber; Design and production: Lucchinetti, Schenkung Dapples, Zürich, Switzerland) with disposable blades. Sections were stained with safranin (Merck, Darmstadt, Germany) (0.5 % in 95 % of ethanol) and mounted in Euparal and observed under an Olympus BX51 (Tokyo, Japan) light microscope and analysed with the Nikon NIS-Elements Basic Research v.2.3 image analysis system (Tokyo, Japan).

Oak ring-porous wood is composed of several types of cells, which are specialised to accomplish their function; vessels, vasicentric tracheids, libriform fibres and axial and ray parenchyma cells (Carlquist 1988). All vessels are sheathed by thin-walled vasicentric tracheids and are responsible for water transport. Most of the EW area is occupied by vessels that can be seen with the naked eye in the transverse plane (diameters more than 200 µm), whereas vessels in LW are much smaller (diameter around 50 µm). LW vessels are distributed solitarily or in wide growth rings, as radially orientated groups, which alternate with groups of thick-walled libriform fibres. Mechanical support is mainly provided by libriform fibres. The distribution of axial parenchyma is either diffuse or in uniseriate diagonal and tangential bands. Oak has two types of rays; uniseriate and broad (up to 30 cells), which are also clearly visible at macroscopic level (Fig. 3).

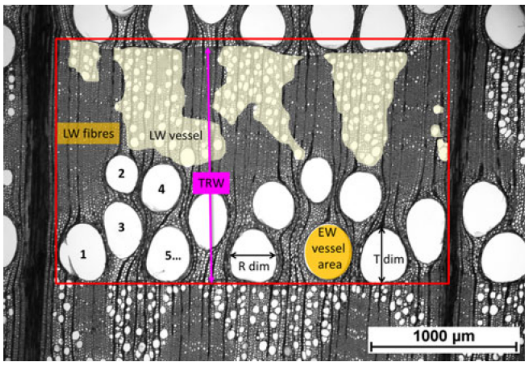


Fig. 3 Schematic illustration of the measured anatomical parameters in each tree-ring. *EW* earlywood, *LW* latewood, *T dim* tangential dimension of EW vessel, *R dim* radial dimension of EW vessel

Table 1 Measured anatomical variables in the tree-rings of W and D oaks

Code of the variable	Description of the measured variable (unit)
TRW	Width of tree-ring (μm)
T ave ves	Average tangential diameter of EW vessels (μm)
R ave ves	Average radial diameter of EW vessels (μm)
EW ves area	Average values of EW vessel area (μm2)
EW ves/Meas area	Conductive area of EW vessels (Percentage of cross-sectional area occupied by EW vessels) 100*(%)
EW ves/EW area	Conductive area in EW (Percentage of EW cross-sectional area occupied by EW vessels) 100*(%)
No of ves/Meas area	EW vessel density (Number of EW vessels per square millimetre of measured area of the tree-ring) ($n°/mm^2$)
No of ves/EW area	EW vessel density in EW (Number of EW vessels per square millimetre of EW measured area) ($n°/mm^2$)
LW portion ves/Fibre	Proportion of LW vessels according to the proportion of fibres in LW (%)
LW portion	Proportion of LW (%)

EW earlywood, *LW* latewood

We analysed the wood structure of the last 39 rings (1970–2008) to avoid juvenile wood, because its anatomical structure and TRW significantly differ from adult wood. In each of the tree-rings, we determined the measurement frame, in which we analysed various anatomical characteristics in earlywood (EW) and latewood (LW) (Table 1). The tangential width of the frame was 4 mm and the radial width was the width of the tree-ring (Fig. 3); however, if multi-layered rays were present, we subtracted their area from the measured area. Visual inspection revealed that the anatomical structure of tree-rings of similar width differed in W and D oaks (Fig. 4).

Anatomical characterisation of tree rings of W and D oaks

Repeated measures analysis of variance (R-M ANOVA) was used to test differences between W and D oaks in all the 10 measured anatomical variables. The common period 1970–2008 was used for this analysis. Normal distribution

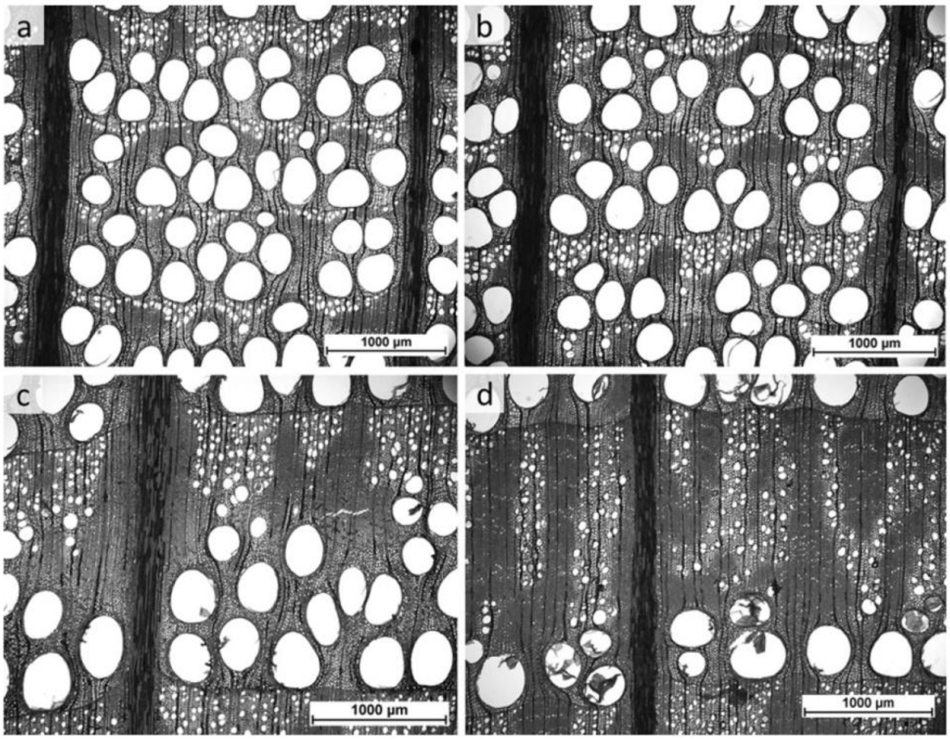


Fig. 4 Similar TRW (**a–d**) with different anatomical structure. **a** Tree-ring of D oak with higher proportion of EW; **b** tree-ring of D oak with higher proportion of LW; **c** tree-ring of W oak with higher proportion of EW; **d** tree-ring of W oak with higher proportion of LW. *EW* earlywood, *LW* latewood

and uniformity of variance of each analysed variable was verified using the Shapiro–Wilk W test and Levene test, respectively (Quinn and Keough 2002).

Influence of river flow on tree growth and anatomical characteristics

The individual measured anatomical series were standardised in the ARSTAN programme (Cook and Holmes 1986) by removing long-term trends using a negative exponential function followed by a cubic smoothing spline with a 50 % cut-off frequency and a response period of 30 years. An autocorrelation filter was applied to the detrended series to remove correlations between consecutive measurements and to obtain a residual series containing only high frequency variations in year-to-year series, which are expected to be mainly related to year-to-year environmental variability. The indexed residual series were then averaged using a biweight robust mean to obtain, both for D and W oaks, residual chronologies of each of the 10 analysed variables.

The common period 1970–2008 was then also considered for multivariate analysis to identify common modes of variability in the obtained residual chronologies. Principal component analysis (PCA) of the covariance matrix of the residual chronologies was used for grouping series with similar year-to-year variations. PCA was conducted using the same time datasets of anatomical variables of W and D oaks consisting of 20 variables in total (10 for each group). These components were rotated orthogonally according to the VARIMAX criterion to redistribute the final explained variance and to obtain more stable and robust patterns. The most representative principal components were selected and the weighting components (rotated component loadings) were examined to identify the pattern of association of each chronology with each component (Kaiser 1992).

Correlation functions between PCA and river flow data were calculated using the DendroClim2002 programme (Biondi and Waikul 2004), whereby the obtained significant PCA eigenvectors were the dependent variables, while the independent variables were the seasonal and annual series of flow data.

Results

Comparison of anatomical parameters in tree-rings of W and D oaks

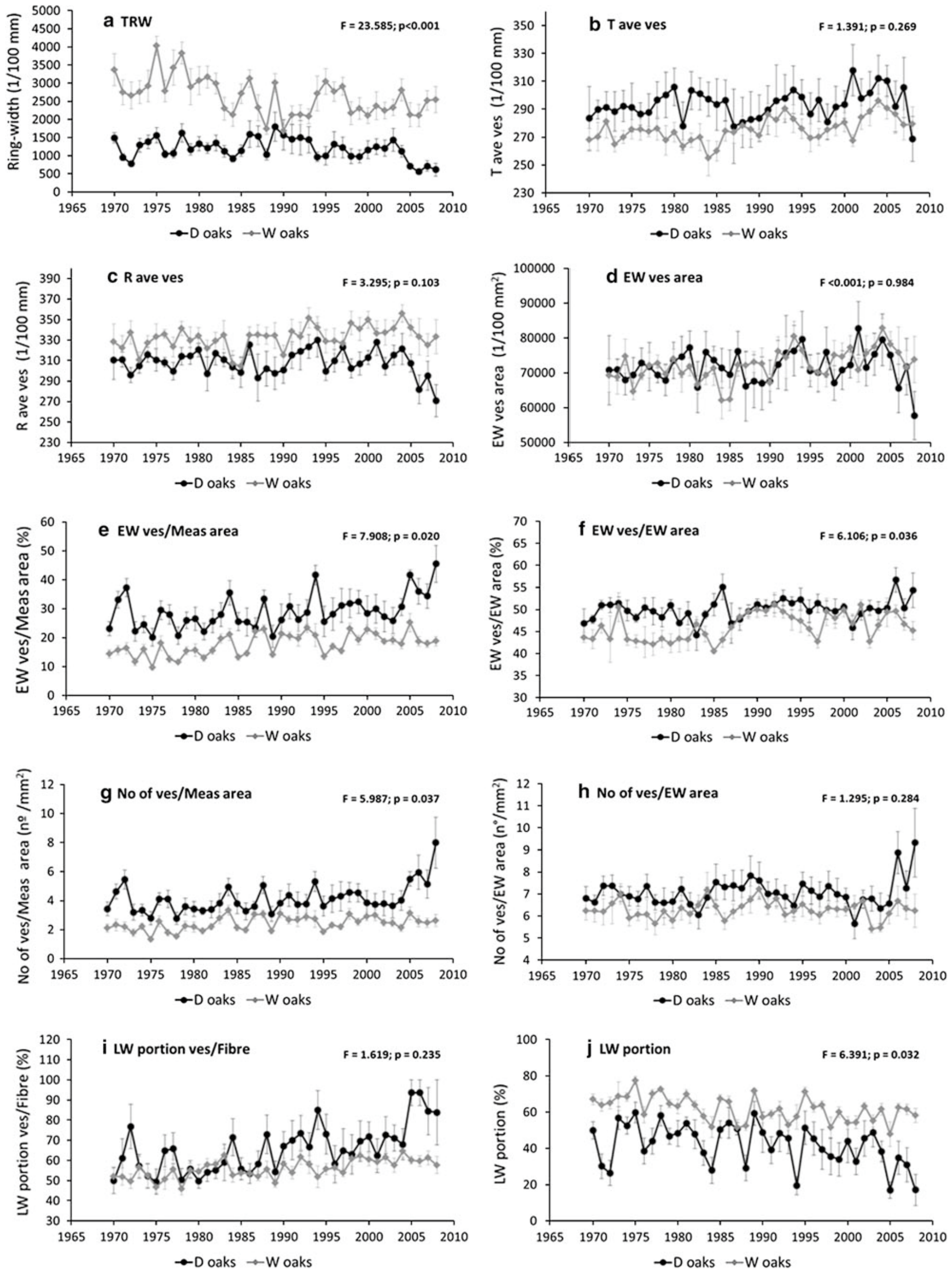
Average values and standard deviation of TRW and measured wood-anatomical variables of W and D oaks are presented in Table 2, whereas the time series of their mean values and standard errors for both groups for the period 1970–2008 are shown in Fig. 5. R-M ANOVA confirmed that differences between the two groups exist in 5 of the 10 analysed variables (Fig. 5).

Tree-rings of W oaks (average ring width = 2,642.05 μm) were about 2.2-times wider than those of D oaks (average ring width = 1,196.31 μm) (Table 2). Average radial and tangential diameters of EW vessels and consequently their cross-sectional area did not differ between the two groups. Average tangential diameter was smaller than the radial one (for 15 μm in D oaks and 56 μm in W oaks, respectively) and average EW vessel area was around 72,000 μm^2 in both groups. The proportion of the conductive area in the EW was higher in D oaks (50.09 %) than in W oaks (46.07 %), although the number of EW vessels in the EW area did not differ between the two groups (Table 2). Since the proportion of LW was higher in W oaks (61.71 %) than in D oaks (42.38 %), the number and total area of EW vessels per measured area was consequently lower. The proportion of vessels in LW did not differ between the groups.

Common and uncommon temporal patterns in measured anatomical variables

PCA analysis revealed differences in year-to-year dynamics among different wood-anatomical characteristics and between W and D oaks. Anatomical characteristics and the relation among the measured variables can be described by four components that together explained 77.2 % of the total variance. PCA analysis demonstrated that the selected anatomical variables in W and D oaks significantly differed in their temporal patterns. Two components of the PCA (PC1 and PC3) were mainly related to D oaks and the other two (PC2 and PC4) to W oaks (Fig. 6).

In both oak groups, TRW and the proportion of LW were positively related (PC1 for D oaks and PC2 for W oaks, respectively). These two anatomical variables were, on the other hand, negatively related to the following anatomical parameters: conductive area of EW vessels and EW vessel density, and in the case of D oaks (PC1) also to the share of vessels in LW (Fig. 6a, b). Furthermore, in both groups, tangential and radial dimensions of EW vessels were positively related (PC3 for D oaks and PC4 for W oaks, respectively) and consequently also average EW vessel area (Fig. 6c, d). These variables were negatively related to EW vessel density, so that high EW vessel density corresponds to lower EW vessel area. To summarise, we found a statistically significant difference between the two groups of oaks in the following anatomical variables: TRW, conductive area of EW vessels, conductive area in EW, EW vessel density and LW proportion.


a TRW
F = 23.585; p<0.001
Ring-width (1/100 mm)
b T ave ves
F = 1.391; p = 0.269
T ave ves (1/100 mm)
c R ave ves
F = 3.295; p = 0.103
R ave ves (1/100 mm)
d EW ves area
F <0.001; p = 0.984
EW ves area (1/100 mm²)
e EW ves/Meas area
F = 7.908; p = 0.020
EW ves/Meas area (%)
f EW ves/EW area
F = 6.106; p = 0.036
EW ves/EW area (%)
g No of ves/Meas area
F = 5.987; p = 0.037
No of ves/Meas area (nº/mm²)
h No of ves/EW area
F = 1.295; p = 0.284
No of ves/EW area (n°/mm²)
i LW portion ves/Fibre
F = 1.619; p = 0.235
LW portion ves/Fibre (%)
j LW portion
F = 6.391; p = 0.032
LW portion (%)
D oaks
W oaks

◀**Fig. 5** Time series of of tree-ring widths and wood-anatomical characteristics of W and D oaks for the period 1970–2008 showing differences in some parameters (TRW, EW ves/Mean area, EW ves/EW area, No of ves/Meas area and LW portion), but similarities in others (T ave ves, R ave ves, EW ves area, No of ves/EW area and LW portion ves/Fibre). *Error bars* indicate standard error. Repeated measures of variance (R-M ANOVA) was used to test for differences between W and D oaks in all the measured anatomical variables. *TRW* tree-ring width, *EW* earlywood, *LW* latewood, *T ave ves* average tangential diameter of EW vessels, *R ave ves* average radial diameter of EW vessels, *EW ves area* average values of EW vessel area, *EW ves/Meas area* conductive area of EW vessels, *EW ves/EW area* conductive area in EW, *No of ves/Meas area* EW vessel density, *No of ves/EW area* EW vessel density in EW, *LW portion ves/Fibre* proportion of LW vessels according to the proportion of fibres in LW

Relation between the measured anatomical variables in each of the studied oak groups and the Krka River flow

Correlation between PCA components and the Krka River flow data at annual and seasonal (from previous autumn to summer) time scales are presented in Fig. 7. PC1 and PC3 were mainly related to D oaks whereas PC2 and PC4 to W oaks. Anatomical variables of D oaks linked to PC1 were positively related to spring (mainly maximum) flow and negatively to summer minimum flow, indicating that tree-rings and latewood were wider if spring flow of the Krka River was lower and minimum summer flow was higher. On the contrary, conductive area of EW vessels and EW vessel density were higher if spring flow was higher and summer flow was lower (Fig. 7a). Anatomical variables of W oaks linked to PC2 were negatively related to the (mean) summer flow showing its positive relation with TRW and latewood widths (LWW), but negative with conductive area of EW vessels, EW vessel density and the share of vessels in LW (Fig. 7b).

We found no relation of PC3 (D oaks) and PC4 (W oaks) with the Krka River flow data, suggesting that in both groups temporal variability patterns of the wood-anatomical variables expressed with these two PC components (i.e., tangential and radial dimensions of EW vessels, average EW vessel area and EW density) were not related with the Krka River flow and were probably influenced by other environmental (climatic) factors (Fig. 7c, d).

Discussion

Comparison of anatomical structure in xylem of W and D oaks

The properties of oak wood are closely related to TRW (e.g., Gasson 1987; Leal et al. 2007, 2008; Rao et al. 1997). Studies on within-tree-variation of wood properties in a radial direction in different oak species have shown that cambial age has an influence on TRW, EW vessel diameter, proportion of fibre, vessel and axial parenchyma in LW and specific gravity (Gasson 1987; Lei et al. 1996). Since cambial age explains more of the variation in wood density than does TRW, although TRW declines with cambial age (Lei et al. 1996; Zhang and Zhong 1991), we excluded the juvenile portion of wood from our analysis.

Tree-rings of W oaks were significantly wider than in D oaks, with a lower proportion of EW. It is well known that in ring-porous oak, LWW tends to increase with increasing TRW, whereas the width of EW (EWW) remains more or less constant (Lebourgeois et al. 2004; Lei et al. 1996;

Table 2 Average values and standard deviation (SD) of measured anatomical variables in W and D oaks

Variable	D Oaks		W Oaks	
	Average	SD	Average	SD
TRW (μm)	1,196.31	593.94	2,642.05	887.52
T ave ves (μm)	293.40	58.11	275.56	55.48
R ave ves (μm)	308.47	60.59	332.44	65.95
EW ves area (μm2)	71,753.86	14,874.27	72,239.30	10,252.00
EW ves/Meas area (%)	28.868	10.595	17.633	5.364
EW ves/EW area (%)	50.085	5.525	46.086	5.823
No of ves/Meas area (n°/mm2)	4.092	1.778	2.242	0.833
No of ves/EW area (n°/mm2)	7.057	1.493	6.293	1.138
LW portion ves/Fibre (%)	65.38	20.87	55.90	9.13
LW portion (%)	42.38	19.85	61.71	10.63

TRW tree-ring width, *EW* earlywood, *LW* latewood, *T ave ves* average tangential diameter of EW vessels, *R ave ves* average radial diameter of EW vessels, *EW ves area* average values of EW vessel area, *EW ves/Meas area* conductive area of EW vessels, *EW ves/EW area* conductive area in EW, *No of ves/Meas area* EW vessel density, *No of ves/EW area* EW vessel density in EW, *LW portion ves/Fibre* proportion of LW vessels according to the proportion of fibres in LW

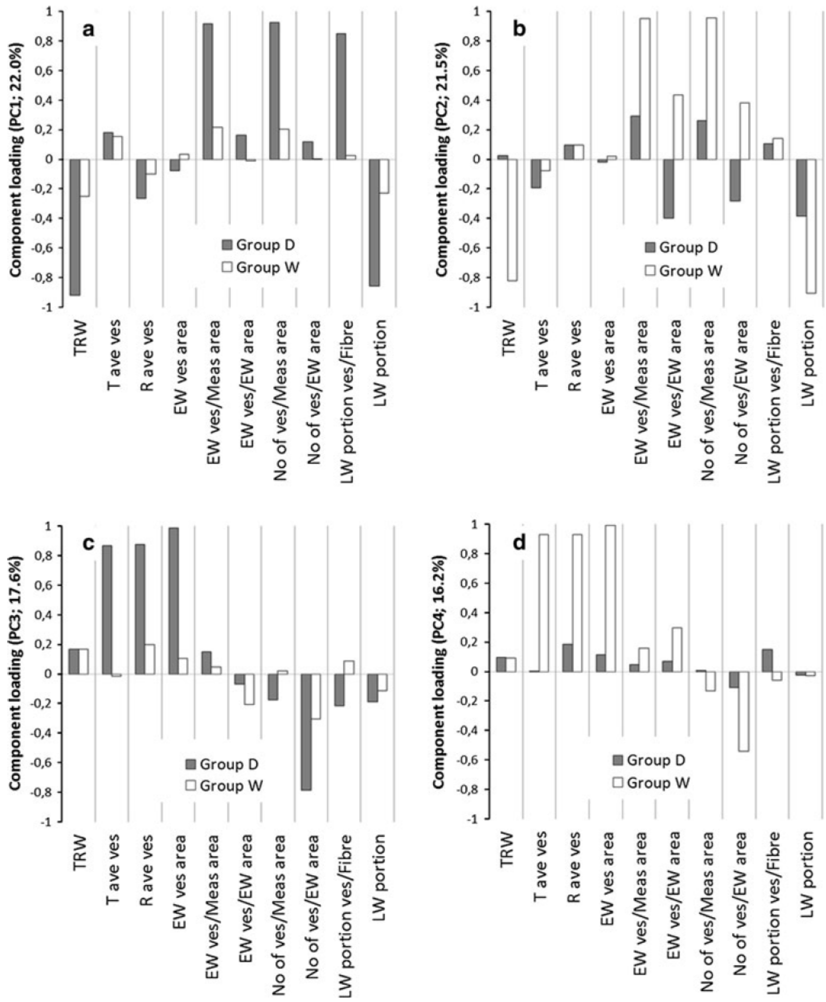


Fig. 6 Anatomical characteristics and relations among the variables are explained by PC1 and PC3 in D oaks (**a**, **c**) and by PC2 and PC4 in W oaks (**b**, **d**). *EW* earlywood, *LW* latewood, *T ave ves* average tangential diameter of EW vessels, *R ave ves* average radial diameter of EW vessels, *EW ves area* average values of EW vessel area, *EW ves/Meas area* conductive area of EW vessels, *EW ves/EW area* conductive area in EW, *No of ves/Meas area* EW vessel density, *No of ves/EW area* EW vessel density in EW, *LW portion ves/Fibre* proportion of LW vessels according to the proportion of fibres in LW

Phelps and Workman 1994; Rao et al. 1997). More precisely, Zhang (1997) noted that with increasing TRW, LWW increases almost linearly, while EWW increases a little at first, but tends to be constant (about 1 mm) when TRW is wider than 3 mm. Since the anatomical structure of EW and LW is very different, their densities also vary, being for about 30 % higher in LW than in EW (Guilley et al. 1999).

We found differences in the anatomical structure of tree-rings in W and D oaks; however, in both groups, anatomical structure of the oak wood proved to be closely related to TRW. In addition to EW proportion, TRW negatively influence the share of total EW conductive area and EW vessel density and, in the case of D oaks, also the proportion of the conductive area in LW, which is in line with the findings of other authors (e.g., Gasson 1987; Phelps and Workman 1994; Rao et al. 1997). The total conductive area in EW was slightly higher in the D oaks, which can be explained by the smaller share of EW in narrow rings, which contain a lower number of vessel rows. It has also been observed in other ring-porous species that in wider rings the proportion of EW vessels with smaller diameters increases, thus reducing the mean EW vessel area (Eilmann et al. 2009; Fonti and García-González 2004; Tardif and Conciatori 2006).

Most studies analysing wood-anatomical features have focused primarily on the structure of EW (more specifically EW vessels), while the structure of LW has only rarely been examined (e.g., Eilmann et al. 2006; Phelps and Workman 1994). Our study shows that the proportion of conductive elements in LW was about 10 % higher in D oaks, although this difference was not statistically significant. In the case of D oaks, narrower rings (<800 μm)

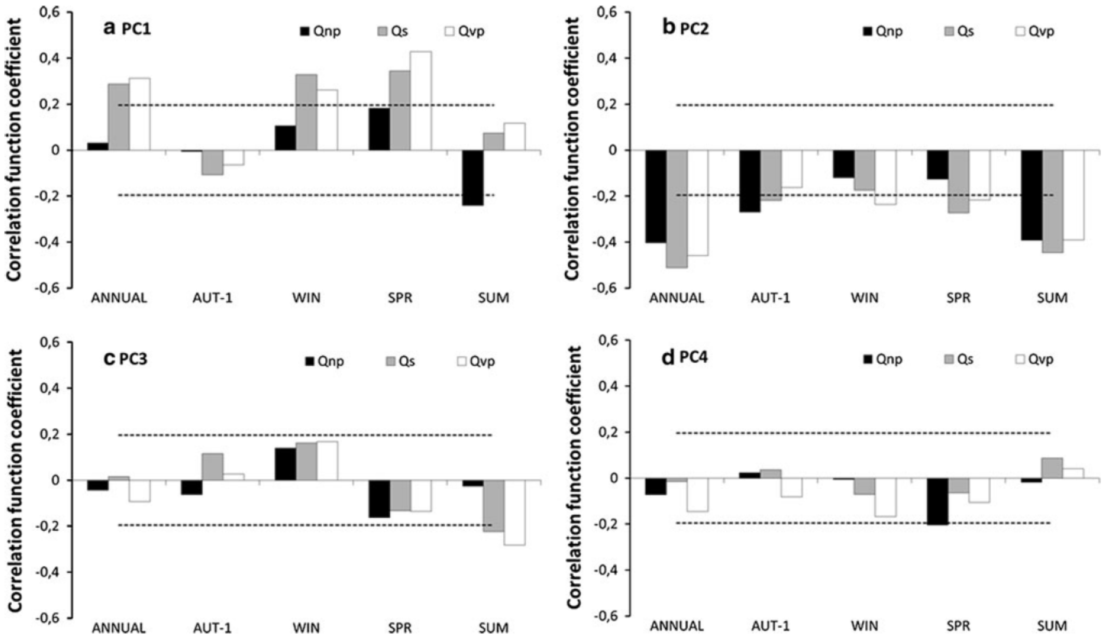


Fig. 7 Correlation between PCA scores and the Krka River flow data at annual and seasonal (from previous autumn to summer) time scales. PC1 and PC3 are mainly related to D oaks (**a**, **c**) whereas PC2 and PC4 to W oaks (**b**, **d**). The horizontal line indicates significance level at 95 %. Q_{np} minimum flow, Q_s mean flow, Q_{vp} maximum flow, *AUT-1* previous autumn, *WIN* winter, *SPR* spring, *SUM* summer, *EW* earlywood, *LW* latewood, *T ave ves* average tangential diameter of EW vessels, *R ave ves* average radial diameter of EW vessels, *EW ves area* average values of EW vessel area, *EW ves/Meas area* conductive area of EW vessels, *EW ves/EW area* conductive area in EW, *No of ves/Meas area* EW vessel density, *No of ves/EW area* EW vessel density in EW, *LW portion ves/Fibre* proportion of LW vessels according to the proportion of fibres in LW

contained only a small portion of LW, which was mainly composed of LW vessels and tracheids. If fibres were present, they were not arranged in radial flames, as typical of oaks. LW was even absent in some cases. The negative relation between the percentage of LW conductive area and TRW that was observed in D oaks has also been reported by Phelps and Workman (1994). These observations suggest that the formation of LW vessels has priority in LW, in comparison with fibres, indicating the precedence of the conductive function over the mechanical, even though LW vessels do not contribute much to conductivity as long as there are conducting EW vessels. However, as a ring-porous species, oak has strongly reduced hydraulic conductivity in early spring because the large vessels in the EW from the past year become embolized either during the summer or during the winter (Bréda and Granier 1996; Hinckley et al. 1979). Early spring, when a new set of EW vessels is forming, is therefore an exception; at that time the ascent of sap takes place via LW vessels, which remain functional for several years (Tyree and Zimmermann 2010). Vessel diameter, area and percentage of conductive area strongly influence the amount of water that can be transported in the living tree, and so the higher proportion of ring occupied by conductive elements, the less tissue is available for supporting, strengthening and storage. A decrease in fibre proportion would then decrease the mechanical properties of LW, but the need for additional strength becomes less crucial as the stem increases in diameter (Rao et al. 1997).

The anatomical structure of wood in *Q. robur*, which is closely related to TRW, with different proportions of xylem elements and their morphological characteristics, defines the hydraulic and mechanical properties of wood and hence affects the survival and efficiency of the living tree (Rao et al. 1997). In terms of wood properties, an increase in the size and density of EW vessels have a negative effect on wood density (Leal et al. 2007) indicating that diminished radial growth considerably negatively affects wood properties and quality of oaks.

Relation between the measured anatomical variables in each of the studied oak groups and the Krka River flow

TRW and LW density have been proven to be closely linked to environmental conditions and are therefore very useful for climate reconstruction (e.g., Friedrichs et al. 2009). In this study, we have demonstrated that certain wood-anatomical variables of *Q. robur* have a potential also in dendrohydrological studies.

The two groups of oaks responded differently to the Krka River flow in the studied period (i.e., 39 years). TRW and LW proportion of D oaks were affected by (mainly maximum) spring and minimum summer flow of the Krka River and were wider if the flow in spring was lower but higher in summer. Since TRW (as well as LW proportion) was negatively related with the conductive area of EW vessels and EW vessel density, these two variables were higher if the spring flow was higher. On the other hand, anatomical variables of W oaks were predominantly related to the (mean) summer flow showing its positive relation with tree-ring and LW proportion, but negative with conductive area of EW vessels, EW vessel density and the share of vessels in LW. Thus, in both oak groups the conductive area of EW vessels and their density were undoubtedly closely (inversely) related to TRW. This could be explained by almost proportional relationship between TRW and LWW (Rao et al. 1997). Hence, if tree-ring is wider, the proportion of LW is larger and consequently the share of EW vessels and their density decreases. Wimmer (2002) considered that only a few wood-anatomical features have proved to be useful for characterising the relationship between tree growth and climate, because they are often inter-correlated with more easily obtainable variables, thus providing little new environmental information. According to Tardif and Conciatori (2006), EW vessel features in ring-porous species may be best used to decipher a discontinuous signal related to tree growth, in particular, for understanding tree physiology. At this point, it should be stressed again that, in addition to our small sample size, most dendroecological studies of *Quercus* spp. have focused on climatic data and not hydrological data and have not been conducted in an area with regular flooding; it is therefore difficult to compare our results with their findings. *Quercus* species are differently adapted to drought (Nardini and Tyree 1999). *Q. robur* is known to be a water-demanding species and is a regular constituent of floodplain forests. It is generally considered to be one of the most flood-tolerant *Quercus* spp. with respect to growth and survival; it can endure prolonged periods of flooding due to a better adjustment of leaf biomass production to the hydraulic conductivity of the root system (Ferner 2009; Prpić 2003; Schmull and Thomas 2000). However, prolonged flooding can cause a dramatic decrease in assimilation and transpiration rates (Ferner 2009).

Radial growth of W oaks was stimulated in the summer period (July–August) if the flow was higher. On the other hand, higher flows of the Krka River did not promote the growth of D oaks although they are growing on a non-flooded area. Few studies have been published on growth patterns of bog *Q. robur* and *Q. sessiliflora*, which are considered to be sensitive indicators of changing ecological conditions because they grow under temporarily extremely wet site conditions (Leuschner et al. 2002; Pilcher 1996; Sass-Klaassen and Hanraets 2006). Suppressed growth of these oaks was probably caused by dramatic hydrological changes resulting in a shortened growth season with an absence of LW (Sass-Klaassen and Hanraets 2006). Site hydrology seems to play an important role in the growth and population dynamics of oaks from such areas (Leuschner et al. 2002; Sass-Klaassen and Hanraets 2006). Similarly, *Q. robur* decline and dieback in European floodplain forests in recent years occur particularly in areas that have already been stressed by a deteriorating hydrological regime (Hager and Buchleitner 2001; Levanič et al. 2011; Vukelić and Rauš 2001). The lowered water table makes the rehabilitation of the forests extremely difficult because the roots of the saplings in early years draw water from the soil layer, which is watered from above. However, when roots grow below these layers in later years, they cannot reach the depressed water table and consequently perish (Haraszthy 2001). The reduced growth of *Q. robur*, often resulting in tree death, may be associated with differences in TRW, EW vessel area and carbon isotope discrimination (Levanič et al. 2011).

Investigating xylem anatomy as a time series at the intra- and inter-annual level has already been demonstrated to be a promising approach in tree biology and climate change research, particularly if complemented by physiological and ecological studies (Fonti et al. 2010). Specific anatomical features, such as EW vessel size and density, have been shown to be reliable ecological indicators that contain environmental information different from that stored in TRW (e.g., Tardif and Conciatori 2006; Fonti et al. 2009, 2010; George et al. 2002; Sass-Klaassen et al. 2011). Interestingly, we found no relation of the Krka River flow with other anatomical variables (size of the EW vessels and their density in EW) of D and W oaks, suggesting that they probably depend on other environmental/climatic factors. Nevertheless, floods are often directly, but very locally, recorded in the cambium, resulting in an extreme reduction in EW vessel area in *Quercus* spp. (George et al. 2002; Sass-Klassen et al. 2010).

Site-specific soil regimes often play a role in limiting moisture availability in oak forests (Charton and Harmon 1973; Estes 1970). Tree-ring time series contain a lot of information about environmental conditions and their impact on the growth of trees. Our study clearly shows that the hydrological information stored in wood-anatomical features may vary, even within the same forest stand, and largely depends on the micro-environment. Reduced wood increments of D oaks suggest that growth conditions are less favourable in the non-flooded areas of Krakovo lowland forest. Decreasing growth curves are among the most obvious growth-related characteristics of the diminishing vitality of trees, which is not only a species-specific but also a site-specific feature (Bigler et al. 2004). The vitality of *Q. robur* from Krakovo forest might vary, implying a link between the health state of oaks from lowland forest in Slovenia and hydrological conditions. Our research suggests that oaks from the non-flooded areas might experience more physiological stress and contain different hydrological information. Thus, trees intended for hydrological or climatological reconstruction must be carefully selected to avoid the possibility of error and potential loss of information in the reconstruction. However, this hypothesis is speculative and for deeper investigations a larger number of sampled trees should be included from several areas in the Krakovo forest. For present study, no data on groundwater were collected but these will be considered in further studies to understand better the way *Q. robur* uses water resources and could complement the results presented in this study by bringing new insight into the survival mechanisms of trees in conditions of changed water availability.

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3.2 OSTALO POVEZOVALNO BESEDILO

3.2.1 Variiranje okoljskega signala v parametrih branik doba (*Quercus robur* L.) z različnim rastnim potencialom

Variations in environmental signals in tree-ring indices in oaks (*Quercus robur* L.) with different growth potential

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V Krakovskem gozdu smo na dveh raziskovalnih ploskvah z različnimi mikroklimatskimi razmerami (W-periodično poplavljani in D-nepoplavljeni) analizirali dve skupini dobov. V raziskavi smo primerjali rastni odziv dreves iz obeh skupin na okoljske spremenljivke, potencialen klimatski signal v strukturi ranega lesa ter morebitne razlike v izotopski diskriminaciji v ranem in kasnem lesu. Za ta namen smo analizirali širino in izotopsko diskriminacijo ogljika v ranem in kasnem lesu. Rezultati so pokazali, da so W dobi rastli statistično značilno bolje skozi celotno proučevano obdobje. Za vse analizirane parametre, z izjemo izotopske diskriminacije ogljika, se je pokazala statistično značilna razlika med D in W dobi. Širine kasnega lesa W dobov so bile v značilni korelaciji s poletnimi (junij-avgust) klimatskimi spremenljivkami, medtem ko je bila izotopska diskriminacija ogljika tesneje povezana s poletnim pretokom Krke. Izotopska diskriminacija ogljika v ranem lesu W dobov je korelirala s pretokom Krke v preteklem poletju in jeseni. Verjetno je nejasnost okoljskega signala pri D dobih posledica manj ugodnih rastnih razmer, kar se odraža v izrazito zmanjšanih debelinskih prirastkih. Naša raziskava predstavlja pomembne razlike v odzivu na okoljske dejavnike med dvema skupinama dobov z različnim zdravstvenim stanjem. V branikah shranjena okoljska informacija lahko variira celo znotraj enega sestoja in je pogojena z mikrorastiščnimi razmerami. Naša raziskava je potrdila domnevo, da z ločeno analizo širin in izotopske diskriminacije ogljika dobimo dodatne informacije v dendroekologiji doba.

Title

Variations in environmental signals in tree-ring indices in oaks (*Quercus robur* L.) with different growth potential

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Abstract

We analysed two groups of *Q. robur*, growing at nearby plots with different micro-location condition (W-wet and D-dry) in the floodplain Krakovo forest, Slovenia. In the study we compared growth response of two different tree groups to environmental variables, the potential climate signal stored in earlywood structure and the potential difference of the information stored in carbon discrimination of earlywood and latewood. For that purpose earlywood and latewood widths and carbon discrimination have been measured. We found out that W oaks were growing significantly better over the whole analysed period. The difference between D and W oaks was significant in all analysed variables with the exception of stable carbon discrimination in latewood. In W oaks, latewood widths correlated with summer (June to August) climatic variables, while carbon isotope discrimination was more connected to Krka River flow during the summer. Earlywood carbon isotope discrimination correlated with summer and autumn River Krka flow of the previous year. In the case of D oaks, environmental signal appears to be vague most probably due to less favourable growth conditions resulting in markedly reduced radial increments. Our study reveals important differences in responses to environmental factors between the two oak groups of different physiological conditions that are preconditioned by environmental stress. Environmental information stored in tree-ring features may vary, even within the same forest stand, and largely depends on the micro-environment. Our analysis confirmed our assumptions that separate EW and LW analysis of widths and Δ provides complementary information in *Q. robur* dendroecology.

Introduction

Pedunculate oak (*Quercus robur* L.) and sessile oak (*Quercus petraea* Matt.) are one of the most widespread and economically important tree species in Europe. Due to the longevity of oaks and their durable wood tree-rings are an important proxy in dendrochronological studies (1). Dendroclimatological studies on oaks from locations in Southeastern Europe have shown that radial growth of oaks is generally promoted by spring and summer precipitation amounts on most sites, while the temperature has more various effects.

Stable isotopes appear to be a particularly valuable tool when studying climate-growth relationships of trees in temperate climate regions. Namely, tree growth is influenced by a complex combination of environmental variables resulting in a lack of a strong climatic signal in tree-ring widths (2-4). The ratio of stable carbon isotopes in leaf tissue is a result of fractionation during CO_2 diffusion through the stomata and carboxylation. Both processes are influenced by plant physiological and environmental conditions (5). Where irradiance and temperature are the limiting factors, the dominant control of stable carbon isotopic composition ($\delta^{13}C$) may be the photosynthetic rate. On the other hand, stomatal conductance dominates in moisture-stressed environments and $\delta^{13}C$ give strong correlations with moisture parameters (6).

In oaks, tree-ring and latewood widths (LW-W) have been mostly used in dendroclimatological studies. So far, only a few studies have included stable carbon isotopes of *Q. robur* tree-rings for Southeastern Europe. Kern et al. (7) found strong correlation between latewood (LW) widths, $\delta^{13}C$ in LW and June precipitation. A comparative study of surviving and dead *Q. robur* trees showed significant differences among tree-ring variables, including carbon discrimination (Δ) (8).

For earlywood (EW), it was shown that its width (9, 10) and $\delta^{13}C$ (2) contain a weaker or even no climatic signal compared to LW. However, the anatomical structure of EW has proven to be a promising environmental proxy (11) and yields great potential to better understand the biochemical processes of carbon isotopes incorporation within the tree (12). In our recent study it was shown that environmental information in wood-anatomical variables of flooded and non-flooded pedunculated oaks may vary within the same forest stand (13).

In this study we examined the potential of tree-ring growth indices and Δ in *Q. robur* for dendroclimatological and dendroecological analysis with a focus on EW. For this purpose we selected two groups of *Q. robur* trees with different growth patterns growing in a floodplain forest in Slovenia. We anticipated that information on environmental sensitivity of EW would be of a particular value in ring-porous trees with narrow tree-rings containing a negligible proportion of LW. In addition, we checked whether climatic information stored in Δ of EW and LW of oaks with different growth potential is additional or redundant information.

Material and methods

Study site characteristics

The study site was located in Krakovo Querco robori–Carpinetum forest (45°54'N, 15 25'E, elevation 150 m), the largest floodplain lowland oak forest complex in Slovenia. It is influenced by the sub-pannonian continental climate. The mean July temperature in the period 1970–2008 measured at the nearby Novo mesto weather station is 20.1°C and the average January temperature is just below zero (–0.1°C). Total annual precipitation is 1,149 mm, where the majority (70%) falls during the growing season (March–October) (Fig 1). Meteorological data for the Novo Mesto station were obtained from the Slovenian Environment Agency.
At the study site hydromorphic soils, pseudogley and amphygley with low infiltration capacity prevail and together with microtopography they greatly influence the runoff, distribution and water movement in the soil. The groundwater level depends on the arrangement of different forms of deposits and differences in permeability of the soil surface. In rainy periods some parts of the forest can remain flooded for several weeks and water slowly evaporates or is absorbed by the soil. Due to a low infiltration capacity of this soil type and in the case of a slightly inclined slope, most of the water on slopes can runoff before absorption, which can have a negative influence on the micro-site hydrological conditions (14).

Apart from rainwater and groundwater levels, the Krka River also affects the hydrological conditions in the Krakovo forest. It belongs to the Karst Rivers and has a rain-snow regime, with runoff peaks in April and November, and minima in August and January (Fig 1). The data for minimal (Qnp), average (Qsp) and maximal (Qvp) monthly rate of Krka River flow for the Podbočje station were obtained from the Slovenian Environment Agency, for the period 1970–2008 (Fig 2).

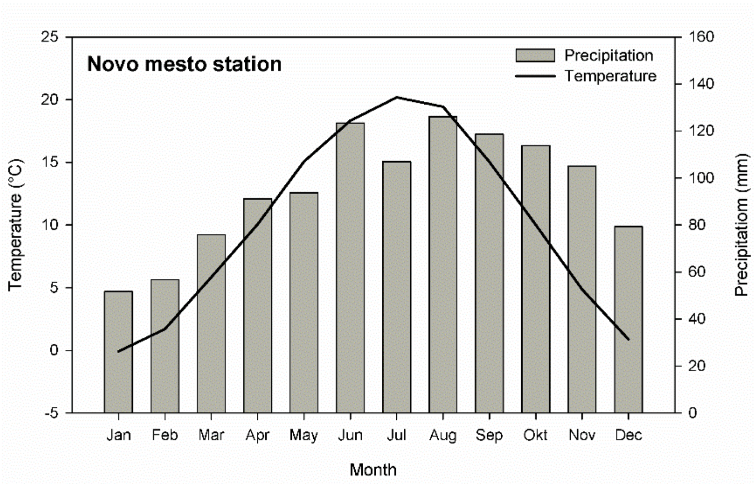

Fig 1. Climatic diagram for Novo mesto meteorological station for the period 1970–2008: The mean monthly temperatures and monthly sum of precipitation are denoted by line and bars, respectively.

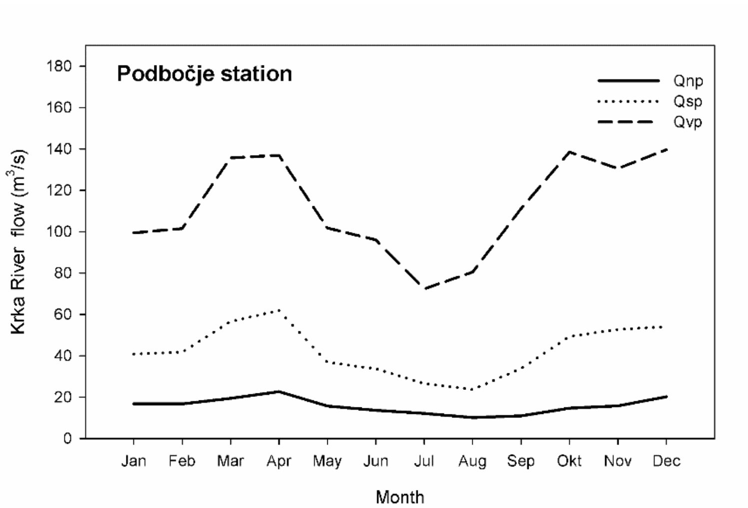

Fig 2. Average monthly flow of the Krka River for the period 1970–2008. The data represent minimal (Qnp), average (Qs) and maximal (Qvp) monthly rates of flow (daily average) in m^3/s.

Tree selection and construction of tree-ring width chronologies

Penduculate oak (*Q. robur* L.) trees were sampled at two research plots. Plots are about 600 m apart and located in the same forest association, but they differ in their hydrological conditions. The first plot is occasionally flooded (W – wet oaks), whereas the second plot (D – dry oaks) remains dry throughout the whole growing season. At each plot six (co)dominant trees were selected and sampled. Selected trees were 80–100 yeas old, with DBH 30–60 cm, without any visible mechanical injures of stems or roots.

During winter 2008–2009, we took 10 mm wide cores about 1.3 m above the ground from each of the trees in order to prepare microscopic slides. We analysed the wood structure of the last 39 rings (1970–2008) in order to avoid juvenile wood, because its anatomical structure and tree-rings significantly differ from adult wood. Ring widths were measured on stained microscope slides (detailed sample preparation and measurements are described in Gričar et al. 2013) and standardized using the ARSTAN program (15) by applying a two-step procedure. Long-term and growth trends were removed using a negative exponential function. This procedure was followed by a 67% cubic smoothing spline with a 50% cut-off frequency to remove any effect of stand competition and disturbance events (16). The autocorrelation was removed by applying an autocorrelation filter to the detrended measurements. Indexed chronologies were combined using bi-weight robust estimation of the mean to obtain residual chronologies.

Isotope measurement

For stable isotope analysis cross-dated tree-rings were divided to EW and LW portions with scalpel under an Olympus SZ-60 binocular microscope (Tokyo, Japan). Samples of EW and LW were separately pooled to create two annualized records. The advantage of pooling is in reducing time of sample preparation and costs. Previous studies show very close $\delta^{13}C$ values of pooled samples and average $\delta^{13}C$ values of individually analysed same trees (17) techniquesα-cellulose was extracted from the samples by following standard techniques (18, 19), homogenized using a Hielscher ultrasonic probe and freeze-dried for 48 hours. Two replicates of 300-350 μg of dry α-cellulose were weighted into tin capsules. Samples were combusted using a PDZ Europa ANCA GSL elemental analyser and CO_2 gas was introduced online to a PDZ Europa 20–20 stable isotope ratio mass spectrometer. Results are expressed using standard delta notation ($\delta^{13}C$) in per mille (‰) relative to the Vienna Pee Dee Belemnite (VPDB) standard. The precision of analysis of a laboratory standard was 0.08‰ (n = 64 internal standards).

Stable carbon isotope values are expressed as isotope discrimination (Δ). By expressing the $^{13}C/^{12}C$ ratio in terms of discrimination of carbon isotopes by C3 plants, we distinguish variations in atmospheric $\delta^{13}CO_2$ due to fossil burning from the effect of plant metabolic processes. Discrimination is expressed as (20):

$$\Delta = \left(\delta^{13}C_{air} - \delta^{13}C_{sample}\right) / \left(1 + \left(\delta^{13}C_{sample} / 100\,0\right)\right) \qquad \text{(eq. 1)}$$

where $\delta^{13}C_{air}$ and $\delta^{13}C_{sample}$ represent the isotopic composition of atmosphere and tree-ring sample, respectively.

Pearson's correlation coefficients were calculated and t-tests were done using program R version 2.8.0. (21). All graphs were produced using the SigmaPlot (version 11) software.

Results

Tree-ring parameters of D and W oaks

Tree-ring widths of oaks from the wetter plot (W oaks) were considerably wider than those of oaks from the drier plot (D oaks) over the whole analysed period (i.e. 1970–2008) (Fig. 3). Average widths of EW and LW significantly differed between D and W oaks; $t = 15.195$, $p < 0.001$ and $t = 16.210$, $p < 0.001$, respectively (Table 1). The difference in growth trends became particularly visible around the year 1990. A tree-ring width decline in D oaks was apparent in LW, where widths were narrower than 0.2 mm in the last analysed years (Fig. 4A) indicating negligible production of LW. EW-W in the two groups of oaks were fairly constant (Fig. 4B). LW and EW chronologies of W oaks exhibited higher mean values and year-to-year variability compared to that of D oaks. In addition, there was weak, but significant difference in Δ in EW ($t = -2.522$, $p < 0.05$) between the oak groups (Fig. 4C), but no difference in Δ in LW ($t = -0.925$, $p > 0.10$) (Fig. 4D).

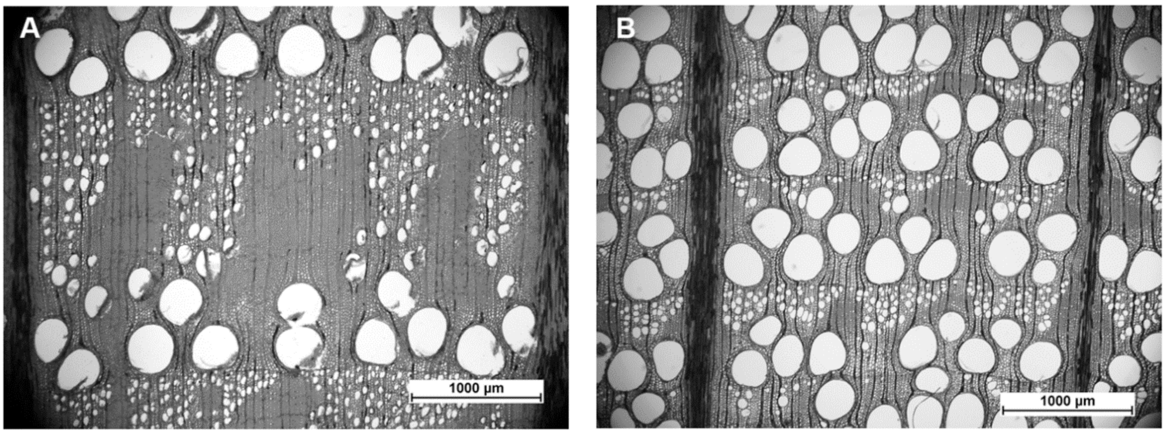

Fig 3. Tree-ring structure of W and D oak. Wide tree-ring of W oak with a large amount of LW (A). Narrow tree-ring of D oak containing only small proportion of LW (B).

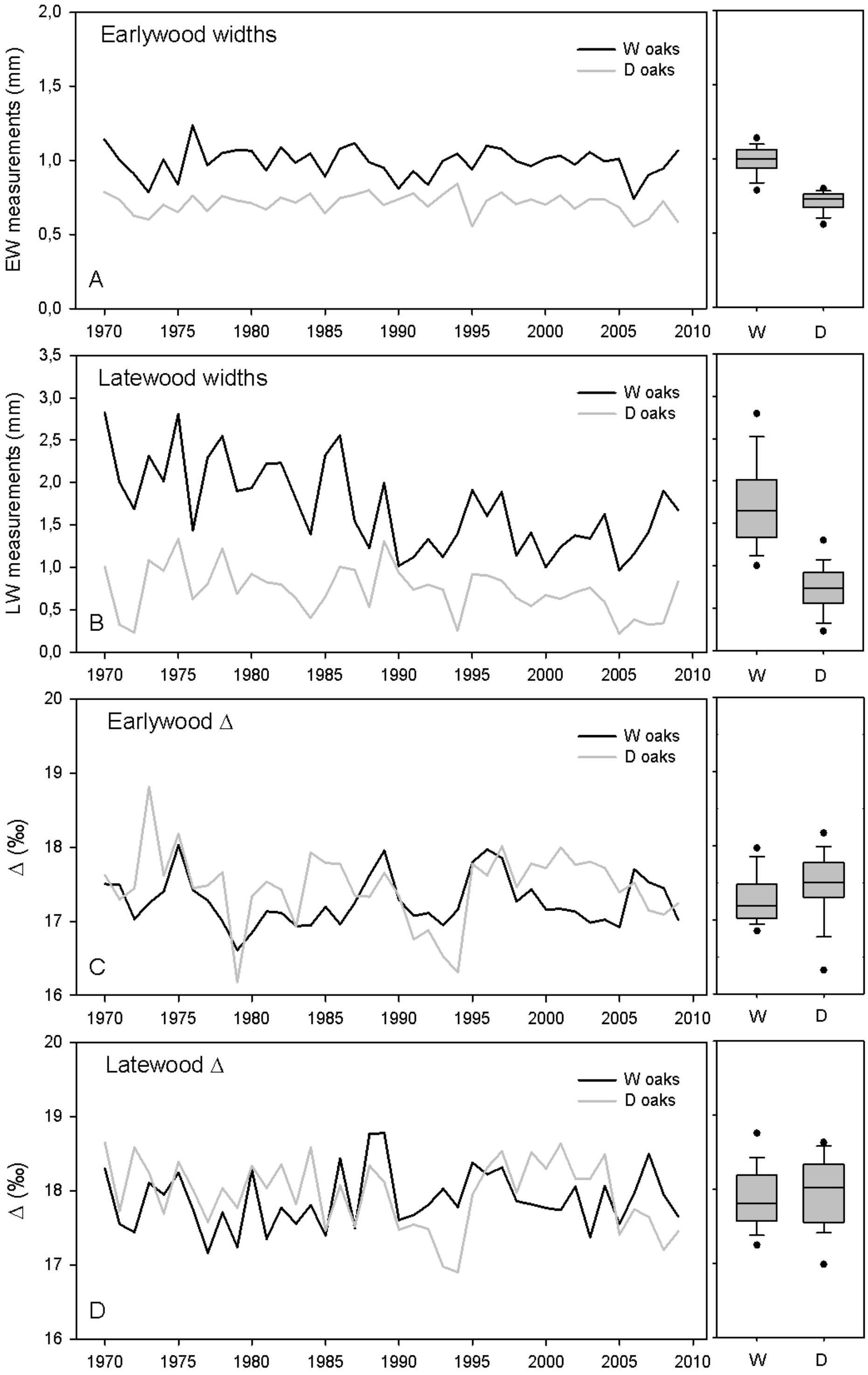

Fig 4. Time series of anlysed tree-ring parameters. Earlywood widths (A), latewood widths (B), earlywood carbon discrimination (C) and latewood carbon discrimination (D) of wet (W) and dry (D) oaks for the period 1970-2008.

Table 1. Descriptive statistics of analysed tree-ring parameters for W and D oaks.

	W oaks				D oaks			
	Avg.	CV (%)	Min	Max	Avg.	CV (%)	Min	Max
EW-W (mm)	0.95	14.0	0.60	1.35	0.58	15.5	0.33	0.76
LW-W (mm)	1.66	30.8	0.89	3.11	0.53	51.3	0.05	1.18
EW-Δ (‰)	17.27	1.9	16.61	18.02	17.47	2.8	16.18	18.81
LW-Δ (‰)	17.88	2.2	17.16	18.78	17.95	.2.6	16.90	18.65

Presentation of average value (Avg.), coefficient of variation (CV), minimum (Min) and maximum (Max) values of earlywood widths (EW-W), latewood widths (LW-W), earlywood carbon isotope discrimination (EW-Δ) and latewood carbon isotope discrimination (LW-Δ). Average value, minimum and maximum values are presented in millimetres [mm], coefficient of variation is presented in percent [%].

In W oaks significant correlation was observed between LW parameters of the previous year and EW parameters of the current year. In both groups highly statistically significant correlation existed between the current year's Δ of EW and Δ of LW and the same is true for the widths (Table 2).

Table 2. Correlation in tree-ring widths and carbon discrimination.

	W oaks		D oaks	
	$EW\text{-}W_{(t)}$	$EW\text{-}\Delta_{(t)}$	$EW\text{-}W_{(t)}$	$EW\text{-}\Delta_{(t)}$
$LW\text{-}W_{(t-1)}$	0.45	-	0.55	-
$LW\text{-}W_{(t)}$	0.15	-	-0.07	-
$LW\text{-}\Delta_{(t-1)}$	-	0.45	-	0.37
$LW\text{-}\Delta_{(t)}$	-	0.57	-	0.65

Correlation between earlywood and latewood widths (LW-W) and carbon discrimination (LW-Δ) of the previous (t-1) and current (t) year.

The relationship between tree-ring parameters, climate and the Krka River flow

The temperature did not affect EW and LW features of D oaks, while in W oaks positive correlation between LW-Δ and warm winter was apparent (Fig. 4A). EW-W of D oaks was correlated with precipitation during the previous year – negatively with summer precipitation and positively with autumn precipitation. LW-W of W oaks was promoted by humid, rainy and cloudy conditions during the current year's summer. EW-W of D oaks responded negatively to the wet and humid summer of the previous year, while the sunny summer and wet autumn of the previous year promoted its width. EW-Δ of D oaks current summer is negatively correlated with summer sunshine duration (Fig. 4D).

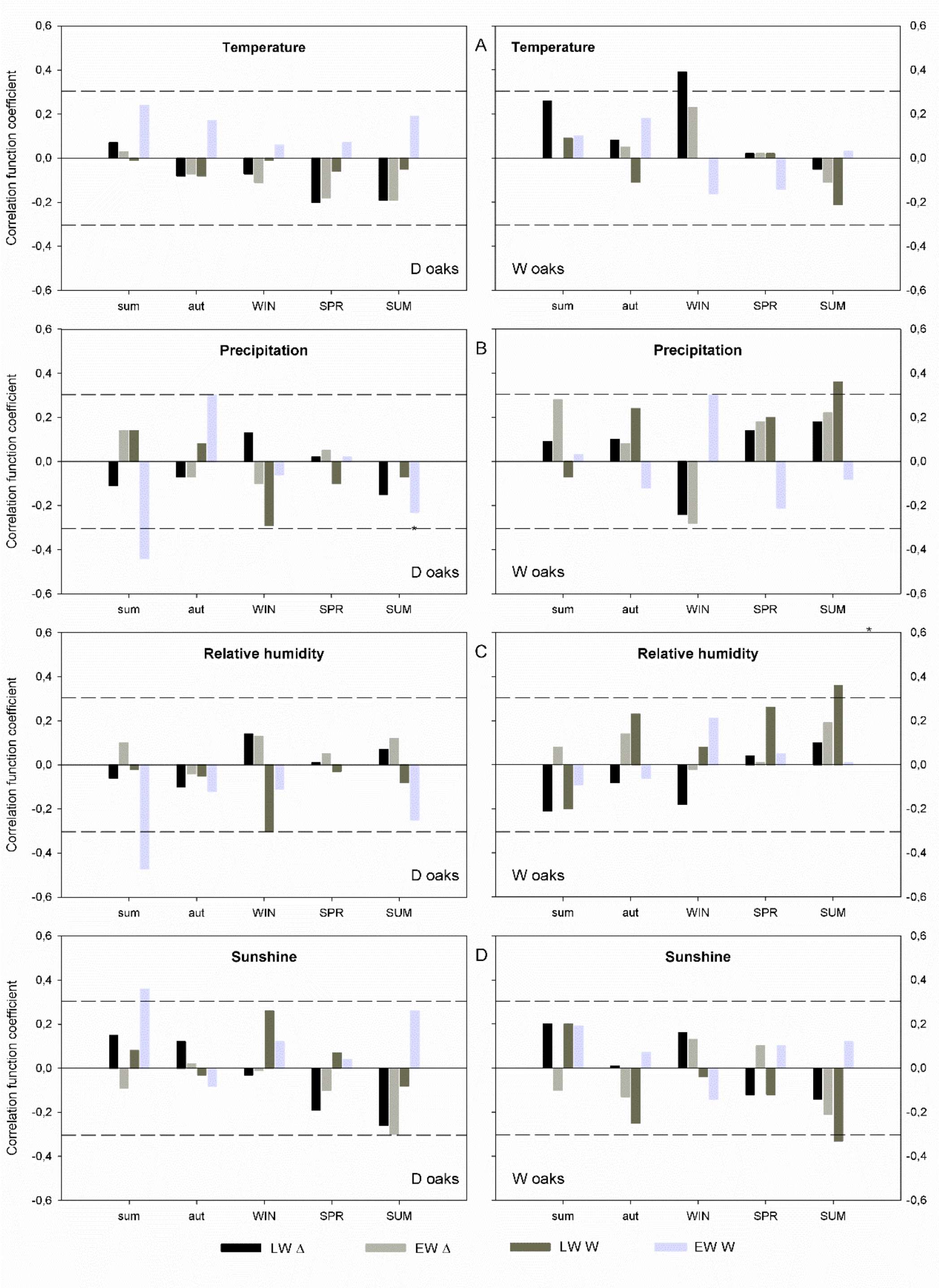

Fig 4. Correlation between analysed tree-ring parameters and meteorological variables. The correlation with **A** temperature, **B** precipitation, **C** relative humidity and **D** sunshine at a seasonal time scale (from previous to current summer). The dashed line denotes statistically significant values at level at 95%. EW-Δ – earlywood discrimination, LW-Δ – latewood discrimination, EW-W – earlywood width, LW-W – latewood width, SUM – summer (from June to August), AUT – autumn (from September to November), WIN – winter (from December to February), SPR – spring (from March to May). Small letters denote the previous year.

In W oaks, high Krka River flow during the summer was positively correlated with EW-Δ, LW-Δ and LW-W. EW-Δ was also positively correlated with a high maximum flow in the previous summer and a high minimum flow during the previous autumn. LW-W was promoted when previous autumn Krka River flow was high, while it negatively affected EW-W. LW-Δ was negatively correlated with low mean Krka River flow in the winter. EW-W of D oaks was narrower when Krka River flow of the previous summer was high. EW-Δ was positively correlated with a high minimum flow during the spring and summer, while LW-Δ was positively correlated only with the spring minimum Krka River flow (Fig. 5).

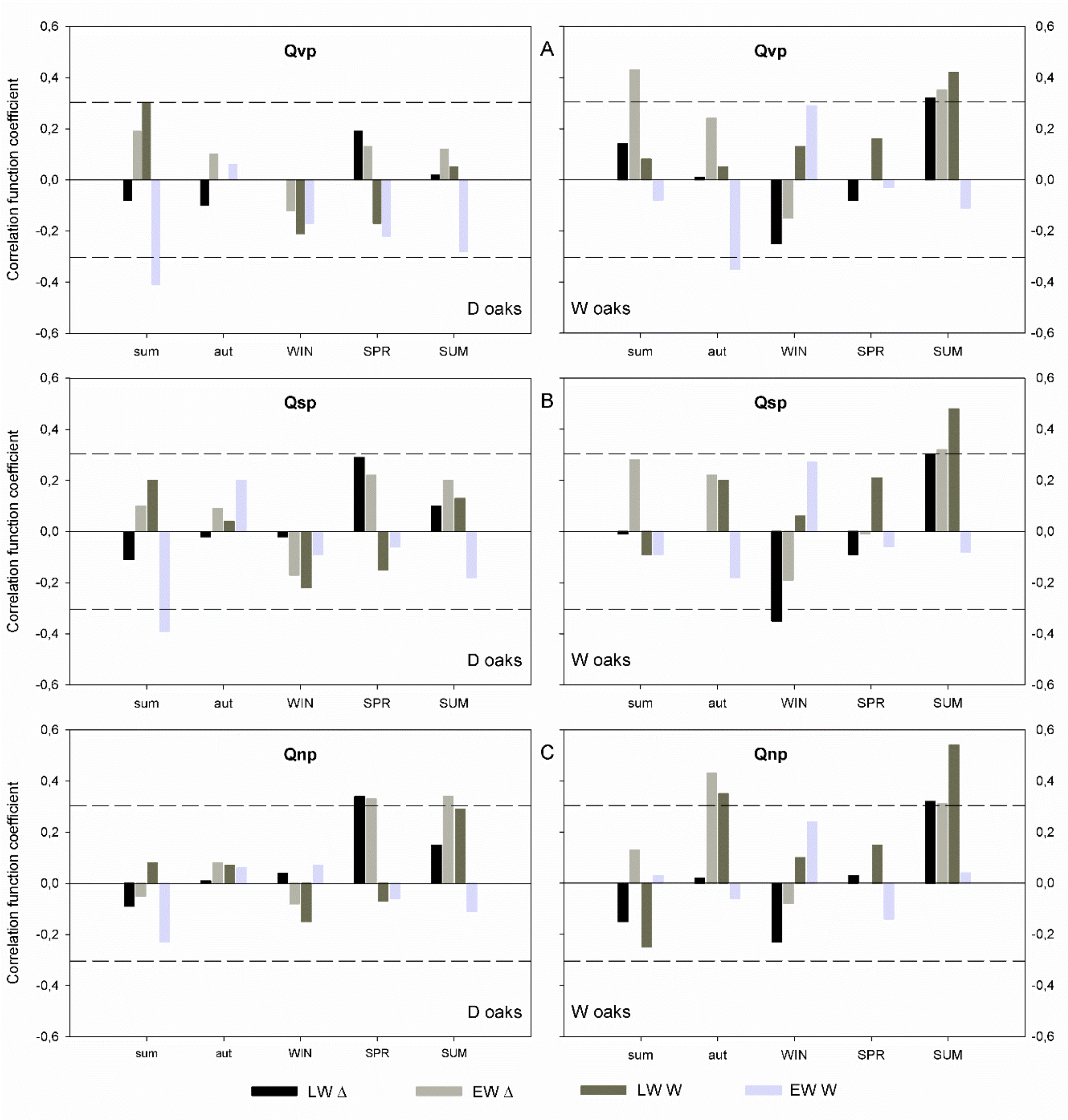

Fig 5. Correlation between analysed tree-ring parameters and the Krka River flow. The correlation with **A** maximum, **B** minimum and **C** mean flow at seasonal time scale. The dashed line denotes statistically significant values at level at 95%. EW-Δ – earlywood discrimination, LW-Δ – latewood discrimination, EW-W – earlywood width, LW-W – latewood width, SUM – summer (from June to August), AUT – autumn (from September to November), WIN – winter (from December to February), SPR – spring (from March to May). Small letters denote the previous year. Qnp – minimum flow, Qsp – mean flow, Qvp – maximum flow. Small letters denote the previous year.

Discussion

Climate and hydrological signals in tree-ring widths and Δ in *Q. robur*

Width and stable isotope composition of EW have rarely been used in dendroclimatological studies, as it is known that EW of deciduous species is mainly synthesized from stored photoasimilates and does not reflect the current year conditions (22, 23). Additionally, it has been shown that no or only weak response to temperature or precipitation is apparent in EW indices (7, 24, 25). However, our aim was to check any environmental information stored in EW, especially in those trees with little or hardly any LW. Our analysis showed that potential climatic information in EW-W of D oaks depends on the previous summer's precipitation, relative humidity and sunshine duration as well as on the previous autumn's precipitation. Current year's climatic and hydrologic conditions appeared to have little influence on analysed EW parameters of D oaks, while LW parameters (with the exception of the correlation between LW-W and winter RH) show no response to climatic variables. The most likely explanation would be that the micro-environmental conditions for radial growth of D oaks are less favourable, indicating that the trees might experience physiological stress. This results in diminished wood increments and a lower proportion of LW. It can be speculated that in such limiting conditions the majority of carbohydrate and nutrient sources are used for EW vessel formation in order to ensure a sufficient water supply for the tree, which is crucial for its survival, as older EW vessels usually become embolized (26). Conductive function has priority over the mechanical as the tree stem increases in diameter because the need for additional strength becomes less crucial (27, 28). Decreasing growth curves are among the most obvious growth-related characteristics of tree decline (29). Tree-ring widths of W oaks, on the other hand, contain similar climatic information as oaks from nearby locations in Southeastern Europe, where a high amount of precipitation and moderate temperatures during the summer appear to be the main climatic factors promoting growth of *Q.* spp. (7, 30, 31). Studied groups of W and D oaks yield different environmental information. In general, EW parameters of D oaks contain climatic information of the previous year, while in the case of W oaks it is stored in LW parameters.

Stable isotopic composition of tree-rings proved to be a valuable tool in dendroclimatological studies in trees at temperate sites, where their growth is influenced by a combination of environmental factors, demonstrating a strong positive correlation with average summer temperature (2, 7, 32), including sunshine hours (2-4), cloud cover and precipitation at sites near species distribution border (3). In the case of W oaks the high summer precipitation and high summer Krka River flow correlated with high LW-Δ. This correlation indicates that moisture stress is one of the most important limiting factors at the analysed site and that stomatal conductance dominates the Δ signal (6). Comparison of tree-ring widths and $\delta^{13}C$ in *Q. robur* from drier and wetter sites in East England revealed higher although not statistically significant correlation of $\delta^{13}C$ with environmental variables at the drier site than at the wetter one. Additionally, $\delta^{13}C$ indices from the LW showed higher correlations with environmental variables and yield considerably more environmental information that the tree-ring width alone (2). In general, tree-ring widths and Δ of W and D oaks in our research provide information on different environmental variables for different time periods. W and D trees do not yield the same nor even similar signals stored in analysed tree-rings variables.

In D oaks, the high minimum Krka River flow during spring was positively correlated with EW-Δ and LW-Δ values, while the summer flow was correlated only with EW-Δ. It has been reported that production of EW cells in *Q. petraea* started at the beginning of April and ceased by the end of May (33, 34). In light of these findings, the positive correlation between EW-Δ of D oaks and the high minimum Krka River flow together with the negative correlation between EW discrimination and sunshine duration during the current summer has no logical explanation. However, high correlation between EW and LW discrimination may indicate that LW of D oaks, which seem to grow in stressed conditions, may be partly synthetized from photosynthetic assimilates produced during the EW formation. It is likely that D oaks suffer from carbon starvation caused by reduced photosynthesis due to a very limited carbon uptake by stomatal closure but continued metabolic demand for carbohydrates (35). Another possible reason for the impact of the summer climatic conditions on analysed EW characteristics would be in ongoing development of EW cells in June. This would also explain the high correlation between the summer (from June to August) Krka River flow and EW-Δ in W oaks.

Environmental sensitivity of *Q. robur* trees with different growth rates

The presented study provides new stable isotope composition data to the tree-ring database in Southeastern Europe, where only few *Q.* spp. stable isotope chronologies exist. Levanič et al. (8) compared tree-ring widths, BAI and Δ of LW in dying and surviving pedunculate oaks. They observed significant differences in all parameters analysed between the two groups. Trees that survived exhibited a relatively constant growth increment and increased Δ values compared to dying trees. Helama et al. (36) compared healthy, declining and dead *Q. robur* trees and noted that healthy oaks had wider increments of EW and LW than declining or dead oaks over their entire life span. Similarly, our results showed that W oaks were growing considerably better over the entire studied period compared to D oaks. LW and EW-Ws of W oaks yield a similar climatic signal as oaks in other studies (7, 31), while D oaks differ in increment widths as well as in their response to environmental conditions. Discrimination of the carbon isotope in W oaks yields little climate information but potential hydrological signal.

It is important to stress that our research is based on the *Q. robur* samples only, while many dendroclimatic studies combine samples of *Q. robur and Q. petraea* in the same chronology (30). It is difficult to identify these two species based solely on their wood anatomical characteristics, whereas ring-width series can be successfully cross-dated; therefore in dendrochronological studies they are often treated as one species, *Q. spp*. (37).However, the ecology of *Q. robur* differs from that of *Q. petraea*; the overall response to climate is modulated by the species ecology and no single climate variable coherently controls growth response of both species (38). Additionally, studies of oak growth and its relation to environmental factors largely depend on the micro-environment (13), which is usually greatly affected by soil properties.

Our analysis confirmed our assumptions that separate EW and LW analysis of widths and Δ provides complementary information in *Q. robur* dendroecology. In general, Δ contains little climate information in contrast to above mentioned studies. However, it reveals important differences in response to environment between the two groups of trees of different physiological conditions that is preconditioned by environmental stress. Environmental

information stored in tree-ring features may vary, even within the same forest stand, and largely depends on the micro-environment. Additionally, in case of W oaks, Δ and anatomical variables (13) appear to be a promising proxy for hydrological studies; however trees should be carefully selected for this purpose. Nevertheless, for a more comprehensive dendrohydrological studies a larger number of trees should be sampled and the groundwater level should be regularly monitored.

Key words

Pedunculate oak, earlywood, latewood, tree-ring width, discrimination, dendrohydrology

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Competing Interests

The authors have declared that no competing interests exist.

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4 POVEZOVALNA RAZPRAVA IN SKLEPI

4.1 POVEZOVALNA RAZPRAVA

4.1.1 Razmerje med kronologijami analiziranih parametrov branik evropskega macesna

Za območje Alp obstaja nekaj macesnovih kronologij širin branik in maksimalne gostote lesa, na podlagi katerih so bile izvedene rekonstrukcije poletnih temperatur (Büntgen U. in sod., 2006; Corona C. in sod., 2010). Redke so kronologije izotopske sestave lesa in prikaz njihovega potenciala za klimatsko rekonstrukcijo oz. izvedene rekonstrukcije, še posebej take, ki temeljijo izključno na analizi macesnovih branik (Kress A. in sod., 2009; Kress A. in sod., 2010). Za območje jugovzhodnih Alp je bila do sedaj narejena le ena kronologija izotopskega razmerja ogljika, in sicer v branikah navadne smreke (*Picea abies*) (Levanič T. in sod., 2008), medtem ko ni bilo objavljene še nobene klimatske rekonstrukcije niti kronologije meritev $\delta^{18}O$ in $\delta^{2}H$ branik. V naši raziskavi smo kronologiji širin branik macesna dodali prve kronologije izotopskega razmerja ogljika ($\delta^{13}C$), kisika ($\delta^{18}O$) in vodika ($\delta^{2}H$) v branikah dreves za območje jugozahodnih Alp. Predstavljene kronologije izotopskega razmerja v branikah evropskega macesna pokrivajo obdobje 1906–2007 in predstavljajo osnovo za proučevanje vpliva okolja/klime na rast evropskega na zgornji gozdni meji in JZ robu njegovega areala.

Ugotovili smo, da so vse tri izotopske kronologije v značilni ($p < 0{,}001$) in pozitivni korelaciji. Najmočneje sta povezani kronologiji $\delta^{18}O$ in $\delta^{2}H$ ($r = 0{,}76$). Ti dve kronologiji sta tudi v korelaciji s širino branik (TRW), medtem ko kronologija $\delta^{13}C$ vrednosti ne korelira s kronologijo širin branik. Močna korelacija med $\delta^{18}O$ in $\delta^{2}H$ ($r = 0{,}76$), ki se je pokazala v naši raziskavi, se glede na potek globalne padavinske premice GMWL (Craig H., 1961) in podobne dejavnike frakcionacije tako pri vodikovih kot kisikovih izotopih zdi logična. Vendar se je v več študijah variiranja izotopske sestave vodnih izotopov v braniki pokazalo, da $\delta^{18}O$ in $\delta^{2}H$ v branikah ne kovariirajo vedno (Hilasvuori E. in Berninger F., 2010; Loader N. J. in sod., 2008). Predvidevamo, da pri analiziranih macesnih prihaja do višje stopnje izmenjave kisika in vodika s ksilemsko vodo v procesu sinteze celuloze kot pri v drugih študijah analiziranih drevesih, in je prav ta dejavnik odgovoren za kovariacijo $\delta^{18}O$ in $\delta^{2}H$.

4.1.2 Klimatski signal v $\delta^{13}C$, $\delta^{18}O$, $\delta^{2}H$ inTRW pri evropskem macesnu

Vsi štirje proučevani parametri macesnovih branik najbolje korelirajo s poletnimi klimatskimi razmerami. Ugotovili smo tudi značilen vpliv ($p < 0{,}05$) temperatur predhodnega leta na $\delta^{13}C$ in širine branik, medtem ko pri vodnih izotopih tega ni zaslediti.

V alpskem okolju je temperatura najpomembnejši dejavnik, ki nadzoruje debelinsko rast dreves (Rossi S. in sod., 2007). Ugotovili smo, da imajo na debelinsko rast macesnov na zgornji gozdni meji v jugovzhodnem delu Alp najmočnejši vpliv temperature pozne pomladi in zgodnjega poletja, od srede maja do vključno julija. Močna povezava med širino branike in temperaturo zgodnjega poletja kaže na neposreden učinek temperature na začetek kambijeve aktivnosti (Gričar J. in sod., 2007; Rossi S. in sod., 2006), ki kulminira okoli poletnega solsticija (Rossi S.in sod., 2006). To so potrdile tudi druge raziskave (Carrer M. in Urbinati C., 2004; Moser L.in sod., 2009; Rossi S.in sod., 2007), medtem ko so raziskave v švicarskih Alpah kot najpomembnejši klimatski dejavnik, ki vpliva na širino macesnovih branik, pokazale julijske in avgustovske temperature (Büntgen U. in sod., 2005).

$\delta^{13}C$ branik na analiziranem območju je močno povezana s temperaturo in trajanjem sončnega obsevanja v juliju in avgustu. Korelacije s padavinami v istem obdobju so sicer značilne, a šibkejše in negativne. Za razliko od širin branik, $\delta^{13}C$-vrednosti niso povezane z junijskimi klimatskimi spremenljivkami. Za to sta možni dve razlagi. 1) Nastanek ranega lesa pri listopadnih vrstah je le delno odvisen od novonastalih fotoasimilantov, v veliki meri pa od v predhodnjem letu shranjenih zalog ogljikovih hidratov (Helle G. in Schleser G. H., 2004; Kozlowski T. T. in Pallardy S. G., 1997a). V tem primeru torej rani les vsebuje informacijo, shranjeno v škrobnih zalogah iz preteklega leta (Kagawa A. in sod., 2006; von Felten S. in sod., 2007). 2) Lahko pa je količina v rani les vgrajene celuloze manjša kot količina celuloze v kasnem lesu (Rossi S. in sod., 2007). Zato je pri izotopski analizi homogeniziranega vzorca njen signal manj pomemben in manj vpliven.

Trajanje sončnega obsevanja skupaj s temperaturo se je pokazalo kot najvplivnejši klimatski dejavnik, ki določa $\delta^{18}O$- in $\delta^{2}H$-vrednosti v macesnovih branikah. Močno korelacijo med vodnimi izotopi in sončnim obsevanjem je težko pojasniti z enim samim neposrednim dejavnikom. Povezava s sončnim obsevanjem je najbrž posredna, preko razmerja med sončnim obsevanjem in vzorci cirkulacije zračnih mas (ter posledično trajektorije vlažnih zračnih mas) in razmerja med sončnim obsevanjem in relativno vlago (gradientom med ambientalnim in internim parnim tlakom).

4.1.3 Prostorski signal $\delta^{13}C$, $\delta^{18}O$, $\delta^{2}H$ in TRW pri evropskem macesnu

Prostorsko korelacijo med posameznimi parametri branik in poletno temperaturo smo proučili s pomočjo povprečnih mesečnih podatkov baze CRU TS 3 (Mitchell T. D. in Jones P. D., 2005), ki jih za obdelavo in izris vzorca prostorskega signala uporablja internetna aplikacija KNMI Climate explorer (van Oldenborgh G. J. in sod., 2004). Izmerjene povprečne temperature junija in julija (JJ) ter v juliju in avgustu (JA) so v značilni in močni ($p < 0{,}001$) korelaciji s CRU TS 3 temperaturnimi podatki. Močan signal ($r > 0{,}6$) se razteza od Velike Britanije do Iberskega polotoka, preko Italije do severne Afrike in na vzhod do Romunije in Ukrajine. Znotraj tega območja se nahaja prostorski vzorec korelacije med $\delta^{13}C$- vrednostmi branik in JA-temperaturami, s centrom signala na širšem območju Alp. Prostorski vzorec za korelacijo med JJ-temperaturami in TRW je podoben, le da je malo šibkejši in rahlo pomaknjen proti jugu. Vzorec najmočnejše ($r > 0{,}6$) prostorske korelacije med $\delta^{13}C$ JA- temperaturami v raziskavi Kress in sodelavcev (2010) je ožji, pomaknjen bolj proti zahodu in s središčem v centralnem delu Alp.

Medtem ko $\delta^{13}C$- in TRW-kronologiji odsevata močan temperaturni signal s središčem nad lokacijami rastišč (JV Alpe), je vzorec prostorske korelacije med temperaturo in izotopsko sestavo kisika ter vodika v branikah macesna povsem drugačen. Odklonjen je proti južni Italiji in zahodnemu Balkanu, signal pa ne seže do Alp ter dlje proti severu in zahodu. To sliko lahko razložimo s pomočjo velikoprostorskih vzorcev pritiska atmosferskega zraka, ki usmerjajo gibanje vlažnih zračnih mas in vplivajo na izotopsko sestavo padavin. Nihanje zračnega pritiska nad Atlantikom (NAO) vpliva na $\delta^{18}O$ zimskih padavin, medtem ko poleti njegov vpliv v Alpah upade in nadzor nad padavinami prevzamejo orografski dejavniki (Field R. D., 2010). Vlažne zračne mase se formirajo nad Jadranom in v vodnih izotopih se shrani signal o temperaturi na mestu evaporacije. Jugozahodni vetrovi jih prinesejo do grebena JV Alp, kjer se sprosti velika količina padavin, preko 2000 mm/leto (Petkovšek Z.

in Trontelj M., 1987). Proučevani macesni, ki uspevajo na rastišču s kraško podlago in velikimi količinami padavin, imajo velik potencial za beleženje informacij o padavinski vodi (Roden J. S. in sod., 2000; White J. W. C. in sod., 1985) ter o karakteristikah zračnih mas, ki prinašajo padavine.

Na podlagi analize štirih različnih proxy podatkov iz macesnovih branik (TRW, $\delta^{13}C$, $\delta^{18}O$ in $\delta^{2}H$) lahko potrdimo prvo postavljeno hipotezo. Širine branik in v $\delta^{13}C$ vsebujejo informacijo o temperaturah in trajanju sončnega obsevanja na širšem območju Alp, s centrom signala na območju rastišča vzorčenih macesnov. TRW vsebuje podatke o razmerah v obdobju zgodnjega poletja, medtem ko se v $\delta^{13}C$ shranjena klimatska informacija navezuje na obdobje med julijem in avgustom. $\delta^{18}O$ in $\delta^{2}H$ prav tako vsebujeta informacijo o klimatskih razmerah v juliju in avgustu, vendar je njun prostorski signal drugačen in se navezuje na mesto nastanka proxy podatka, ki ga v tem primeru predstavlja evaporacija vodne pare nad Jadranskim morjem.

4.1.4 Rekonstrukcija izbrane klimatske spremenljivke

V naši raziskavi so se kot najmočnejše pokazale korelacije med potekom $\delta^{13}C$-vrednostmi in temperaturo ter sončnim obsevanjem. Ob predpostavki, da poletna temperatura in sončno obsevanje močno in enoznačno kovariirata skozi daljše časovno obdobje in ob odsotnosti dodatnih informacij, bi lahko sklepali, da je tako temperatura kot sončno obsevanje primeren klimatski dejavnik za rekonstrukcijo (McCarroll D. in Pawellek F., 2001). Variiranje temperature v prostoru je zvezno in enakomerno, medtem ko so sončno obsevanje, oblačnost in tudi padavine bolj lokalno pogojeni dejavniki. Meritve temperature so precej bolj točne in natančne kot meritve sončnega obsevanja, prav tako so na voljo dolgi časovni nizi izmerjenih temperatur (Auer I. in sod., 2007; Böhm R. in sod., 2001). Zanesljivi dolgi seti trajanja fotosintetsko aktivnega obsevanja so redkokje na voljo, kar nas sili k uporabi posrednih podatkov, kot so npr. število ur sončnega obsevanja ali oblačnosti. Vsa ta dejstva so v korist odločitvi za rekonstrukcijo temperature. Vendar je, kadar primerjamo korelacije klimatskih dejavnikov med seboj in s proučevanim parametrom branike, potrebna previdnost. O močnih korelacijah med $\delta^{13}C$-vrednostmi branik in poletnimi temperaturami sicer poročajo tudi drugi avtorji (Hilasvuori E. in sod., 2009; Kress A.in sod., 2010; Sidorova

O. V. in sod., 2010), hkrati pa so nekatere dendroklimatološke raziskave postavile pod vprašaj rekonstrukcije temperatur na podlagi $\delta^{13}C$-vrednosti branik tudi v primeru, ko je njihova korelacija z lokalnimi temperaturnimi seti zelo močna (Gagen M. in sod., 2011; Young G. H. F. in sod., 2010).

4.1.4.1 Kalibracija in verifikacija rekonstrukcije klimatske spremenljivke

Da bi preverili primernost rekonstrukcije klime na podlagi $\delta^{13}C$, smo izvedli kalibracijsko-verifikacijski test na podlagi dveh ločenih obdobij (1884–1945 in 1946–2006), za kateri imamo podatke o temperaturi in sončnem obsevanju. Rezultati so pokazali, da temperatura ni najbolj primeren dejavnik za rekonstrukcijo. Delež pojasnjene variance za sončno obsevanje je v obeh obdobjih podoben, za temperaturo pa je v zadnjem obdobju (1946–2006) precej nižji kot v prvi polovici (1884–1945) kalibracijskega obdobja. Čeprav so vrednosti zmanjšanja napake (RE) višje pri temperaturni kot pri rekonstrukciji sončnega obsevanja, to ne drži za bolj občutljiv koeficient učinkovitosti rekonstrukcije (CE). Nizka vrednost CE nakazuje na razliko v izmerjenih in napovedanih vrednostih. V našem primeru se ta razlika verjetno pojavi zato, ker krivulji poteka temperature in sončnega obsevanja po letu 1983 ne potekata več sinhrono in $\delta^{13}C$-vrednosti namesto temperaturi sledijo poteku sončnega obsevanja. Če je močna korelacija med temperaturo in $\delta^{13}C$ posredna preko fotosintetsko aktivnega sevanja in je naraščanje temperatur posledica vpliva povišanih koncentracij toplogrednih plinov, potem je razhod v poteku temperature in trajanja sončnega obsevanja natanko to, kar bi pričakovali. Glede na dostopne podatke o sončnem obsevanju na podlagi korelacijske analize ni mogoče dokončno sklepati, ali je dominantni signal $\delta^{13}C$-vrednosti proučevanih branik temperatura ali sončno obsevanje. Razen za zadnjih nekaj desetletij ni opaznih daljših obdobij razmika med temperaturami in sončnim obsevanjem na meteorološki postaji Villacher Alpe.

Zanesljivost rekonstrukcije JA-temperatur na podlagi $\delta^{13}C$-vrednosti lahko do določene mere preverimo z uporabo dolgih povprečnih temperatur na območju vzhodnih Alp (Böhm R.in sod., 2001), s katerimi so temperature iz Villacher Alp v tesni korelaciji ($r = 0{,}93$, $p < 0{,}001$) in tudi v enako močni korelaciji s $\delta^{13}C$ ($r = 0{,}56$), kot so temperature na meteorološki postaji Villacher Alpe. Daljše kalibracijsko in verifikacijsko obdobje, ki ga omogoča temperaturni niz Böhm in sodelavcev (2001), naj bi zmanjšalo vpliv razmika, ki ga opažamo v zadnjih desetletjih. V našem primeru pa je še vedno razvidno, da prihaja do očitne precenitve temperatur, kadar za kalibracijo temperatur v obdobju 1763–1884 uporabimo obdobje 1885–2006. To je razvidno iz vrednosti CE, ki je zelo blizu vrednosti nič. Tudi če odstranimo problematično obdobje (1983–2006) in razvijemo kalibracijo na krajšem obdobju (1885–1982), razmik ostane. Vrednosti CE in RE so v tem primeru še slabše, saj ni več razlike med povprečnima temperaturama kalibracijskega in verifikacijskega obdobja. V primerjavi naše temperaturne rekonstrukcije z rekonstrukcijo na podlagi dokumentarnih dokazov (Dobrovolný P. in sod., 2010) so jasno vidne anomalije in dolga obdobja razhajanja obeh setov. Naša rekonstrukcija podcenjuje temperature v obdobju 1630–1660, v obdobju 1600–1840 pa jih močno precenjuje. Primerjava z rekonstrukcijo poletnih temperatur Trachsel in sodelavcev (2012) pokaže podoben vzorec.

Glede na dobro korelacijo δ^{13}C-vrednosti branik z izmerjenimi urami sončnega obsevanja, neujemanje s preverjenimi temperaturnimi rekonstrukcijami ter glede na teoretsko ozadje sklepamo, da je trajanje sončnega obsevanja primernejši dejavnik za rekonstrukcijo.

4.1.4.2 Rekonstrukcija trajanja sončnega obsevanja in primerjava z obstoječima temperaturnima rekonstrukcijama

Če δ^{13}C-vrednosti res odražajo spremembe v trajanju sončnega obsevanja, lahko primerjava δ^{13}C-krivulje in temperaturnih rekonstrukcij razkrije obdobja, ko so bile temperature in sončno obsevanje manj povezani, kot se to kaže v kalibracijskem obdobju. V primerjavi s temperaturnimi rekonstrukcijami (Dobrovolný P.in sod., 2010; Trachsel M.in sod., 2012) so jasno razvidna obdobja, ko temperatura in δ^{13}C-kronologija potekata sinhrono, in obdobja, ko se razhajata. Obdobje 1570–1590 izstopa kot izrazito hladno in oblačno, medtem ko so bila poletja okoli leta 1600 sončna in precej topla. Med leti 1625 in 1650 ni bilo posebej hladno, a zelo oblačno. Od druge polovice 17. stoletja dalje je temperatura upadala, postajalo pa je vedno bolj sončno. To hladno obdobje se konča z začetkom prve polovice 18. stoletja, ki je bilo zelo sončno in tudi toplo. V preostanku 18. stoletja so opazna neznačilna nihanja temperature in sončnega obsevanja blizu povprečnih vrednosti, z izjemo krajšega sončnega obdobja okoli leta 1790. V obdobju 1800–1825 je bilo hladno in oblačno, nato pa sledi dviganje poletnih temperatur, ne pa tudi porast v trajanju sončnega obsevanja med leti 1825–1940 in od leta 1983 dalje.

Glede na dobro korelacijo δ^{13}C-vrednosti branik z izmerjenimi urami sončnega obsevanja, neujemanje s preverjenimi temperaturnimi rekonstrukcijami ter glede na teoretsko ozadje sklepamo, da je trajanje sončnega obsevanja primeren dejavnik za rekonstrukcijo. Tri leta izstopajo s posebej visokimi izotopskimi vrednostmi in napovedanimi vrednostmi sončnega obsevanja: 2006, 1911 in 1705. Jeseni 2006 je bilo izjemno toplo in suho (Luterbacher J. in sod., 2007). Poletje leta 1911 je bilo vroče in suho, z najmanjšo količino padavin glede na dosegljive podatke o padavinah. Rekonstrukcija precenjuje trajanje sončnega obsevanja, kar je verjetno posledica vpliva suše na frakcionacijo listnih rež. Leto 1705 so Dobrovolny in sodelavci (2010) rekonstruirali kot izjemno toplo, medtem ko je v rekonstrukciji Catsy in sodelavcev (2005) poletje leta 1706 z vključenim junijem izpostavljeno kot eno izmed najbolj vročih in sušnih. Druga leta s sončnim poletjem so še 1696, 1719, 1600 in 1601. V letih 1913 in 1840 so δ^{13}C-vrednosti nenavadno nizke. Poletje 1913 je bilo zelo hladno in deževno. Čeprav trajanje sončnega obsevanja v Villacher Alpah ni bilo izrazito nizko, so bile morda razmere na rastišču dreves drugačne. Poletje leta 1840 je bilo hladno, vendar v regionalnem merilu ne pretirano deževno (Casty C. in sod., 2005). Poleg omenjenih dveh se kot oblačna pojavljajo še poletja v letih 1580, 1582, 1804, 1850 in 1868.

S predstavljeno rekonstrukcijo trajanja sončnega obsevanja smo potrdili drugo postavljeno hipotezo, da z uporabo iz branik pridobljenih proxy podatkov lahko rekonstruiramo klimatsko spremenljivko za obdobje pred instrumentalnimi meritvami. Poudariti pa moramo, da je pri interpretaciji predstavljene rekonstrukcije potrebna previdnost, saj temelji na majhnem vzorcu dreves iz le dveh rastišč in tudi negotovost okoli letnih vrednosti je zelo velika. Za rekonstrukcijo smo uporabili podatke o številu sončnih ur, ki je najboljši razpoložljiv podatkovni niz, vendar je le približek vrednosti fotosintetsko aktivnega sevanja, ki vpliva na potek fotosinteze. Poleg naštetega tudi uporaba obratne regresije vedno pripelje do podcenjevanja variabilnosti klime v preteklosti, moč njenega vpliva pa je proporcionalna z vrednostjo nepojasnjene variance (McCarroll D. in sod., 2010).

4.1.5 Odziv dreves z različnim rastnim potencialom na okoljske dejavnike

Na podlagi analize branik doba (*Quercus robur* L.) smo proučevali, ali se drevesa z dveh mikro lokacij (periodično poplavljano in izjemoma poplavljano) v istem sestoju, vendar z različnimi mirkorastiščnimi pogoji in posledično različnim zdravstvenim statusom, razlikujejo v odzivu na okolje. Zanimalo nas je, ali poleg splošno uveljavljene analize širin branik tudi drugi parametri branike vsebujejo klimatski oziroma drug okoljski signal ter kakšne okoljske informacije lahko dobimo iz slabše rastočih dreves, ki proizvedejo zelo malo ali celo nič kasnega lesa.

4.1.5.1 Razlike med skupinama v posameznih parametrih branik

V naši analizi smo našli in potrdili razlike med zgradbo branik dobov, rastočih na redno poplavljanem (W dobi) in izjemoma poplavljenem (D dobi) rastišču. Skupini hrastov se med seboj razlikujeta v širini branike, deležu prevodnega območja trahej ranega lesa, deležu prevodnega območja v ranem lesu, gostoti trahej ranega lesa, širini (in deležu) kasnega lesa, širini ranega lesa ter diskriminaciji ogljikovega izotopa v ranem lesu. Med povprečnim tangencialnim in radialnim premerom trahej ranega lesa, povprečnimi vrednostmi območja trahej ranega lesa, gostoto trahej ranega lesa v ranem lesu, deležem trahej kasnega lesa glede na delež vlaken v kasnem lesu ter izotopski diskriminaciji v kasnem lesu nismo odkrili statistično značilnih razlik. V obeh skupinah dreves je lesnoanatomska zgradba v tesni korelaciji s širino branike. Ta je v negativni povezavi z deležem ranega lesa, prevodnim območjem in gostoto trahej ranega lesa ter v primeru D hrastov tudi z deležem prevodnega območja v kasnem lesu. Podobno so ugotovili tudi drugi raziskovalci (Gasson P., 1987; Rao R. V. in sod., 1997). Delež prevodnega območja v ranem lesu je rahlo večji pri D hrastih, kar pojasnimo z manjšim deležem ranega lesa v ožjih branikah, ki imajo manjše število kolobarjev trahej. V obeh skupinah hrastov je širina branike v negativni korelaciji s prevodnim območjem in gostoto trahej ranega lesa. Ta odnos lahko razložimo s skoraj sorazmernim odnosom med širino branike in širino kasnega lesa (Rao R. V.in sod., 1997). Torej, če je branika širša, je delež kasnega lesa večji in posledično sta delež in gostota trahej EW manjša.

Večina dosedanjih lesnoanatomskih študij je bila usmerjenih v proučevanje parametrov ranega lesa s poudarkom na trahejah, medtem ko je bila struktura kasnega lesa le redko analizirana (Eilmann B. in sod., 2006; Phelps J. E. in Workman E. C., 1994). Odkrili smo, da imajo D hrasti rahlo večji delež (10%) prevodnih elementov v kasnem lesu kot W hrasti, vendar razlike niso statistično značilne. Pri D hrastih je pri ožjih branikah (< 800μm) prisoten le majhen delež kasnega lesa, ki ga v glavnem sestavljajo traheje kasnega lesa in traheide. V primeru prisotnosti libriformskih vlaken, le-ta niso razporejena v za hrast značilne radialne plamene. V nekaterih primerih je kasni les pri D hrastih popolnoma izostal. O negativnem razmerju med deležem prevodnega območja kasnega lesa in širino branike sta poročala tudi Phelps in Workman (1994). V nasprotju z lesnoanatomskimi raziskavami, ki so osredotočene na rani les, izotopske raziskave lesa hrastov temeljijo skorajda izključno na proučevanju kasnega lesa, saj je rani les listopadnih dreves v glavnem zgrajen iz zalog preteklega leta (Helle G. in Schleser G. H., 2004; Kagawa A.in sod., 2006). Okoljski signal tekočega leta v ranem lesu je zato zelo šibek ali pa ga sploh ni (Fonti P. in García-González I., 2008; Kern Z. in sod., 2013), zaradi česar predvsem dendroklimatološke raziskave rani les navadno izpuščajo iz analize. Vendar pa se je analiza ranega lesa izkazala kot obetajoče orodje v dendroekoloških raziskavah (Fonti P. in sod., 2010) in nosi potencial za boljše razumevanje biokemičnih procesov v drevesu (Kimak A. in Leuenberger M., 2015). Prav tako je signal v ranem lesu zanimiv v primeru, ko imajo proučevana drevesa drastično zmanjšan delež kasnega lesa.

4.1.5.2 Odziv na klimo

Pri D dobih je potencialna klimatska informacija shranjena v ranem lesu in se navezuje na količino padavin, relativno vlažnost in trajanje sončnega obsevanja v preteklem poletju kot tudi na količino padavin v preteklem poletju. Na analizirane parametre ranega in kasnega lesa (z izjemo korelacije med širino kasnega lesa in zimsko relativno vlažnostjo) klimatske razmere tekočega leta ne vplivajo. Glede na strukturo in količino kasnega lesa sklepamo, da so mikrorastiščni pogoji D dobov v Krakovskem gozdu manj ugodni za rast in posledično so drevesa izpostavljena fiziološkemu stresu. Na to nakazujejo majhni debelinski prirastki in zmanjšan delež kasnega lesa, ki so med najbolj očitnimi kazalci propadanja dreves (Bigler C. in sod., 2004). V takšnih neugodnih razmerah je možno, da se večina ogljikovih hidratov in hranil porabi za nastanek trahej ranega lesa. Nastanek prevodnih elementov je za drevo pomembnejši kot nastanek libriformskih vlaken, kar kaže na večjo prednost prevodne funkcije pred mehansko (Phelps J. E. in Workman E. C., 1994; Rao R. V. in sod., 1997). Traheje kasnega lesa v primerjavi s prevodnostjo trahej ranega lesa le malo prispevajo k celotni prevodnosti. Izjema je zgodnja pomlad, ko je zmožnost prevajanja vode pri hrastu kot venčasto porozni vrsti močno zmanjšana, kar je posledica poletne ali zimske embolije (Bréda N. in Granier A., 1996; Hinckley T. M. in sod., 1979). Premer trahej, območje in delež prevodnega območja močno vplivajo na količino vode, ki se lahko transportira v drevesu. Večji ko je delež prevodnih elementov v braniki, manj tkiva je na voljo za mehansko podporo in zalogo hranil. Stres se tako kaže v zmanjšanih debelinskih prirastkih in manjšem deležu kasnega lesa. V nasprotju s tem pa širine branik W dobov s periodično poplavljenega rastišča izkazujejo podoben odziv na klimatske dejavnike kot hrasti z bližnjih srednjeevropskih lokacij, kjer sta visoka poletna količina padavin ter zmerna poletna temperatura glavna dejavnika, ki pozitivno vplivata na debelinsko rast hrasta (Čufar K. in sod., 2008b; Čufar K. in sod., 2014; Kern Z. in sod., 2013). V primerjavi z našimi rezultati se kot največja pokaže razlika v odzivu na temperature, ki ga pri D dobih sploh ni, medtem ko se pri W dobih pokaže le vpliv zimske temperature na izotopsko diskriminacijo v kasnem lesu.

Analiza izotopske sestave branik se je že izkazala kot zelo primerna metoda v dendroklimatoloških študijah na zmernih rastiščih, kjer na rast drevesa vplivajo številni dejavniki, kar se navadno kaže v pomanjkanju stabilnega in robustnega klimatskega signala. Izotopska sestava branik navadno tudi na takih rastiščih pokaže močno korelacijo s pomembnimi dejavniki, kot je povprečna poletna temperatura (Kern Z. in sod., 2013; Robertson I. in sod., 1997; Young G. H. F. in sod., 2012) vključno s trajanjem sončnega obsevanja (Loader N. J. in sod., 2008) ter oblačnostjo in padavinami na robu areala obravnavane vrste (Hilasvuori E. in Berninger F., 2010). V primeru W dobov sta visoka količina poletnih padavin in visok pretok Krke v korelaciji z visoko Δ v kasnem lesu. Tudi ta korelacija nakazuje, da je sušni stres med najpomembnejšimi omejujočimi dejavniki na analiziranem rastišču in da torej v Δ prevladuje signal prevodnosti listnih rež (McCarroll D. in Loader N. J., 2004). Primerjava širin branik in $\delta^{13}C$ dobov, rastočih v vzhodni Angliji, je razkrila višje, a statistično neznačilne korelacije $\delta^{13}C$ z okoljskimi spremenljivkami na sušnejših rastiščih v primerjavi z bolj vlažnimi rastišči. Prav tako se je pokazalo, da $\delta^{13}C$ kasnega lesa vsebujejo močnejši in obsežnejši klimatski signal kot same širine kasnega lesa (Robertson I. in sod., 1997). V primerjavi z omenjeno raziskavo širine in Δ W dobov iz naše raziskave ne vsebujejo informacije o isti okoljski spremenljivki. V širinah kasnega lesa je prisotna predvsem informacija o klimatskih razmerah v tekočem letu, medtem ko je hidrološka informacija o pretoku reke Krke shranjena tudi v izotopski diskriminaciji ranega in kasnega lesa.

4.1.5.3 Odziv na pretok Krke

Lesnoanatomske značilnosti in Δ so pri W dobih v glavnem povezane s poletnim pretokom Krke. To se kaže preko pozitivne korelacije s širino branike, deležem kasnega lesa ter Δ tako ranega kot tudi kasnega lesa, po drugi strani pa z negativno korelacijo s prevodnim območjem in z gostoto trahej ranega lesa ter deležem trahej v kasnem lesu. Hidrološke razmere v preteklem letu vplivajo na analizirane parametre ranega lesa. Visok pretok Krke v preteklem poletju in jeseni je v pozitivni korelaciji z Δ ranega lesa, medtem ko je visok jesenski maksimalni pretok v negativni korelaciji s širino kasnega lesa. Pozitivne korelacije W dobov z visokim pretokom Krke ter visoko količino padavin v poletju nakazujejo, da je sušni stres eden izmed bolj pomembnih dejavnikov, ki vpliva na rast analiziranih dobov, ter da prevodnost listnih rež močneje vpliva na signal Δ kot sam potek fotosinteze (McCarroll D. in Loader N. J., 2004).

Odziv D dobov na hidrološke razmere je bolj nepričakovan. Medtem ko debelinsko rast W dobov stimulira večji poletni pretok Krke, ta na D hraste ne vpliva, čeprav rastejo na sušnejšem, nepoplavljenem območju. Na širino branike in delež kasnega lesa D hrastov vpliva maksimalni spomladanski in minimalni poletni pretok Krke. Branika je bila širša ob nižjem spomladanskem in višjem poletnem pretoku Krke. Tudi Δ je v pozitivni korelaciji z visokim minimalnim pretokom Krke. Visok spomladanski pretok vpliva na Δ ranega in kasnega lesa, medtem ko je s poletnim pretokom povezana samo Δ ranega lesa. Glede na časovni potek nastanka ranga lesa, ki poteka nekje od začetka aprila do konca maja (Gričar J., 2010; Horáček P. in sod., 2003), korelacija med okoljskimi spremenljivkami v poletju in parametri ranega lesa nima smiselne razlage. Vendar pa visoka korelacija med Δ ranega in kasnega lesa lahko nakazuje, da je pri D dobih, ki rastejo v bolj stresnih razmerah, nastanek kasnega lesa vsaj delno odvisen od produktov fotosinteze, ki nastajajo v času formacije ranega lesa. Verjetno je, da so D dobi podvrženi t. i. »stradanju ogljika«, do katerega pride zaradi zmanjšane fotosintetske aktivnosti pri dalj časa močno priprtih listnih režah in istočasno neprekinjeni porabi ogljikovih hidratov za potrebe metabolizma (McDowell N. in sod., 2008). Druga možna razlaga korelacije ranega lesa s poletnimi razmerami, ki bi pojasnila tudi visoke korelacije med poletnim pretokom Krke ter širinami in Δ ranega lesa W dobov, je v nadalje potekajočem razvoju celic ranega lesa v juniju.

V raziskavi nismo našli povezave med pretokom Krke in ostalimi anatomskimi značilnostmi (velikost trahej ranega lesa in njihova gostota v ranem lesu) D in W hrastov. Sklepamo, da nanje vplivajo drugi okoljski dejavniki. Raziskovanje kronologij izotopskega razmerja v branikah ter lesnoanatomskih časovnih serij na medletni in sezonski resoluciji se je do sedaj že potrdilo kot možen pristop v raziskavah na področju biologije dreves v povezavi s klimatskimi spremembami, v kombinaciji s fiziološkimi in z ekološkimi študijami (Fonti P. in sod., 2010; McCarroll D. in Loader N. J., 2004). Specifični lesnoanatomski parametri, kot je velikost trahej ranega lesa in njihova gostota, so se pokazali kot zanesljiv ekološki indikator, ki vsebuje drugačno okoljsko informacijo kot ustaljeno uporabljene širine branik (Fonti P. in sod., 2010; Sass-Klaassen U. in sod., 2011; Tardif J. C. in Conciatori F., 2006).

4.1.5.4 Občutljivost dobov z različnim rastnim potencialom na okoljske dejavnike

Z našo raziskavo razširjamo precej skopo mrežo podatkov o izotopski sestavi branik dreves z območja jugovzhodne Evrope, za katero je objavljenih le nekaj hrastovih izotopskih kronologij (Kern Z. in sod., 2013; Levanič T. in sod., 2011). Poudariti velja, da naša raziskava temelji izključno na vzorcih doba (*Quercus robur* L.), medtem ko številne dendroklimatološke študije združujejo vzorce doba in gradna v skupno kronologijo (Čufar K. in sod., 2014). Razločevanje med tema dvema vrstama le na podlagi anatomije lesa je nezanesljivo, četudi je sinhronizacija njunih kronologij širin branik navadno uspešna. Posledično sta v dendrokronologiji dob in graden obravnavana kot ena vrsta, *Quercus spp.*

Vendar pa je njuna ekologija različna in temu je prilagojen njun odziv na klimo. Friedrichs in sod. (2008) so pokazali, da noben posamezen klimatski dejavnik ne vpliva enako na debelinsko rast doba in gradna. Dodati je potrebno, da na odziv hrastov na klimo v veliki meri vplivajo tudi lastnosti tal. Večina študij hrastovih branik je osredotočenih na njihovo odvisnost od klimatskih dejavnikov, ne pa tudi hidroloških. Poleg tega se te raziskave od naše razlikujejo v vrstni sestavi analiziranih dreves (*Quercus spp.*) ter talnih lastnostih rastišča in v tem oziru je naše rezultate z njimi težko primerjati.

Rezultati naše analize kažejo potencial analiziranih parametrov branik doba za njihovo uporabo v hidroloških študijah. Jasno se pokaže tudi, da v braniki shranjena okoljska informacija lahko značilno variira celo znotraj enega sestoja in da je močno odvisna od mikrolokacije ter z njo pogojenega zdravstvenega stanja dreves. Zmanjšan prirastek D hrastov kaže, da so rastni pogoji manj ugodni v nepoplavljenem delu Krakovskega gozda, saj je upadajoča prirastna krivulja ena izmed najbolj očitnih značilnosti upadanja vitalnosti dreves (Bigler et al. 2004). Z analizo različnih parametrov branik dveh skupin dobov v Krakovskem gozdu ter analizo korelacije z okoljskimi dejavniki in različnim odzivom nanje potrjujemo tretjo postavljeno hipotezo, da so D dobi izpostavljeni dolgotrajnim stresnim razmeram in predvidevamo, da v takih okoliščinah drevesa vse notranje zaloge porabijo za preživetje, medtem ko je njihov odziv na okoljske dejavnike zabrisan. Zatorej morajo biti drevesa, določena za hidrološko ali klimatološko rekonstrukcijo, pazljivo izbrana. S tem se izognemo morebitnim napakam in izgubi informacij pri rekonstrukciji. Za podrobnejšo raziskavo bi potrebovali večje število v vzorec vključenih dreves iz različnih predelov Krakovskega gozda, zato je naša raziskava preliminarna. Prav tako bi bilo potrebno na proučevanih ploskvah dolgoročno spremljati raven podtalnice. S tem bi bolje razumeli mehanizme črpanja vode pri dobu in bi lahko dopolnili predstavljene rezultate z novim vpogledom v preživetvene mehanizme dreves v spremenjenih hidroloških razmerah.

4.2 SKLEPI

Izotopske kronologije macesnovih branik na zgornji gozdni meji v jugovzhodnih Alpah so med seboj značilno in pozitivno povezane. $\delta^{18}O$ in $\delta^{2}H$ sta v značilni, a šibkejši korelaciji s TRW, medtem ko $\delta^{13}C$ s TRW kronologijo ni povezana. Najmočnejša je korelacija med kronologijama vodnih izotopov. Ta tesna povezanost je najverjetneje posledica visoke stopnje izmenjave kisika in vodika s ksilemsko vodo v procesu sinteze celuloze, s čimer se zmanjša vpliv ostalih frakcionacijskih faktorjev. Proučevani macesni na rastiščih s kraško podlago in veliko količino padavin imajo velik potencial za arhiviranje signala padavinske vode.

Na širino macesnove branike najmočneje vplivajo temperature in trajanje sončnega obsevanja v pozni pomladi in zgodnjem poletju, to je od srede maja do julija. Za razliko od širin branik, na izotopske kronologije vplivajo ne vplivajo klimatske razmere v juniju, pač pa julijske in avgustovske. Sklepamo, da je odsotnost povezave z junijskimi razmerami povezana z lastnostjo listopadnih vrst, pri katerih je nastanek ranega lesa v veliki meri odvisen od v preteklem letu nastalih škrobnih zalog, ki vsebujejo drugačen signal. Druga možna razlaga je, da je količina v rani les vgrajene celuloze manjša kot količina celuloze v kasnem lesu in je zato njen signal manj vpliven.

Posebna vrednost v doktorski disertaciji predstavljenih serij proxy podatkov macesnovih branik je v njihovem prostorskem signalu. Pokrivajo območje Jadrana in širšega območja jugovzhodnih Alp, ki predstavlja vezni člen med centralnimi Alpami in Dinaridi. Na podlagi analize literature je razvidno, da je tem območju zelo malo objavljenih kronologij širin branik in njihove izotopske sestave, prav tako pa tudi na njih temelječih klimatskih rekonstrukcij. S hkratnim proučevanjim različnih parametrov branike smo potrdili prvo hipotezo, da kombinacija različnih proxy podatkov da boljšo časovno in prostorsko opredeljeno informacijo kot posamezni podatki.

$\delta^{13}C$ macesnovih branik je v močni korelaciji tako s temperaturo kot tudi trajanjem sončnega obsevanja. Kljub daljšim časovnim nizom instrumentalnih meritev in zveznosti temperaturnega signala v prostoru in času se je pokazalo, da je trajanje sončnega obsevanja najpomembnejši klimatski dejavnik, ki vpliva na $\delta^{13}C$ macesnovih branik v naši raziskavi, in zato primernejši klimatski dejavnik za rekonstrukcijo kot temperatura. Pri klimatskih rekonstrukcijah je pomembna izbira pravega klimatskega dejavnika v pravem časovnem oknu. Primerjava obstoječih temperaturnih rekonstrukcij z našo 520 let dolgo rekonstrukcijo trajanja sončnega obsevanja razkriva obdobja razhajanja in sinhronega poteka krivulj temperatur in sončnega obsevanja. S predstavljeno rekonstrukcijo trajanja sončnega obsevanja smo potrdili drugo postavljeno hipotezo, da z uporabo iz branik pridobljenih proxy podatkov lahko rekonstruiramo klimatsko spremenljivko za obdobje pred instrumentalnimi meritvami.

V lesnoanatomskih parametrih ranega lesa D dobov je shranjena informacija o klimatskih razmerah v preteklem poletju, medtem ko klimatskega signala v kasnem lesu skoraj ni zaznati. Pretok Krke se je pokazal kot značilni dejavnik, povezan s širino branike, deležem kasnega lesa in diskriminacijo ogljikovega izotopa (Δ). Glede na korelacijo parametrov ranega lesa s poletnim pretokom Krke ter korelacijo med Δ ranega in kasnega lesa sklepamo, da je pri dobih s sušnejših rastišč (D dobi) nastanek kasnega lesa vsaj delno odvisen od produktov fotosinteze, nastalih v času formacije ranega lesa. D dobi so verjetno podvrženi t.i. stradanju ogljika kot posledice dolgotrajno zmanjšane fotosintetske aktivnosti ob neprekinjeni porabi ogljikovih hidratov za potrebe metabolizma. Glede na zelo slabe debelinske prirastke, majhno količino in strukturo kasnega lesa ter korelacije s klimatskim in hidrološkimi razmerami sklepamo, da D dobi rastejo v stresnih razmerah, v katerih se vse zaloge porabljajo za preživetje drevesa, klimatski signal pa je zabrisan.

Širine branik W dobov iz periodično poplavljenega rastišča se odzivajo podobno kot hrasti iz bližnjih srednjeevropskih lokacij. Visoka pozitivna korelacija parametrov kasnega lesa s količino padavin in pretokom Krke v poletju nakazuje, da je pomanjkanje vode en izmed bolj pomembnih dejavnikov, ki vplivajo na debelinsko rast dobov na analiziranem rastišču.

Z analizo različnih parametrov branik dveh skupin dobov v Krakovskem gozdu ter analizo korelacije z okoljskimi dejavniki in različnim odzivom nanje potrjujemo tretjo postavljeno hipotezo, da so D dobi izpostavljeni dolgotrajnim stresnim razmeram in predvidevamo, da v takih okoliščinah drevesa vse notranje zaloge porabijo za preživetje, medtem ko je njihov odziv na okoljske dejavnike zabrisan.

Predstavljamo potencial analiziranih parametrov branik doba za njihovo uporabo v dendrohidroloških študijah. Hkrati poudarjamo, da morajo biti drevesa za tovrstne raziskave pazljivo izbrana, saj se s tem lahko izognemo morebitnim napakam in izgubi informacij.

Glavna razlika med tradicionalno dendrokronološko analizo širin branik in v našem delu predstavljenim pristopom je v večjem številu pridobljenih podatkov iz analize posameznih branik ter v podrobnejši prostorski in časovni ločljivosti informacij o odzivu rastlin na spreminjajoče se okolje. S predstavljenimi rezultati dopolnjujemo razmeroma skopo mrežo izotopskih kronologij branik na območju jugozahodne Evrope.

5 POVZETEK / SUMMARY

5.1 POVZETEK

Poznavanje spreminjanja klimatskih dejavnikov v preteklosti je pomembno z vidika razumevanja in uvrščanja aktualnih klimatskih sprememb v širši časovni kontekst. Ker so nizi inštrumentalnih meritev, ki nam omogočajo vpogled v pretekle klimatske razmere, razmeroma kratki (do 250 let), za ta namen lahko uporabimo nadomestne ali t. i. proxy podatke, shranjene v različnih naravnih arhivih. Med vsemi poznanimi nosilci proxy podatkov so branike dreves med najnatančnejšimi in najdragocenejšimi naravnimi arhivi informacij o okolju v preteklosti. Dendrokronologija je relativno mlada veda in dendrokronološke analize so bile sprva namenjene datiranju lesenih objektov. Z razvojem tehnologije in uvajanjem novih metod so se hkrati razvijale tudi njene podvede, kot sta npr. dendroklimatologija in dendroekologija.

Na območju Slovenije je do sedaj manjkala rekonstrukcija klimatskih razmer, ki bi temeljila na hkratni analizi večih parametrov posameznih branik. Prav tako je bilo opravljenih le zelo malo raziskav, ki bi vključevale izotopsko sestavo branik. Pri izdelavi disertacije smo sledili naslednjim ciljem: 1. predstaviti prve dolge kronologije stabilnih izotopov v branikah ter njihov potencial za rekonstrukcijo klimatskih razmer v preteklosti, 2. vpeljati analizo izotopske sestave branik, ki je na našem območju do sedaj še ni bilo, 3. ugotoviti najpomembnejše klimatske parametre, ki značilno vplivajo na odziv dreves (strukturo branike) ter rekonstruirati izbrano klimatsko spremenljivko in 4. s hkratnim proučevanjem različnih informacij, zajetih v parametrih branike želimo dobiti boljši vpogled v odnos med delovanjem okoljskih dejavnikov in fizioloških procesov v drevesu ter oceniti primernost različno vitalnih dreves za rekonstrukcijo klimatskih dejavnikov.

Naša raziskava temelji na analizi branik evropskega macesna (*Larix decidua* Mill.) in doba (*Quercus robur* L.). Macesen je eden od glavnih gradnikov gozdne meje, medtem ko je dob vrsta kolinskega pasu in značilna vrsta poplavnih ravnic evropskih rek. Obema proučevanima vrstama je skupna dolgoživost ter obstojen les z jasno definiranimi branikami, kar predstavlja prednost za izvedbo dendrokronološke analize.

Macesen smo vzorčili na dveh lokacijah na zgornji gozdni meji – na širšem območju Vršiča in Dleskovške planote. 29 drevesom smo na prsni višini (1,3 m od tal) s prirastoslovnim svedrom odvzeli po dva tanka (0,5 cm) in en debel (1,2 cm) izvrtek. V Krakovskem gozdu smo na vsaki od dveh 600 m oddaljenih ploskvah z različnimi mikrorastiščnimi razmerami vzorčili 6 dreves z debelim in tankim svedrom. Po opravljeni standardni pripravi tankih izvrtkov smo z uporabo ATRICS sistema za zajem slike ter WinDENDRO in PAST4 programske opreme za analizo podatkov dobili kronologije širin branik. Tem smo v ARSTAN programu odstranili vpliv sestoja in vpliv rastnega trenda. Debele izvrtke smo razrezali na posamezne branike, pri dobih še ločeno na rani in kasni les. Iz razrezanih vzorcev smo izolirali α-celulozo za analizo izotopske sestave kisika ($\delta^{18}O$) in ogljika ($\delta^{13}C$) ter celulozni nitrat za analizo izotopske sestave vodika ($\delta^{2}H$). Pred nadaljnjo analizo kronologij izotopske sestave ogljika smo odpravili še trend spremenjenega razmerja med lažjim (^{12}C) in težjim (^{13}C) ogljikovim izotopom v atmosferi. Pred pripravo vzorcev za izotopsko analizo smo debele izvrtke dobov z drsnim mikrotomom še narezali na 5 do 6 cm dolge rezine. Pobarvane in fiksirane mikroskopske preparate smo analizirali z uporabo svetlobnega mikroskopa in Nikon NIS-Elements Basic Research sistemom za analizo slike.

V doktorski disertaciji predstavljamo nove kronologije širin branik (TRW) ter prve kronologije $\delta^{13}C$, $\delta^{2}H$ in $\delta^{18}O$ v celulozi branik evropskega macesna na gozdni meji v jugovzhodnih Alpah za obdobje 1907–2006. Vse tri izotopske kronologije so značilno in pozitivno povezane med seboj ($p < 0{,}001$). Kronologija TRW je v najtesnejši korelaciji z junijsko temperaturo, medtem ko na izotopsko zgradbo branik najbolj vplivajo razmere v juliju in avgustu. Poletne temperature in število sončnih ur so najmočneje povezani z vsemi štirimi kronologijami, padavine pa imajo manjši vpliv. Analiza prostorske korelacije je pokazala, da je v kronologijah vodnih izotopov shranjena informacija o temperaturnih razmerah na območju Jadrana. Ta predstavlja izvorno območje vlažnih mas, ki prinašajo padavine nad proučevano območje. Močan signal sega v južno Italijo in na zahodni del Balkana. TRW in $\delta^{13}C$-kronologiji vsebujeta potencial za rekonstrukcijo poletnih temperatur na širšem območju južne in zahodne Evrope zahodno od Karpatov. Potrdili smo prvo postavljeno raziskovalno hipotezo, da v braniki poleg širine branike lahko analiziramo še številne druge parametre, ki vsebujejo dodatne informacije, s čimer dobimo obsežnejšo informacijo. Hkratno proučevanje različnih proxy podatkov da boljšo časovno in prostorsko opredeljeno informacijo kot posamezni podatki.

Izkazalo se je, da na podlagi $\delta^{13}C$-kronologije lahko rekonstruiramo trajanje sončnega obsevanja, kljub temu da je korelacija s temperaturami močnejša. Kronologija branik namreč bolje sledi poteku kronologije sončnega obsevanja. Rekonstruirane temperature pred dvajsetim stoletjem so višje kot izmerjene temperature, prav tako njihov potek ne sovpada z objavljenimi temperaturnimi rekonstrukcijami. V tem delu tako predstavljamo prvo, 520 let dolgo rekonstrukcijo trajanja sončnega obsevanja v vzhodnih Alpah. Primerjavo z razvojem regionalnih temperatur so razkrila obdobja, ko so bile temperature in sončno obsevanje manj povezani, kot se to kaže v kalibracijskem obdobju. Hladnemu in oblačnemu obdobju 1570–1590 so sledila sončna in precej topla poletja okoli leta 1600. Sledilo je obdobje med leti 1625 in 1650, ko ni bilo posebej hladno, bilo pa je oblačno. Od druge polovice 17. stoletja dalje je postajalo vedno bolj hladno in hkrati bolj sončno. Z začetkom 18. stoletja se hladno obdobje zamenja s sončnim in toplim. Sledijo neznačilna nihanja temperature in sončnega obsevanja okoli povprečnih vrednosti. Po hladnem in oblačnem obdobju 1800–1825 so se temperature, z vmesnim oblačnim obdobjem 1825–1940, začele dvigati. Z rekonstrukcijo trajanja sončnega obsevanja smo potrdili drugo hipotezo, da z uporabo proxy podatkov, pridobljenih iz branik, lahko rekonstruiramo izbrane klimatske spremenljivke v obdobju pred instrumentalnimi meritvami.

Z analizo branik doba smo proučevali, ali se drevesa iz dveh rastišč z različnimi mikrorazmerami in posledično različnim zdravstvenim stanjem razlikujejo v odzivu na okolje. Potrdili smo razlike med zgradbo branik dobov s periodično poplavljenih (W dobi) in izjemoma poplavljenih (D dobi) rastišč. Skupini se med seboj razlikujeta v širini branike, prevodnem območju trahej ranega lesa, prevodnem območju v ranem lesu, gostoti trahej ranega lesa, širini in deležu kasnega lesa, širini ranega lesa ter diskriminaciji ogljikovega izotopa v ranem lesu. Značilnih razlik nismo odkrili med povprečnim tangencialnim in radialnim premerom trahej ranega lesa, povprečnimi vrednostmi območja trahej ranega lesa, gostoto trahej ranega lesa v ranem lesu, deležem trahej kasnega lesa glede na delež vlaken v kasnem lesu ter diskriminaciji ogljikovega izotopa v kasnem lesu. Izkazalo se je, da je pri D dobih potencialna informacija o klimatskih razmerah shranjena v ranem lesu in se navezuje na obdobje preteklega poletja. V kasnem lesu D dobov smo odkrili le odziv na visok najnižji spomladanski pretok Krke v tekočem letu. Glede na strukturo in količino kasnega lesa sklepamo, da so D dobi izpostavljeni dolgotrajnim stresnim razmeram, v katerih se vse zaloge porabijo za preživetje, odziv na okoljske dejavnike pa je nejasen. Po drugi strani pa W dobi izkazujejo podoben odziv na klimatske dejavnike kot hrasti iz bližnjih srednjeevropskih lokacij, kjer sta visoka količina poletnih padavin in zmerna poletna temperatura glavna dejavnika, ki pozitivno vplivata na debelinsko rast hrasta. Njihova debelinska rast, anatomska sestava branik in diskriminacija ogljikovega izotopa je v značilni korelaciji tudi s pretokom reke Krke. Rezultati raziskave so pokazali, da v braniki shranjena okoljska informacija lahko značilno variira celo znotraj enega sestoja ter da je močno odvisna od mikrolokacije. Potrdili smo tretjo hipotezo, da s proučevanjem kombinacije več parametrov branike lahko ocenimo zdravstveno stanje dreves in njihovo primernost za rekonstrukcijo izbranih okoljskih dejavnikov.

5.2 SUMMARY

Having knowledge about past climate changes is important from the viewpoint of understanding and categorizing current climate changes into a wider time context. As the series of instrumental measurements which enable our insight into past climatic conditions are relatively short (up to 250 years), alternative or so-called proxy data, stored in different natural archives, may be used for this purpose. Among all known holders of proxy data, tree-rings are one of the most accurate and valuable natural archives of information about the environment in the past. Dendrochronology is a relatively young science and dendrochronological analyses were primarily intended for dating wood objects. With the advance of technology and the implementation of new methods, its subsciences, e.g. dendroclimatology and dendroecology, also evolved.

Up until now, the Slovenian area lacked a reconstruction of climatic conditions based on simultaneous analysis of several parameters of individual tree-rings. Also, only some research including the isotope composition of tree-rings has been done in the past. While preparing this dissertation, the following goals were observed: 1. to present the first comprehensive chronologies of stable isotopes in tree-rings and their potential for the reconstruction of past climatic conditions, 2. to determine the most important climatic parameters that characteristically influence tree-ring structure, 3. to reconstruct a selected climate parameter, and 4. to evaluate, through simultaneous study of different tree-ring parameters, the suitability of trees with different vitalities intended for the reconstruction of climate factors. Our research is based on the analysis of European larch (La*rix decidua* Mill.) and pedunculate oak (Q*uercus robur* L.) tree-rings. Larch is one of the main building species of the upper timberline, while penduncolate oak is a species of lowlands and a typical species of European rivers' floodplains. Both studied species share the characteristics of longevity and durable wood with clearly defined tree-rings, which is an advantage when performing the dendrochronological analysis.

Larch was sampled at two locations at the upper timberline – in the wider area of the Vršič mountain pass and the Dleskovška plateau. On each of the 29 trees two thin increment cores

(0.5 cm) and one thick increment core (1,2 cm) were taken at breast height (1.3 m above ground). In Krakovo forest, at each of the two plots with different micro-plot conditions, which are 600 m apart from each other, we sampled 6 trees with a thick and a thin drill. After a standard preparation of thin increment cores, we used the ATRICS system to capture the images and the WinDENDRO and PAST4 software to carry out data analysis to obtain the tree-ring width chronology. We then proceeded and removed the influence of structure and growth trend in the ARSTAN program. Thick increment cores were cut into individual tree-rings, and with penduculate oak also separately into earlywood and latewood. α-cellulose was isolated from the cut samples for the analysis of the isotope composition of oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$), and cellulose nitrate for the analysis of the isotope composition of hydrogen ($\delta\,^{2}H$). Prior to further analysis of the chronology of the isotope composition of carbon, we rectified the trend of the altered relationship between the lighter (^{12}C) and heavier (^{13}C) carbon isotope in the atmosphere. Prior to the preparation of samples for isotope analysis, the thick increment cores of penduculate oaks were cut into 5–6 cm long slices using a sledge microtome. The coloured and fixed microscopic specimens were analyzed using an optical microscope and the Nikon NIS-Elements Basic Research system for image analysis.

This doctoral dissertation presents new tree-ring width (TRW) chronologies and first chronologies of $\delta^{13}C$, $\delta\,^{2}H$ and oxygen $\delta\,^{18}O$ isotopes in the cellulose of European larch tree-rings at the upper timberline in Southeastern Alps for the period 1907–2006. All three isotope chronologies are characteristically and positively correlated ($p < 0.001$). The TRW chronology is most closely correlated with June temperatures, while the conditions in July and August influence mostly the isotope composition of tree-rings. Summer temperatures and the number of sun hours are the most closely correlated with all four chronologies, while the effect of precipitation is smaller. The analysis of spatial correlation showed that water isotope chronologies store information about temperature conditions in the Adriatic area. This is the originating area of air masses which bring precipitation to the studied area. A strong signal reaches into Southern Italy and Western Balkans. The TRW and $\delta^{13}C$ chronologies hold the potential for reconstructing summer temperatures in the wider area of Southern and Western Europe west of the Carpathians. We confirmed our first research hypothesis stating that apart from tree-ring width, it is possible to analyze several other parameters within the tree-ring, which give us additional information and we can thus expand our overall information. Researching different proxy data simultaneously provides information that is, compared to individual data, better temporally and spatially defined.

It has been shown that it is possible to reconstruct the duration of solar irradiation based on the $\delta^{13}C$ chronology despite the fact that the correlation with temperatures is stronger. Tree-rings chronology better follows the flow of solar irradiation chronology. Reconstructed temperatures from 20 years ago are higher than measured temperatures, and their course does not coincide with published temperature reconstructions. This dissertation paper presents the first 520-year-long reconstruction of solar irradiation duration in the Western Alps. The comparison to the development of regional temperatures was brought to light by periods in which temperatures and solar irradiation were less closely linked compared to the calibration period. The cool and cloudy period 1570–1590 was followed by sunny and relatively warm summers circa 1600. After this came the period 1625–1650, which was not particularly cold, however was cloudy. From the second half of the 17th century onwards the climate became ever colder and at the same time sunnier. With the start of the 18th century, the cold period is replaced by a sunny and warm period. What follows are uncharacteristic temperature and solar irradiation oscillations close to average values. After the cold and cloudy period 1800–1825 the temperatures start to rise, with the exception of a cloudy period 1825–1940. With the reconstruction of the solar irradiation duration, the second hypothesis was confirmed stating that it is possible to reconstruct select climate parameters from a period prior to instrumental measurements with the use of proxy data acquired from tree-rings.

Pedunculate oak tree-rings analysis was used to research whether trees from two different plots with different micro-conditions and subsequently different health conditions also differ in their response to the environment. We confirmed the differences in pedunculate oak tree-rings constructions found in periodically flooded (W oaks) and exceptionally flooded (D oaks) plots. The groups differ in tree-ring widths, the conducting area in the earlywood tracheids, the conducting area in the earlywood, the thickness of earlywood tracheids, the latewood width and portion, the earlywood width and the discrimination of carbon isotope in earlywood. No distinctive differences were found between the average tangential and radial diameters of earlywood tracheids, the average values of the earlywood tracheid area, the density of the earlywood tracheids in earlywood, the portion of the latewood tracheids according to the portion of fibers in latewood and the discrimination of the carbon isotope in latewood. It has been demonstrated that D oaks store potential information about climatic conditions in their earlywood and is tied to the period of the previous summer. In D oaks latewood we discovered only a response to the high lowest spring Krka River flow in the current year. According to the structure and quantity of latewood we can conclude that D oaks are exposed to long-term stressful circumstances where all stocks are used for survival and the response to environmental factors is not clearly expressed. On the other hand, W oaks show a similar response to climate factors as oaks in close-by Central European locations, where a high amount of summer precipitation and moderate summer temperatures are the main factors influencing the thickness increment of oak. Their thickness increment, the anatomical construction of tree-rings and carbon isotope discrimination are characteristically correlated also with the flow of the Krka River. Research results have shown that environmental information stored in tree-rings may characteristically vary even within a single structure and that it is importantly dependent on the micro-location. The third hypothesis was thus proven stating that through researching a combination of multiple tree-ring parameters, the health condition of trees and their suitability for reconstructing environmental factors may be evaluated.

6 VIRI

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