The potential of tree-ring cellulose content as a novel supplementary proxy in dendroclimatology

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Abstract. Cellulose content (CC [%]) in tree rings is usually utilized as a tool to control the quality of the α-cellulose extraction from tree-rings in the preparation of stable isotope analysis in wooden tissues. Reported amounts of CC [%] are often limited to mean values per tree. For the first time, CC [%] series from two high Alpine species, Larix decidua Mill. (European Larch, LADE) and Pinus cembra L. (Swiss stone pine, PICE) are investigated in modern wood samples and Holocene wood remains from the Early and Mid-Holocene. Modern CC [%] series reveal a species-specific low-frequency trend independent from their sampling site over the past 150 years. Climate-cellulose relationships illustrate the ability of CC [%] to record temperature in both species, but for slightly different periods within the growing season.

The Holocene CC [%] series illustrate diverging low-frequency trends in both species, independent of sampling site characteristics (latitude, longitude and elevation). Moreover, potential age trends are not apparent in the two coniferous species. The arithmetic mean of CC [%] series in the Early and Mid-Holocene indicate low CC [%] succeeding cold events. In conclusion, CC [%] in tree rings show high potential to be established as novel supplementary proxy in dendroclimatology.

1 Introduction

Highly-resolved proxy records from the tree-ring archive contribute significantly in reconstructing and understanding past climate variability on versatile temporal and spatial scales (Büntgen et al., 2011; Esper et al., 2002; Mann et al., 1998). The annually-resolved and calendar-dated tree-ring records are particularly useful in disentangling natural climate variability from the anthropogenic imprint on the global climate and its impact on the climate system, which enables the improvement of models in view of projecting future climate change (Keel et al., 2016; Keller et al., 2016).

Most dendroclimatological reconstructions are based on tree-ring width (TRW) and maximum latewood density (MXD) records (Büntgen et al., 2006; Ljungqvist et al., 2016; Trouet et al., 2009). However, the determination of stable isotope ratios of carbon (δ^{13}C), oxygen (δ^{18}O) and hydrogen (δD) isotopes in tree components and their ability to reconstruct past climate has gradually opened a new field of dendroclimatology during the past four decades (Frank et al., 2015; Leuenberger, 1998; McCarroll and Loader, 2004; Treydte et al., 2006). In addition, the potential of stable isotope records in tree rings has been increased by improvements in both sample preparation and measurement techniques (Boettger et al., 2007; Filot et al., 2006; Laumer et al., 2009; Loader et al., 2015). Even though the isotopic composition has been determined in various components of a tree, e.g. bulk wood samples, leaves, methoxyl groups of lignin and leaf waxes (Anhäuser et al., 2017; Borella et al., 1999;

Kahmen et al., 2011; Kimak et al., 2015), the most preferred component is α-cellulose extracted from tree-rings (McCarroll and Loader, 2004).

The α-cellulose is the dominant component in tree-rings and its extraction follows a standardized procedure (Boettger et al., 2007). The woody annual growth layers of trees, i.e. the tree rings, are composed of lignin (phenolic polymer), hemicellulose (heterogenous polysaccharides) and cellulose (β-1,4 glucan) in a ratio of 1:1:2 (Freudenberg, 1965; Hu et al., 1999). Thereof, the part of cellulosic material which is insoluble in a 17% sodium hydroxide (NaOH) solution is defined as α-cellulose (Burton and Rasch, 1931; Cross and Bevan, 1912). The various tree-ring components are known to differ in their isotopic composition, whereas α-cellulose reveals to be the most stable component as the polymer is of long-term stability (Boettger et al., 2007; Leavitt and Danzer, 1993; McCarroll and Loader, 2004).

The quantity of the α-cellulose content (CC [%]), generally calculated from the dry weight and the α-cellulose weight of a wood sample, is usually determined and used as a tool to test the quality of the cellulose extraction and the purity of the cellulose (Burton and Rasch, 1931).

However, variations in the amount of α-cellulose and the determined CC [%] (also: cellulose yield; used rather simultaneously in literature) are scarcely mentioned in dendroclimatological literature. Merely few publications provide numbers on the mean extracted CC [%] (Cullen and Macfarlane, 2005; Gaudinski et al., 2005; Leavitt and Danzer, 1993; Loader et al., 1997). While reported results on the α-CC [%] are scarce in literature, the importance of the CC [%] calculation as a tool for the determination of degradation state in subfossil wood is addressed within several studies (Brenninkmeijer, 1983; Loader et al., 2003 and references therin; Schleser et al., 1999).

Schleser et al. (1999) point out that the determination of isotopic ratios in cellulose from woody plant tissues often assumes constancy in the CC [%], which may hold true for modern samples from living trees. However, samples from subfossil or fossil wood have presumably experienced degradation processes with potential influence on the α-CC [%]. The artificial degradation of samples revealed a discrimination of $\delta^{13}C$ values up to 0.3‰ with decreasing CC [%], where the change in degradation behavior over time splits into initially increased degradation of cellulose with a strong depletion in $\delta^{13}C$, followed by an inverse effect comprising an enrichment in $\delta^{13}C$ with advancing degradation (Schleser et al., 1999).

Loader et al. (2003) discusses the use of whole wood versus α-cellulose in subfossil tree samples as the various wood components (lignin, hemicelluloses, cellulose) experience variable degrees of decomposition changing the ratio of the individual components within a tree ring. The polysaccharide components, i.e. cellulose and hemicelluloses, decompose more rapidly than lignin, which leads to altered ratio of cellulose and lignin in whole wood samples which in turn modifies the isotopic composition of whole wood samples (Borella et al., 1998; Loader et al., 2003; Schleser et al., 1999). Therefore, cellulose is the preferred wood component in subfossil wood studies; however, the effect of partial decay of cellulose on the isotopic signature of tree rings and its implications for paleoclimate reconstructions is insufficiently investigated, although results from naturally degraded subfossil wood show no evidence for a change in the isotopic composition of cellulose due to degradation (Loader et al., 2003).

While studies on CC [%] and its implication for dendroclimatological studies are scarce, even less is known on the influence of environmental conditions on the annual variability of the α-CC [%] (Genet et al., 2011). A study on CC [%] in roots revealed influences of topography and soil moisture status on root CC [%] (Hales et al., 2009). However, variations in CC [%] over time may as well be related to tree metabolism and the availability in

non-structural carbon (NSC), which occurs in the form of starch and sugars, where the sugars provide the nutrients for cell wall components such as cellulose (Haigler et al., 2001; Hoch et al., 2002). Studies on NSC concentrations at the Alpine tree-line provide evidence that trees growing in the tree-line ecotone are not depleted in carbon; on the contrary, NSC concentrations are found to increase with elevation, implying that the limitation of tree growth at the upper tree-line does not result from insufficient nutrition, but rather thermal conditions, i.e. temperature, which limits the sink activity and thereby growth (Hoch et al., 2002; Hoch and Körner, 2003, 2009, 2012; Körner, 2003). Applied to the CC [%] of a tree ring, it can be hypothesized that temperature acts as the main control, as it is steering the sink activity, i.e. the tissue formation.

This manuscript presents results of a pilot study, where time series of modern and subfossil tree ring cellulose are established, allowing a novel insight into a tree-ring component which is commonly used in dendroclimatology, but hardly examined itself in its variability over time and in the factors driving its variability. The current study is embedded in a project which aims at reconstructing Holocene climate variability for the past 9000 years. It offers the opportunity to investigate the α-CC [%] and its variations in long-living trees from two high-Alpine coniferous tree species (Larix decidua Mill. and Pinus cembra L.) in Holocene wood remains found in glacier forefields and peat bogs in the European Alps as well as in modern trees sampled at the current Alpine tree line. The case study aims at (i) identifying common trends in time series of α-CC [%] in tree rings from Early- and Mid-Holocene subfossil and modern wood material; (ii) investigating dependencies of CC [%] in relation to their sampling site (latitude, longitude, elevation), their age and the species; (iii) analyzing climate-CC [%] relationships and finally (iv) determining the ability to utilize CC [%] as a supplementary proxy in dendroclimatological reconstructions.

2 Material and methods

2.1 Samples and sampling sites

Holocene wood remains and modern tree-ring material are sampled with the intent to reconstruct Holocene climate variability in a multi-proxy approach. Thereby, samples from two Alpine tree-line species, namely the Swiss stone pine (Pinus cembra L.) and the European larch (Larix decidua Mill.) are collected as they represent the typical tree-line species present at modern sampling sites in form of living trees, and in glacier forefields and peat bogs as subfossil wood remains (Joerin et al., 2008; Nicolussi, et al., 2009; Nicolussi and Patzelt, 2000a). The sampling sites are located along a SW-NE transect in the Central European Alps (46°02'N - 47°03'N, 7°33'E - 12°15' E; Fig. 1, Table 1 and 2). The modern tree-ring material has been cored at three upper tree-line sites close in the proximity of glacier sites during fieldwork in 2015 (Figure 2, Table 1). At each site, four trees per species have been cored; however, at the sites UAZR and FPCR, only one of the two species is present. Cores are taken at breast height from four radial sections; two in parallel to the slope, one up- and one down-slope.

The Holocene wood remains stem from glacier forefields, peat bogs and small lakes, which have been continuously collected over the last two decades (Joerin et al., 2006, 2008; Nicolussi, et al., 2009; Nicolussi et al., 2005; Nicolussi and Patzelt, 2000b). Wood material of the EACC (Eastern Alpine Conifer Chronology) has been merged and updated with subfossil samples of the same species and altitude collected by continued sampling in glacier forefields (Nicolussi and Schlüchter, 2012).

2.2 Tree ring width and dating

Total tree-ring widths (TRW) is measured for all samples. At least two radii are measured per tree with a precision of 0.001 mm by using a LINTAB device in connection to TSAPWin software (Rinn, 1996). For each sample, a mean TRW series is established by crossdating and averaging the radii per tree. In case the inner part

5 of the stem is missing in a tree-ring series, the tree-ring number between the pith and the first tree-ring measured is estimated (pith-offset (PO), Table 3). For the modern series, dating is simple as the exact sampling date is known. For the Holocene wood remains, the mean series are compared to local tree-ring series and reference chronologies (Nicolussi 2009, 2012). Crossdating is achieved by calculating statistical parameters, and applying visual controls.

10 ### 2.3 Sample preparation

Samples for CC [%] analysis are prepared as 5-year tree-ring blocks per tree, thus no pooling between trees is applied. For the Holocene samples, radial wedges are cut out of stem disks by using a bandsaw. Subsequently, blocks are prepared from the wedges and the cores under the microscope by using a commercial scalpel. The dry weight of each block is scaled and is defined to be between 20 mg and 50 mg, as a 60% weight loss is expected

15 during α-cellulose extraction. Further, the incipient degradation in samples might have additionally reduced the CC [%] in the samples. The blocks are further sliced into smallest slices of wood material by first slicing in parallel to the fiber direction, and subsequently against the fiber direction. The resulting size of the cut sample is < 1 mm. Again, the dry weight of the cut sample is scaled to estimate the loss during the cutting procedure. For the following chemical treatment, the samples are packed into ANKOM Filter Bags (Type F57), heat-sealed and

20 labelled individually.

2.4 Cellulose extraction

The cellulose extraction is based on a modified Jayme-Wise procedure (Boettger, 2007; Borella, 1999; Leuenberger, 1998). The bleaching (delignification) of samples in a 1% $NaClO_2$ + CH_3COOH solution with a pH value of 4 is conducted at 70°C for at least 30 hours to remove lignin. In a second step, samples are treated in

25 a 17% NaOH solution at 25°C for 45 minutes to extract the pure α-cellulose (Burton and Rasch, 1931; Cross and Bevan, 1912). Subsequently, samples are neutralized in a 1% HCl solution using 25% HCl and washed intensely with heated and cold distilled water until the pH of the washing water is ~ 7. Samples are dried in an oven at 50°C; the duration of the drying is thereby dependent on the sample size in the individual filter bags.

30 ### 2.5 Calculation of CC [%]

The CC [%] is usually calculated from the dry weight of wood and the cellulose weight of the sample:

$$CC\ [\%] = \left(\frac{cellulose\ weight}{dry\ weight}\right) \times 100\% \tag{1}$$

In order to optimize the approximation of the CC [%], the current study includes the weighting of 1. dry weight, 2. dry weight and α-cellulose weight, whereas the 1. dry weight defines the weight of the 5-year tree ring block,

35 the 2. dry weight the weight of the wood sample after cutting. Thereby, the potential loss during the cutting process is determined and considered in the CC [%] calculation resulting in a precise estimation:

$$CC\ [\%]' = \left(\frac{cellulose\ weight}{2.\ dry\ weight}\right) \times 100\% \tag{2}$$

A remaining source of error is the collection of dry α-cellulose from the ANKOM filter bags. The described slicing of wood samples facilitates the removal of cellulose from filter bags compared to ground wood samples and thereby reduces the loss of cellulose when being collected from the filter bags. The resulting loss is examined for 42 samples, where the filter bag including the cellulose and the emptied filter bag are scaled in addition to the cellulose weight to give an accurate estimate of the observed loss.

2.6 Outlier detection and correction

Outliers in CC [%] are observed for Holocene wood remains, where the outermost rings have experienced a higher degree of degradation, e.g. due to abrasion at the glacier sites or the exposition of wood to environmental influences and associated weathering of the sample. The differential grades of degradation lead to pronounced decreases in CC [%], appearing within the outermost rings, but also along cracks between individual rings and seldom on the innermost parts of trees, which complicate the comparison of individual time series. Therefore, detection and elimination of outliers is performed by visual and statistical application of boxplots (Tukey, 1977). Tukey (1977) defines outliers as samples, which lie outside the whiskers, whereby the extension of the whisker is limited by the 1,5 inner-quartile range (IQR). In addition to the boxplots, visual comparisons of suspicious samples with other cellulose series is applied; thereby outliers close to the whiskers revealed to be true values and the loss of samples by outlier detection and elimination could be minimized to the most extreme values.

2.7 Meteorological data

CC [%] series of modern sampling sites are correlated with meteorological data from the HISTALP database to investigate the relationship between climate and CC [%] (Auer et al., 2007). Data is obtained in form of Coarse Resolution Subregional Means (CRSM), which are calculated as arithmetic means from homogenized individual station series for five defined subregions within the Greater Alpine Region (GAR), which have been identified by EOF-based regionalization. Auer et al. (2007) recommend CRSM-series especially for lower frequency analysis for all climate parameters. Series from FPCR and UAZR are correlated to meteorological data from the NW subregion, and from VRR to data from the SW region, respectively, due to their geographical location.

For these subregions, monthly anomalies (calculated for the reference period 1961-1990) of temperature (T [°C], 1760-2008), precipitation (prec [mm], 1800-2008), cloud cover (cloud [%], 1840-2008) and sunshine (sun [h], 1880-2008), including the arithmetic seasonal (MAM, JJA, SON, DJF), semi-annual (AMJJAS, ONDJFM) and annual means are extracted. For further analysis, 5-year mean values are calculated for the annually resolved climate data as CC [%] series were established in 5-year resolution and which thereby integrate the mean climatic signal over five consecutive years.

Pearson's correlation coefficient is calculated between the mean CC [%] series and the monthly, seasonal and annual values for the four climate variables for the time period 1865-2008 (1880-2008 in case of sunhine).

3 Results

As the precise calculation of the CC [%] depends on error estimates for the dry weight of samples and the cellulose weight, two steps in the calculation of CC [%] are here improved: the precision of dry weight and the loss estimation in cellulose weight.

The mean sample loss during the cutting process amounts to 2.6 ± 1.7 % of the dry weight of an individual sample, where mean loss values range from 0.3 % up to 11.1 % per tree (data not shown here). Besides, very few individual samples experienced losses between 10 % and 30 % caused by wood pieces bouncing off during cutting or loss of material due to powdery wood substance as a result of degradation. Hence, for the precise

5 calculation of CC [%], a determination of sample weight after cutting or milling is essential.

Second, a further reduction of error is achieved by the estimation of α-cellulose loss when the extracted cellulose is removed from the ANKOM filter bags, as there is the potential of small fibers getting caught in the filter bag. The investigation of 42 filter bags and the contained CC [%] revealed a mean loss of α-cellulose of 0.378 ± 0.163 mg (3.2 ± 1.4 %) (Table 4). Therefore, the calculation of the CC [%] results in the minimum CC [%] of the

10 sample, as losses of cellulose in the order of 3.2 ± 1.4 % of the cellulose weight are to be expected, which would result in slightly increased CC [%] value. However, as the unpacking is accomplished equally for all samples, a systematic error is assumed which affects the CC [%] calculation in the same manner. It can be assumed that the systematic error does not influence the variability of the individual CC [%] series.

The improvements in the CC [%] calculation justify the use of the term "CC [%]" rather than "cellulose yield",

15 as sources of error are reduced and well estimated, and resulting calculations are close to the true values.

3.1 Modern CC [%] series

For a better understanding of CC [%] variations, modern CC [%] series are analyzed in their temporal variability per species and site. Trees sampled at UAZR consist solely of PICE samples, as the tree-line at that site is only composed of one tree species. Measured tree-ring width series vary in length between 150 and 205 years; the

20 pith offset estimation varies between 32 and 82 for the individual trees (Table 3). The mean segment length (MSL) amounts to 173 years and the period covered by all four samples spans from 1860 to 2014 A.D. (Table 1). The mean cellulose content per tree varies between 31.6 % and 39.7 %, where UAZR-1 (33.2 %) and UAZR-2 (33.2 %) show distinctly lower mean CC [%] values as UAZR-3 (38.6 %) and UAZR-4 (39.7 %). These differences are a result of low minimum values for UAZR-1 and UAZR-2, which are up to 10 % lower than for

25 the other two trees (Table 5). Further, the time series of CC [%] for UAZR -1 and -2 show more variability in their series compared to UAZR-3 and -4, which show a high common variability and merely small fluctuation in their CC [%]. This is further confirmed by the calculated Pearson's correlation coefficient (r) with highest values for UAZR-3 and -4 (r=0.64) (Table 6). A significant correlation is also found between UAZR-2 and -3 (r=0.36), whereas the remaining correlation coefficients are non-significant (p > 0.05). The arithmetic mean calculated

30 from four samples in their common period (1860-2015 AD) indicates an increase in CC [%] over time by ~ 5 % (red line, Fig. 3, top left).

In contrast to UAZR, the sampling site FPCR consist solely of LADE trees, where tree ring series contain between 155 and 335 years; the MSL amounts to 208 years, raised by the sample FPCR-1 (335 years), whereas the residual tree-ring series consist of 155 and 170 years (Table 1, Table 3). The four tree samples cover a

35 common period from 1860 to 2015 A.D. (Table 1). The mean CC [%] per tree varies between 28.3 % and 32.9 %, showing overall lower mean CC [%] values than observed for the PICE trees at UAZR (Table 5). Further, the range of CC [%] per individual tree is large, as LADE trees show low minimum CC [%] values between 15.2 % and 21.8 % as well as high maximum values of up to 51.6 % (Table 5). In their common period, trees at FPCR show a decrease in CC [%] from 1860 to 1945 A.D. and a strong increase of nearly 20 % from 1980 to 2014

40 A.D, which is common among the individual trees (Fig. 3, top right). Pearson's correlation coefficient illustrates

the common variability among the samples FPCR-1, -5 and -6, showing high correlation coefficient between 0.67 and 0.79, whereas non-significant and lower correlation coefficients are found for FPCR-3 (Table 6).

The sampling site VRR located in the Val Roseg in Eastern Switzerland, and in the proximity of the Tschierva glacier, is the only sampling site where both tree species, LADE and PICE are found, thereby allowing a direct

5   comparison of species under same climatic growing conditions.

PICE trees at VRR exhibit tree-ring series of 170 to 255 years, with a common period between 1845 and 2015 A.D. and a MSL of 209 years (Table 1, Table 3). Mean CC [%] varies between 32.9 % and 37.2 %, where the samples VRR-1 and VRR-1-2 (same tree) reveal a range of > 20%, whereas the other PICE trees at VRR exhibit sizes between 13 % and 16.3 % (Table 5). Minimum values are around 20 % for VRR-1 (and 1-2) and around 30

10 % for all the other trees, and maximum values exceed 40 % with maximum values > 45 % (Table 5). Calculated correlation coefficients are almost all significant (p < 0.05) and are between 0.41 and 0.75 (Table 6). The arithmetic mean series for VRR-PICE displays an increase in mean cellulose content of > 10 % in the common period (Fig. 3, bottom left).

The LADE tree-ring series at the same location, with a common growth period between 1865 and 2015 A.D. and

15 MSL of 219 years, consist of two times 150 years, 190 and 385 years (Table 1, Table 3). Mean CC [%] is between 26.3 % and 36.0 % with minimum values ranging from 16.3% to 30.4 %, whereas the maximum values per tree show a lower spread between 39.4 % and 46.3 % (Table 5). As for PICE, LADE CC [%] series reveal significant correlation coefficients among the individual trees (Table 6). The arithmetic mean series shows a decrease in CC [%] of approximately 5% in the period 1865-1980 A.D., followed by a strong increase of more

20 than 10 % during the recent three decades (Fig. 3, bottom right).

The investigation of individual tree-ring CC [%] series from three sampling sites (UAZR, FPCR, VRR) and two coniferous tree species (PICE, LADE) display common variability of trees from the same species, independent from the sampling site. Individual CC [%] per site and species show in general good agreement, represented in their correlation coefficients, which allows the establishment of arithmetic mean chronologies for their common

25 growth periods (comparable time periods for all sites).

Common trends in the tree species, independent of the sampling site, indicate a common influence from environmental factors on CC [%]. All PICE series reveal a continuous increase of CC [%] over time, whereas the LADE samples show a decrease over the common period, replaced by a rapid increase over the past three decades (Fig. 4, left). The close relationship between the site series of the same tree species is also revealed by

30 the calculated Pearson's correlation coefficient, where r=0.86 for LADE and r=0.76 for PICE mean series.

3.2 Climate-cellulose relationships

To test the influence of environmental conditions on the CC [%], Pearson's correlation coefficient is calculated between the mean CC [%] series per site and species and the climate variables temperature (cf. Fig 4, right), precipitation, sunshine as well as cloud cover (Fig. 5-6, Fig. S1-2).

35 A temperature signal is clearly recorded in both tree species, independent from the sampling site (Fig. 5). Both PICE sites show significant correlations with temperature throughout the year, with high correlation during the growing season and interestingly in October (r=0.67 for UAZR-PICE, r=0.65 for VRR-PICE). Seasonal temperature averages result in highest correlations for PICE; correlations for the summer season (JJA) result in r=0.68-0.71 and for autumn season (SON) in r=0.72-0.75 for UAZR-PICE and VRR-PICE, respectively. In

40 LADE, correlations with temperature are lower than for PICE and significant correlations are found mostly

during the growing season (May-August). Seasonal averages in temperature anomalies result in highest correlations for the summer season (JJA) for both FPCR-LADE (r=0.55) and VRR-LADE (r=0.69).

Precipitation is best recorded in PICE at UAZR during the early growing season, revealing highest correlation coefficients in May (r=0.68). In contrast, LADE mean series at both sites and PICE at VRR mostly show non-

5   significant correlations with precipitation records (Fig. 6).

The influence of cloud cover is highest in PICE trees during the early as well as towards the end of the growing season (Fig. S1). Significant positive correlations are found in MAM for UAZR-PICE (r=0.49), and for JJA in both UAZR-PICE (r=0.41) and VRR-PICE (r=0.42). Interestingly, the correlation coefficient for cloud cover and LADE mean series reveals significant negative correlations during the winter season (DJF, r=-0.56 (FPCR-

10 LADE), r=-0.63 (VRR-LADE)), especially in January for FPCR-LADE (r=-0.57) and in February for VRR-LADE (r=-0.46).

High, significant correlations with sunshine are observed for the late autumn and winter season, whereas negative correlations are found during summer season, being significant at both PICE sites in August, where VRR-PICE reveals significant negative correlations with sunshine from May to August (Fig. S2). For UAZR-

15 PICE, highest correlation with sunshine are found in January (r=0.86) as well as in February (r=0.76) and the winter season (DJF, r=0.87), whereas for VRR-PICE, highest correlations are found for November and December (r=0.84 and r=0.82, respectively) as well as for the winter season (r=0.79). The pattern is similar for FPCR; significant positive correlations occur in late autumn and winter (N, D, J, D) and are highest in January (r=0.62). In contrast, VRR-LADE shows highest correlations in late autumn (Nov) and in February (r=0.66 and

20 r=0.55, respectively).

In summary, the results of the Pearson's correlation coefficient analysis between HISTALP data and mean CC [%] per species and site indicate partly significant influence of environmental factors (temperature, precipitation, cloud cover, sunshine duration) on the CC [%] in tree rings and the potential to reconstruct past climate from this novel proxy. However, the comparison of the two coniferous species further indicates a species-specific response

25 of CC [%] on the environmental conditions.

3.3 Holocene CC [%] series

In a further step, CC [%] series are established for LADE and PICE tree ring samples from glacier forefields, peat bogs and small lakes for the period from 8550 to 3500 years b2k (Fig. 7). To investigate trends in the CC [%], the individual series are averaged per species (PICE in blue, LADE in green) and an arithmetic mean is also

30 calculated over all series (black), which is smoothed by a spline (orange) to illustrate long-term changes (Fig. 7). The arithmetic mean series shows the high variability in the CC [%] over time, fluctuating between 22 % and 40 %. Further, the series exhibits interesting low frequency trends with rapidly decreasing CC [%] in the periods 8250-7950 years b2k (Δ CC [%] ~ 5 %), 6250-5950 years b2k (Δ CC [%] ~ 2 %), 5650-5450 years b2k (Δ CC [%] ~ 4 %), 5300-5000 years b2k (Δ CC [%] ~ 5.5 %) and 4500-4000 years b2k (Δ CC [%] ~ 6 %). Besides

35 these phases of rapid decreases, CC [%] is increasing on a multi-centennial scale between 7350 and 6250 years b2k (Δ CC [%] ~ 4.5 %) and more rapidly after low CC [%] phases, e.g. in the periods 7950-7750 years b2k (Δ CC [%] ~ 3.5 %), 5450-5300 years b2k (Δ CC [%] ~ 7 %) and 4000-3650 years b2k (Δ CC [%] ~ 8.5 %). The calculated arithmetic mean series per species exhibit mostly lower mean values in the LADE series (green) and more positive values for PICE (blue). LADE shows extremely low CC [%] between 4700 and 4200 years b2k,

40 and extreme high values between 4000 and 3900 years b2k. The PICE average series shows extremely low

values around 8000 years b2k as well as around 4000 years b2k; otherwise both the LADE and PICE series mostly fluctuate between 30 % and 40 %.

For most of the investigated time period, the sample replication amounts to 3 trees; two gaps at 7300 and 6300 years b2k are solely covered by a single tree; partly a replication of ≥ 4 trees is achieved (Fig. 7, bottom).

Fig. 7 further displays identified cold phases (light-blue bars) analyzed by Wanner et al. (2011), where the darker bars illustrate the cold phases which were identified by three different phenomena (cold phases, glacier advances, Bond cycles) and the lighter areas mark the entire length of the cold phases; additional cold period are marked as blue vertical lines (Wanner et al., 2011, 2015). Interestingly, the strong decreases in cellulose content emerge mostly after the indicated cold phases.

As modern wood samples already displayed differential trends in the CC [%], mean series of LADE and PICE and their low-frequency trends are investigated closer in Fig. S3, where the contribution of individual trees per species is revealed in the sample replication (Fig. S3 (d)). The mean series reveal distinct offsets over time (Fig. S3 (a)). In the periods 8100-7800 years b2k and 5600-4600 years b2k, the smoothed values exhibit a common long-term trend, whereas in the remnant phases, series even show opposed trends. The difference between the two arithmetic mean series emphasizes that the differences between the species is not constant over time (Fig. S3 (b)).

Although the Holocene samples stem from various sites, the phases 8900-8200 years b2k and 7500-6700 years b2k are dominated by glacier forefield sites at Mont Miné and Tschierva glacier, respectively (Fig. S3 (c)). For both intervals, the two coniferous species are present, and as the sampling site is equal, similar as well as divergent trends in the species result presumably from the impact of environmental factors on CC [%].

In order to confirm non-existing influence of the sampling site location on CC [%] series also in Holocene samples, the arithmetic mean of CC [%] per site and species is calculated and compared to latitude, longitude and elevation of the individual sites (Fig. S4). Calculated linear regressions (dotted lines) per species do not indicate a dependence of CC [%] and sampling site locations, as linear regressions are non-significant ($p > 0.05$).

To further exclude non-climatic influences on CC [%], individual CC [%] series per tree are aligned according to their biological age by taking the estimated pith offset into account (Fig. S5). Both PICE (upper panel) and LADE (lower panel) do not reveal a distinct low-frequency trend in the age-aligned series.

4 Discussion

The determination of α-CC [%] is a common procedure in tree-ring laboratories that measure stable isotope ratios in tree-ring cellulose. However, to our current knowledge, existing records of CC [%] were simply used as a tool to ascertain the quality of the extracted cellulose; the CC [%] is, if mentioned in literature, given as a mean value and its temporal variability is not regarded any further.

The investigation of CC [%] in modern tree samples of two high-Alpine coniferous species (LADE, PICE) at three sites in the Swiss Alps reveals common variations in the CC [%] within the tree species, independent of the sampling site, pointing out a common environmental driver. While PICE trees show a continuous increase over the investigated common period, larch trees are characterized by a decrease throughout the 20th century followed by a strong increase in CC [%] from the 1980s until today.

Although the two species are both found at the upper tree-line and known to be adapted to the harsh environmental conditions, LADE is characterized as the light-demanding pioneer species which is often found in

open settings, e.g. on glacier forefields, whereas PICE is, under undisturbed conditions, the highest rising species in the inner sections of the Alps and with that adapted to short vegetation periods (Ellenberg, 1996). Further, both species are coniferous; however, LADE trees are also deciduous, as they lose their needles at the end of the growing season. Therefore, differences in the tree metabolism are to be expected.

5 The investigation of the climate-cellulose relationships by correlating mean CC [%] series with climate variables (temperature, precipitation, cloud cover, sunshine duration) extracted from the HISTALP database (Auer et al., 2007) over a common period of 140 years (1865-2008 A.D.; 1880-2008 for sunshine) reveal interesting correlation coefficients between CC [%] and climate. Temperature is recorded in both species, revealing highest correlations during summer season, and additionally for the autumn season in PICE trees. An increase in CC [%]

10 in trees with increasing temperatures may be explained by the enhanced sink activity in trees at the upper tree-line. In the attempt to establish a functional explanation of the Alpine tree-line, Körner (1998) compiled five hypotheses, where the climate-driven tree-line is either driven by stress, disturbance, reproduction, carbon balance or growth limitation (Körner, 1998) Since then, several publications have shown that concentrations of non-structural carbon (NSC) in trees at the upper tree-line increase with elevation, thereby indicating no

15 evidence that trees at high elevations experience a carbon shortage, but rather implying a lowered sink activity at the upper tree-line resulting in a growth-limitation (Hoch et al., 2002; Hoch and Körner, 2009, 2012; Körner, 2003). As photosynthetic activity is less sensitive to cold temperatures than growth, it may still be active at 0°C, where temperatures are too cold for a tree to grow (minimum temperatures for tree growth are ~ 5-7 °C) (Hoch and Körner, 2012; Körner, 1998; Tranquilini, 1979). Therefore, the C acquisition in a tree can still be active,

20 although low temperatures prevent the tissue formation. In consequence, the concentration of NSC in trees at the upper tree-line is increasing, as the source is still active, but the sink is not. In conclusion, colder temperatures during the growing season reduce the tissue formation in tree rings, resulting in a less CC [%] in the tree ring as the source activity is low. Regarding the investigated species in this study, the LADE as a pioneer species, exploring the upper tree-line, highly benefits from the increase in temperature associated with global warming

25 and which is especially pronounced since the 1980s (Rebetez and Reinhard, 2008). As temperatures during the growing season increase and the growing season is potentially prolonged, the sink activity and the CC [%] in tree rings is increased.

In PICE trees, the cellulose constant is slowly and steadily increasing over time, potentially reacting more slowly to increased temperatures, prolonged growing seasons and rising mean annual temperatures. In contrast, LADE

30 series from modern trees exhibit a rapid increase over the past 30 years, obviously benefitting from increased temperatures during the growing season. The CC [%] in Holocene wood remains and modern wood samples could therefore serve as a novel additional proxy in multi-proxy approaches, offering the opportunity to test the temperature sensitivity of diverse tree species, before combining them into chronologies. A combination of differently reacting tree species allows a complementation of temperature-CC [%] relationships, which may

35 result in more robust climate reconstructions.

Moreover, the PICE trees at two modern sampling sites showed highly significant correlation coefficients with sunshine duration especially during the winter season. As conifers are known to be photosynthetically active throughout the year as long as temperatures permit photosynthetic activity (Hoch et al., 2002 and references therin), PICE trees may potentially benefit from increased sunshine duration and be able to increase the carbon

40 acquisition during the winter season. Therefore, the CC [%] in PICE trees may even represent an annual signal. On the other hand, significant correlations found for sunshine duration and cloud cover with LADE CC [%]

series may represent artefacts, as LADE trees lose their needles in autumn and carbon acquisition is only possible during the growing season.

The low-frequency trends exhibited in the mean series of Holocene α-CC [%] in the period from 9000-3500 years b2k illustrate the potential of CC [%] as an additional proxy. The arithmetic mean CC [%] series shows pronounced decreases after known cold events in the Early and Mid-Holocene, whereas a continuous increase is observed between 7350 and 6250 years b2k, which could be the result of increased temperatures and more favorable growing conditions for trees at the upper tree-line. However, the investigation of the individual species also illustrates differences in variations between LADE and PICE approving the observed differences in species within modern samples. A complete understanding of CC [%] on tree species and environmental conditions will help to further improve the robustness of this novel proxy.

5 Conclusion and outlook

For the first time, α-CC [%] and its temporal variability of the two coniferous Alpine tree-line species Larix decidua Mill. (European larch, deciduous) and Pinus cembra L. (Swiss stone pine, evergreen) have been investigated on a centennial scale for modern samples from living trees and on a multi-millennial scale for the Early and Mid-Holocene including the transition to the Late Holocene. The established CC [%] series revealed species-specific long-term trends, independent of the location of the sampling site along the Swiss Alps in both past and present tree samples. First investigations of climate-cellulose relationships display the ability of CC [%] to record temperature at the Alpine tree-line.

As higher α-CC [%] values have been observed for low-elevation tree-ring material (unpublished data), the influence of elevation ought to be examined along an altitudinal gradient to quantify the decrease in α-cellulose and thereby verify the hypothesis that not carbon, but temperature is the limiting factor resulting in limited sink activity which results in turn in lower CC [%] values.

In a succeeding study, the site- and species-sensitivity of multiple proxies (TRW, CC%, δD, δ13C, δ18O) and the correlations between the individual proxies will be examined in relation to the environmental conditions per site to quantify their ability for multi-millennial reconstructions based on a multi-proxy approach from tree rings (Ziehmer et al., in prep.). Thereby, the effects of variations in α-CC [%] on triple isotope records will be tested.

Moreover, α-CC [%] series ought to be established in annual resolution to establish a robust calibration and verification of the CC [%]-climate relationship as the reduced resolution of our samples (5-year tree-ring blocks) prohibits the proper analysis of the climatic response of cellulose and a robust calibration against instrumental data. The existence of numberless CC [%] series in dendro-laboratories all over the world will allow a broad investigation of CC [%] in various species from wide-spread sampling sites in temporal resolution reaching from intra-annual to decadal tree-ring samples.

A complete comprehension of environmental factors controlling the α-CC [%] in diverse tree species will result in an improved understanding of physiological and biochemical processes in forming annual growth layers, and thus serve as an additional proxy in dendroclimatology.

Data availability

At present, data can be obtained upon request. As agreed among the project participants, datasets will be made available to the public after the official completion of the Alpine Holocene Tree Ring Isotope Records (AHTRIR) project.

5 **Author contribution**

M. M. Z. and M. L. designed the study. The preparation of the experiments as well as the statistical analysis and interpretation were carried out by M. M. Z. The drafting of the manuscript was accomplished by M. M. Z. with contributions from all co-authors.

Competing interest

10 The authors declare that they have no conflict of interest.

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References

Anhäuser, T., Greule, M., Polag, D., Bowen, G. J. and Keppler, F.: Mean annual temperatures of mid-latitude
20 regions derived from ??2H values of wood lignin methoxyl groups and its implications for paleoclimate studies, Sci. Total Environ., 574, 1276–1282, doi:10.1016/j.scitotenv.2016.07.189, 2017.

Auer, I., Reinhard, B., Jurkovic, A., Lipa, W., Orlik, A., Potzmann, R., Sch, W., Ungersb, M., Matulla, C., Briffa, K., Jones, P., Efthymiadis, D., Brunetti, M., Nanni, T., Maugeri, M., Mercalli, L., Mestre, O., Moisselin, J.,
25 Begert, M. and Gerhard, M.: HISTALP – historical instrumental climatological surface time series of the Greater Alpine Region Abstract :, , 46(July 2006), 17–46, doi:10.1002/joc, 2007.

Boettger, T., Haupt, M., Knöller, K., Weise, S. M., Waterhouse, J. S., Rinne, K. T., Loader, N. J., Sonninen, E., Jungner, H., Masson-Delmotte, V., Stievenard, M., Guillemin, M. T., Pierre, M., Pazdur, A., Leuenberger, M.,
30 Filot, M. S., Saurer, M., Reynolds, C. E., Helle, G. and Schleser, G. H.: Wood Cellulose Preparation Methods and Mass Spectrometric Analyses of $\delta 13 C$, $\delta 18 O$, and Nonexchangeable $\delta 2 H$ Values in Cellulose , Sugar and Starch: An Interlaboratory Comparison, Anal. Chem., 79(12), 4603–4612, doi:10.1021/ac0700023, 2007.

Borella, S., Leuenberger, M., Saurer, M. and Siegwolf, R.: C analysis of tree rings: Pooling, milling, and cellulose extraction, J. Geophys. Res., 103(D16), 19519, doi:10.1029/98JD01169, 1998.

Borella, S., Leuenberger, M. and Saurer, M.: O in tree rings: Wood-cellulose comparison and method dependent
5   sensitivity, J. Geophys. Res., 104(D16), 19267, doi:10.1029/1999JD900298, 1999.

Brenninkmeijer, C. A. M.: Deuterium, oxygen-18 and carbon-13 in three rings and peat deposits in relation to climate, 1983.

Büntgen, U., Frank, D. C., Nievergelt, D. and Esper, J.: Summer temperature variations in the European Alps,
10   A.D. 755-2004, J. Clim., 19(21), 5606–5623, doi:10.1175/JCLI3917.1, 2006.

Büntgen, U., Tegel, W., Nicolussi, K., McCormick, M., Frank, D., Trouet, V., Kaplan, J. O., Herzig, F., Heussner, K.-U., Wanner, H., Luterbacher, J. and Esper, J.: 2500 years of European climate variability and human susceptibility., Science (80-.)., 331(6017), 578–82, doi:10.1126/science.1197175, 2011.

Burton, J. O. and Rasch, R. H.: The determination of the alpha-cellulose content and copper number of paper, Bur. Stand. J. Res., 6(4), 603, doi:10.6028/jres.006.037, 1931.

Cross, C. F. and Bevan, E. J.: Researches on cellulose III (1905-1910), , 173 [online] Available from:
20   http://www.worldcat.org/title/researches-on-cellulose-iii-1905-1910/oclc/575996290?referer=di&ht=edition,
1912.

Cullen, L. E. and Macfarlane, C.: Comparison of cellulose extraction methods for analysis of stable-isotope ratios of carbon and oxygen in plant material., Tree Physiol., 25(5), 563–569, doi:10.1093/treephys/25.5.563,
25   2005.

Ellenberg, H.: Vegetation Mitteleuropas mit den Alpen in ökologischer, dynamischer und historischer Sicht: 170 Tab, 1996.

30   Esper, J., Cook, E. R. and Schweingruber, F. H.: Low-frequency signals in long tree-ring chronologies for reconstructing past temperature variability., Science, 295(5563), 2250–3, doi:10.1126/science.1066208, 2002.

Filot, M. S., Leuenberger, M., Pazdur, A. and Boettger, T.: Rapid online equilibration method to determine the D/H ratios of non-exchangeable hydrogen in cellulose, Rapid Commun. Mass Spectrom., 20(22), 3337–3344,
35   doi:10.1002/rcm.2743, 2006.

Frank, D. C., Poulter, B., Saurer, M., Esper, J., Huntingford, C., Helle, G., Treydte, K., Zimmermann, N. E., Schleser, G. H. and Ahlström, A.: Water-use efficiency and transpiration across European forests during the Anthropocene, Nat. Clim. Chang., 5(6), 579–583, doi:10.1038/nclimate2614, 2015.
40

Freudenberg, K.: Lignin: Its Constitution and Formation from p-Hydroxycinnamyl Alcohols: Lignin is duplicated by dehydrogenation of these alcohols; intermediates explain formation and structure., Science, 148(3670), 595–600, doi:10.1126/science.148.3670.595, 1965.

5 Gaudinski, J. B., Dawson, T. E., Quideau, S., Schuur, E. a G., Roden, J. S., Trumbore, S. E., Sandquist, D. R., Oh, S.-W. and Wasylishen, R. E.: Comparative Analysis of Cellulose Preparation Techniques for Use with 13 C, 14 C, and 18 O Isotopic Measurements, Anal. Chem., 77(22), 7212–7224, doi:10.1021/ac050548u, 2005.

Genet, M., Li, M., Luo, T., Fourcaud, T., Cl??ment-Vidal, A. and Stokes, A.: Linking carbon supply to root cell-
10 wall chemistry and mechanics at high altitudes in Abies georgei, Ann. Bot., 107(2), 311–320, doi:10.1093/aob/mcq237, 2011.

Haigler, C. H., Ivanova-Datcheva, M., Hogan, P. S., Salnikov, V. V, Hwang, S., Martin, K. and Delmer, D. P.: Carbon partitioning to cellulose synthesis., Plant Mol. Biol., 47(1–2), 29–51, doi:10.1023/A:1010615027986,
15 2001.

Hales, T. C., Ford, C. R., Hwang, T., Vose, J. M. and Band, L. E.: Topographic and ecologic controls on root reinforcement, J. Geophys. Res. Solid Earth, 114(3), 1–17, doi:10.1029/2008JF001168, 2009.

20 Hoch, G. and Körner, C.: The carbon charging of pines at the climatic treeline: a global comparison., Oecologia, 135(1), 10–21, doi:10.1007/s00442-002-1154-7, 2003.

Hoch, G. and Körner, C.: Growth and carbon relations of tree line forming conifers at constant vs. variable low temperatures, J. Ecol., 97(1), 57–66, doi:10.1111/j.1365-2745.2008.01447.x, 2009.
25
Hoch, G. and Körner, C.: Global patterns of mobile carbon stores in trees at the high-elevation tree line, Glob. Ecol. Biogeogr., 21(8), 861–871, doi:10.1111/j.1466-8238.2011.00731.x, 2012.

Hoch, G., Popp, M. and Körner, C.: Altitudinal increase of mobile carbon pools in Pinus cembra suggests sink
30 limitation of growth at the Swiss treeline, Oikos, 98(March), 361–374, doi:10.1034/j.1600-0706.2002.980301.x, 2002.

Hu, W. J., Harding, S. a, Lung, J., Popko, J. L., Ralph, J., Stokke, D. D., Tsai, C. J. and Chiang, V. L.: Repression of lignin biosynthesis promotes cellulose accumulation and growth in transgenic trees., Nat.
35 Biotechnol., 17(8), 808–812, doi:10.1038/11758, 1999.

Joerin, U. E., Stocker, T. F. and Schlüchter, C.: Multicentury glacier fluctuations in the Swiss Alps during the Holocene, The Holocene, 16(5), 697–704, doi:10.1191/0959683606hl964rp, 2006.

Joerin, U. E., Nicolussi, K., Fischer, A., Stocker, T. F. and Schlüchter, C.: Holocene optimum events inferred from subglacial sediments at Tschierva Glacier, Eastern Swiss Alps, Quat. Sci. Rev., 27(3–4), 337–350, doi:10.1016/j.quascirev.2007.10.016, 2008.

5 Kahmen, A., Dawson, T. E., Vieth, A. and Sachse, D.: Leaf wax n-alkane ??D values are determined early in the ontogeny of Populus trichocarpa leaves when grown under controlled environmental conditions, Plant, Cell Environ., 34(10), 1639–1651, doi:10.1111/j.1365-3040.2011.02360.x, 2011.

Keel, S. G., Joos, F., Spahni, R., Saurer, M., Weigt, R. B. and Klesse, S.: Simulating oxygen isotope ratios in tree ring cellulose using a dynamic global vegetation model, Biogeosciences, 13(13), 3869–3886,
10 doi:10.5194/bg-13-3869-2016, 2016.

Keller, K. M., Lienert, S., Bozbiyik, A., Stocker, T. F., Churakova, O. V., Frank, D. C., Klesse, S., Koven, C. D., Leuenberger, M., Riley, W. J., Saurer, M., Siegwolf, R., Weigt, R. B. and Joos, F.: 20th-century changes in carbon isotopes and water-use efficiency: Tree-ring based evaluation of the CLM4.5 and LPX-Bern models,
15 Biogeosciences Discuss., (December), 1–48, doi:10.5194/bg-2016-515, 2016.

Kimak, A., Kern, Z. and Leuenberger, M.: Qualitative Distinction of Autotrophic and Heterotrophic Processes at the Leaf Level by Means of Triple Stable Isotope (C-O-H) Patterns, Front. Plant Sci., 6(November), 1008, doi:10.3389/fpls.2015.01008, 2015.

Körner, C.: A re-assessment of high elevation treeline psoitions and their explanation, Oecologia, 115(4), 445–459, 1998.

Körner, C.: Carbon limitation in trees, J. Ecol., 91(1), 4–17, doi:10.1046/j.1365-2745.2003.00742.x, 2003.

Laumer, W., Andreu, L., Helle, G., Schleser, G. H., Wieloch, T. and Wissel, H.: A novel approach for the homogenization of cellulose to use micro-amounts for stable isotope analyses, Rapid Commun. Mass Spectrom., 23(13), 1934–1940, doi:10.1002/rcm.4105, 2009.

30 Leavitt, S. W. and Danzer, S. R.: Method for batch processing small wood samples to holocellulose for stable-carbon isotope analysis, Anal. Chem., 65(1), 87–89, doi:10.1021/ac00049a017, 1993.

Leuenberger, M.: Stable isotopes in tree rings as climate and stress indicators, vdf Hochschulverlag AG., 1998.

35 Ljungqvist, F. C., Krusic, P. J., Sundqvist, H. S., Zorita, E., Brattström, G. and Frank, D.: Northern Hemisphere hydroclimate variability over the past twelve centuries, Nature, 532(7597), 94–98, doi:10.1038/nature17418, 2016.

Loader, N. J., Robertson, I., Barker, A. C., Switsur, V. R. and Waterhouse, J. S.: An improved technique for the
40 batch processing of small wholewood samples to α-cellulose, Chem. Geol., 136(3–4), 313–317, doi:10.1016/S0009-2541(96)00133-7, 1997.

Loader, N. J., Robertson, I. and McCarroll, D.: Comparison of stable carbon isotope ratios in the whole wood, cellulose and lignin of oak tree-rings, Palaeogeogr. Palaeoclimatol. Palaeoecol., 196(3–4), 395–407, doi:10.1016/S0031-0182(03)00466-8, 2003.

Loader, N. J., Street-Perrott, F. A., Daley, T. J., Hughes, P. D. M., Kimak, A., Levanič, T., Mallon, G., Mauquoy, D., Robertson, I., Roland, T. P., van Bellen, S., Ziehmer, M. M. and Leuenberger, M.: Simultaneous Determination of Stable Carbon, Oxygen, and Hydrogen Isotopes in Cellulose, Anal. Chem., 87(1), 376–380, doi:10.1021/ac502557x, 2015.

Mann, M. E., Bradley, R. S. and Hughes, M. K.: Global-scale temperature patterns and climate forcing over the past six centuries, Nature, 392(6678), 779–787, doi:10.1038/nature02478, 1998.

McCarroll, D. and Loader, N. J.: Stable isotopes in tree rings, in Quaternary Science Reviews, vol. 23, pp. 771–
15   801, Elsevier., 2004.

Nicolussi, K., Kaufmann, M., Melvin, T. M., van der Plicht, J., Schiessling, P. and Thurner, A.: A 9111 year long conifer tree-ring chronology for the European Alps: a base for environmental and climatic investigations, The Holocene, 19(2009), 909–920, doi:10.1177/0959683609336565, 2009.

Nicolussi, K. and Patzelt, G.: Discovery of early-Holocene wood and peat on the forefield of the Pasterze Glacier, Eastern Alps, Austria, The Holocene, 10(2), 191–199, doi:10.1191/095968300666855842, 2000a.

Nicolussi, K. and Patzelt, G.: Untersuchungen zur holozänen Gletscherentwicklung von Pasterze und
25   Gepatschferner (Ostalpen), Zeitschrift für Gletscherkd. und Glazialgeol., 36(1–2), 1–87, 2000b.

Nicolussi, K. and Schlüchter, C.: The 8.2 ka event-Calendar-dated glacier response in the Alps, Geology, 40(9), 819–822, doi:10.1130/G32406.1, 2012.

30   Nicolussi, K., Kaufmann, M., Patzelt, G., Plicht Van Der, J. and Thurner, A.: Holocene tree-line variability in the Kauner Valley, Central Eastern Alps, indicated by dendrochronological analysis of living trees and subfossil logs, Veg. Hist. Archaeobot., 14(3), 221–234, doi:10.1007/s00334-005-0013-y, 2005.

Rebetez, M. and Reinhard, M.: Monthly air temperature trends in Switzerland 1901-2000 and 1975-2004, Theor.
35   Appl. Climatol., 91(1–4), 27–34, doi:10.1007/s00704-007-0296-2, 2008.

Rinn, F.: Tsap V 3.6 Reference manual: computer program for tree-ring analysis and presentation, Rinntech, Heidelb., 1996.

40   Schleser, G. H., Frielingsdorf, J. and Blair, A.: Carbon isotope behaviour in wood and cellulose during artificial aging, Chem. Geol., 158(1–2), 121–130, doi:10.1016/S0009-2541(99)00024-8, 1999.

Tranquilini: Physiological Ecology of the Alpine Timberline., 1979.

Treydte, K. S., Schleser, G. H., Helle, G., Frank, D. C., Winiger, M., Haug, G. H. and Esper, J.: The twentieth
5   century was the wettest period in northern Pakistan over the past millennium., Nature, 440(7088), 1179–1182, doi:10.1038/nature04743, 2006.

Trouet, V., Esper, J., Graham, N. E., Baker, A., Scourse, J. D. and Frank, D. C.: Persistent positive North Atlantic oscillation mode dominated the Medieval Climate Anomaly., Science, 324(5923), 78–80,
10   doi:10.1126/science.1166349, 2009.

Tukey, J. W.: Exploratory Data Analysis, Addison-Wesley Publishing Company., 1977.

Wanner, H., Solomina, O., Grosjean, M., Ritz, S. P. and Jetel, M.: Structure and origin of Holocene cold events,
15   Quat. Sci. Rev., 30(21–22), 3109–3123, doi:10.1016/j.quascirev.2011.07.010, 2011.

Wanner, H., Mercolli, L., Grosjean, M. and Ritz, S. P.: Holocene climate variability and change; a data-based review, J. Geol. Soc. London., 172, 254–263, doi:10.1144/jgs2013-101, 2015.

site code		FPCR	UAZR	VRR
sampling site		Val d'Hérens, Mont Miné glacier	Haslital, Unteraar glacier, north shore Grimsel reservoir	Val Roseg
site character	site type	near timberline	near timberline	near timberline
	latitude °N	46°04' N	46°34' N	46°26' N
	longitude °E	7°33' E	8°17' E	9°51' E
	aspect	WSW	SSO	E
	elevation (m a.s.l.)	1930-2000	1968-1986	2127-2190
	number of trees	4	4	8
	period A.D.	1680-2015	1810-2015	1630-2015
	period A.D. with replication ≥ 4	1860-2015	1865-2015	1845-2015 (PICE) / 1865-2015 (LADE)
	mean segment length [years]	208	173	209 (PICE) / 219 (LADE)

Table 1. Site characteristics of modern sites. Coordinates, aspect and elevation describe their geographical distribution from west to east. Four trees are sampled per site and species, respectively, resulting in eight trees cored at VRR, where both species are present at the tree-line. Site characteristics are complemented by the maximum
5 **growth period and the period covered by a sample replication of four trees in years A.D. as well as the mean segment length (MSL) in years.**

site code	sampling site	site character				
		site type	latitude °N	longitude °E	aspect	elevation (m a.s.l.)
ahmo	Ahrntal, Moaralm	peat bog	47°02' N	12°05' E	SE	1995
ahst	Ahrntal, Starklalm	peat bog	47°03' N	12°07' E	S	2080
bih	Paznaun, Bielerhöhe	peat bog	46°55' N	10°06' E	N	1930-2020
eba	Ötztal, Ebenalm	lake/peat bog	47°01' N	10°57' E	NO	2060-2170
g	Ötztal, Gurgler Zirbenwald	peat bog	46°51' N	11°01' E	NW	2060
gdm	Kaunertal, Daunmoränensee	lake/peat bog	46°53' N	10°43' E	O	2295
ggua	Ötztal, Gurgler Alm	peat bog	46°51' N	11°00' E	W	2150-2200
gli	Kaunertal, Ombrometer	peat bog	46°53' N	10°43' E	NO	2135-2160
gp	Kaunertal, Gepatschferner	glacier foreland	46°52' N	10°44' E	W	2060-2275
hib	Defereggental, Hirschbichl	lake/peat bog	46°54' N	12°15' E	E	2140
kofl	Ahrntal, Kofler Alm	peat bog	46°57' N	12°06' E	S	2165-2190
mazb	Vinschgau, Marzoneralm/B	peat bog	46°35' N	10°57' E	N	2125
mazc	Vinschgau, Marzoneralm/C	peat bog	46°35' N	10°57' E	N	2120
maze	Vinschgau, Marzoneralm/E	peat bog	46°35' N	10°57' E	N	2125
mm	Val d'Hérens, Glacier du Mont Mine	glacier foreland	46°02' N	7°33' E	NNE	1980-2010
mort	Morteratschgletscher	glacier foreland	46°25' N	9°56' E	W	2040-2050
rt	Rojental	lake/peat bog	46°48' N	10°28' E	SO	2400
tah	Passeier, Timmeltal	peat bog	46°54' N	11°08' E	S	1975-2260
tsc	Val Roseg, Tschiervagletscher	glacier foreland	46°24' N	9°53' E	NW	2115-2210
ua	Haslital, Unteraargletscher	glacier foreland	46°34' N	8°13' E	E	1950
ulfi	Ultental, Fiechtsee	lake/peat bog	46°28' N	10°50' E	N	2110
uwba	Ultental, Weißbrunnalm	peat bog	46°28' N	10°49' E	NE	2330
zer	Mattertal, Zermatt, Findelengletscher	glacier foreland	46°03' N	7°47' E	N	2300-2330

Table 2. Site characteristics of Holocene sites. Given are the site codes as well as the geographic location expressed by latitude, longitude and aspect. Elevation of sampling sites is comparable with modern sampling sites, occasionally exceeding the current tree-line.

sample	species	start [years b2k (A.D.)]	end [years b2k (A.D.)]	years	PO	PO (5-year)	number of samples (5-year)
UAZR-1	PICE	135 (1865 A.D.)	- 14 (2014 AD)	150	50	10	30
UAZR-2	PICE	140 (1860 A.D.)	- 14 (2014 AD)	155	82	17	31
UAZR-3	PICE	165 (1835 A.D.)	- 14 (2014 AD)	180	61	13	36
UAZR-4	PICE	190 (1810 A.D.)	- 14 (2014 AD)	205	32	7	41
FPCR-1	LADE	320 (1680 A.D.)	- 14 (2014 AD)	335	150	30	67
FPCR-3	LADE	155 (1845 A.D.)	- 14 (2014 AD)	170	30	6	34
FPCR-5	LADE	155 (1845 A.D.)	- 14 (2014 AD)	170	45	9	34
FPCR-6	LADE	140 (1860 A.D.)	- 14 (2014 AD)	155	55	11	31
VRR-1	PICE	205 (1795 A.D.)	- 14 (2014 AD)	220	62	13	44
VRR-1-2	PICE	195 (1805 A.D.)	- 14 (2014 AD)	205	77	15	41
VRR-3	PICE	175 (1825 A.D.)	- 14 (2014 AD)	190	12	3	38
VRR-4	PICE	155 (1845 A.D.)	- 14 (2014 AD)	170	49	10	34
VRR-5	PICE	240 (1760 A.D.)	- 14 (2014 AD)	255	13	3	51
VRR-6	LADE	135 (1865 A.D.)	- 14 (2014 AD)	150	6	2	30
VRR-7	LADE	135 (1865 A.D.)	- 14 (2014 AD)	150	13	3	30
VRR-8	LADE	370 (1630 A.D.)	- 14 (2014 AD)	385	27	6	77
VRR-9	LADE	175 (1825 A.D.)	- 14 (2014 AD)	190	25	5	38

Table 3. Individual tree characteristics per modern sampling site. Given are the tree species, start and end date of the α-cellulose content series in years b2k and years A.D. in brackets, respectively. The length of the individual series is enlisted in years and the estimation of the pith offset (PO) in annual resolution and calculated as number of 5-year blocks. The number of 5-year cellulose blocks covering the length of the cellulose series is shown.

sample	bag incl. sample [mg]	emptied bag [mg]	expected sample weight [mg]	sample weight [mg]	loss [mg]	loss [%]
1	138.250	126.552	11.698	11.473	0.225	1.9
2	138.112	128.480	9.632	9.307	0.325	3.4
3	129.500	116.654	12.846	12.293	0.553	4.3
4	147.350	134.918	12.432	11.980	0.452	3.6
5	129.060	118.279	10.781	10.405	0.376	3.5
6	133.301	123.375	9.926	9.731	0.195	2.0
7	147.471	132.364	15.107	14.758	0.349	2.3
8	136.276	123.247	13.029	12.486	0.543	4.2
9	147.790	134.199	13.591	13.011	0.580	4.3
10	136.433	125.743	10.690	10.341	0.349	3.3
11	132.212	121.153	11.059	10.708	0.351	3.2
12	133.778	119.853	13.925	13.553	0.372	2.7
13	145.170	133.002	12.168	11.667	0.501	4.1
14	140.314	128.114	12.200	11.804	0.396	3.2
15	118.253	108.009	10.244	10.211	0.033	0.3
16	127.516	115.089	12.427	12.004	0.423	3.4
17	124.031	111.314	12.717	11.733	0.984	7.7
18	123.409	113.444	9.965	9.704	0.261	2.6
19	127.960	119.475	8.485	8.228	0.257	3.0
20	154.157	136.382	17.775	17.348	0.427	2.4
21	138.214	126.419	11.795	11.321	0.474	4.0
22	114.291	101.147	13.144	12.823	0.321	2.4
23	136.587	125.258	11.329	11.305	0.024	0.2
24	121.803	107.166	14.637	14.383	0.254	1.7
25	130.931	116.707	14.224	13.969	0.255	1.8
26	138.189	126.357	11.832	11.503	0.329	2.8
27	128.379	114.667	13.712	13.430	0.282	2.1
28	132.578	120.533	12.045	11.402	0.643	5.3
29	131.704	119.411	12.293	11.726	0.567	4.6
30	113.811	102.525	11.286	10.972	0.314	2.8
31	120.800	109.288	11.512	11.310	0.202	1.8
32	116.480	102.682	13.798	13.321	0.477	3.5
33	125.114	112.714	12.400	12.036	0.364	2.9
34	119.056	107.090	11.966	11.530	0.436	3.6
35	121.489	109.201	12.288	11.951	0.337	2.7
36	106.796	92.591	14.205	13.770	0.435	3.1
37	126.301	111.446	14.855	14.389	0.466	3.1
38	127.248	113.117	14.131	13.642	0.489	3.5
39	156.524	143.516	13.008	12.529	0.479	3.7
40	130.215	121.632	8.583	8.347	0.236	2.7
41	144.788	140.079	4.709	4.362	0.347	7.4
42	123.144	109.257	13.887	13.681	0.206	1.5

Table 4. Calculated sample loss during unpacking of extracted α-cellulose from ANKOM Filter Bags (Type F57) for 42 samples (sample tree MM-602, PICE). The expected sample weight is calculated by subtracting the scaled weight of the emptied filter bag from the weight of the filled bag. The difference between the expected weight of α-cellulose and the scaled weight of unpacked cellulose defines the loss, which is given here in [mg] and [%].

site	sample number	species	mean CC [%]	σ CC [%[	minimum CC [%]	maximum CC [%]	range CC [%]
UAZR	UAZR-1	PICE	33.2	3.9	26.7	42.6	15.9
	UAZR-2	PICE	31.6	3.4	24.8	36.8	12.0
	UAZR-3	PICE	38.6	1.6	34.8	42.8	8.0
	UAZR-4	PICE	39.7	2.5	34.3	46.4	12.1
FPCR	FPCR-1	LADE	29.0	6.5	18.3	48.9	30.6
	FPCR-3	LADE	29.1	6.9	15.2	38.5	23.3
	FPCR-5	LADE	32.9	6.7	20.9	51.6	30.7
	FPCR-6	LADE	28.3	5.6	21.8	46.6	24.8
VRR	VRR-1	PICE	32.9	5.2	21.2	41.9	20.7
	VRR1-2	PICE	33.0	4.5	20.8	42.0	21.2
	VRR-3	PICE	34.3	3.2	29.0	42.0	13.0
	VRR-4	PICE	37.2	3.9	29.2	45.5	16.3
	VRR-5	PICE	35.4	3.3	30.2	45.7	15.5
	VRR-6	LADE	30.6	5.4	19.9	39.5	19.6
	VRR-7	LADE	36.0	4.4	30.4	44.5	14.1
	VRR-8	LADE	26.3	6.0	16.3	39.4	23.1
	VRR-9	LADE	32.5	5.5	26.4	46.3	19.9

Table 5. General statistics of the individual α-cellulose content series per tree from the modern sites. Given are the arithmetic mean values, the standard deviation as well as maximum and minimum α-cellulose content values as well as the range per tree. To simplify the comparison between species, the species per site and tree individuum are enlisted once more.

	UAZR-1	UAZR-2	UAZR-3	UAZR-4			FPCR-1	FPCR-3	FPCR-5	FPCR-6
UAZR-1	1					FPCR-1	1			
UAZR-2	-0.10	1				FPCR-3	0.31	1		
UAZR-3	0.35	0.36	1			FPCR-5	0.74	0.18	1	
UAZR-4	0.08	0.32	0.64	1		FPCR-6	0.79	0.39	0.67	1

	VRR-1	VRR-1-2	VRR-3	VRR-4	VRR-5		VRR-6	VRR-7	VRR-8	VRR-9
VRR-1	1									
VRR-1-2	0.75	1								
VRR-3	0.66	0.44	1							
VRR-4	0.75	0.62	0.63	1						
VRR-5	0.75	0.41	0.70	0.68	1					
VRR-6							1			
VRR-7							0.78	1		
VRR-8							0.35	0.54	1	
VRR-9							0.55	0.61	0.60	1

Table 6. Pearson's correlation coefficient calculated for the individual trees per site and species calculated in their common growth period (sample replication ≥ 4 trees). Bold numbers indicate that correlations coefficients are significant (p < 0.05).

Figures
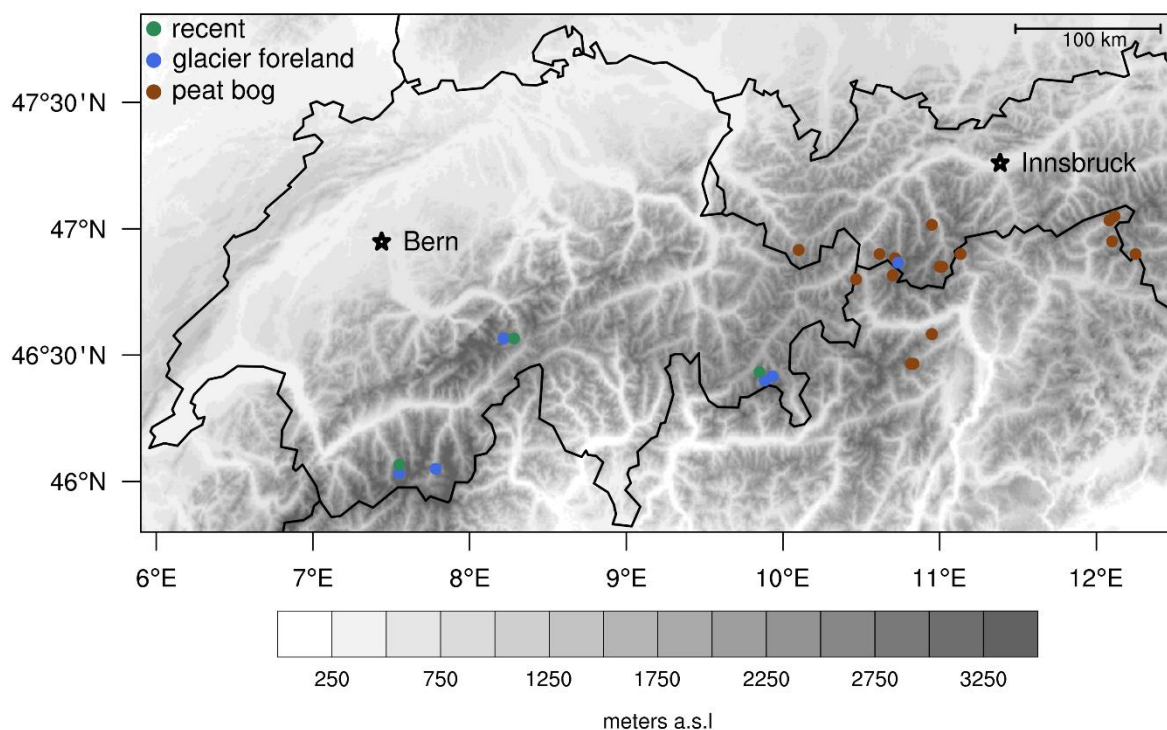
Fig. 1. **Sampling sites of Holocene wood remains and living tree individuals. The sites are located along a SW-NE**
5   **transect along the central European Alps and contain glacier forefields (blue), peat bogs and small lakes (maroon),**
where Holocene wood remains are collected, and modern sites, where living trees were cored (green). The modern
sites are situated in the proximity of glacier forefield sites, thereby resembling similar growth conditions and serving
as a connection between Holocene and present-day tree growth and environmental conditions.

Fig. 2. Location of modern sampling sites at the Alpine tree-line at Mont Miné glacier (FPCR, left), in front of
5   **Unteraar glacier (UAZR, center) and in the Val Roseg close to the Tschierva glacier (VRR, right). (Source: K.**
Nicolussi)
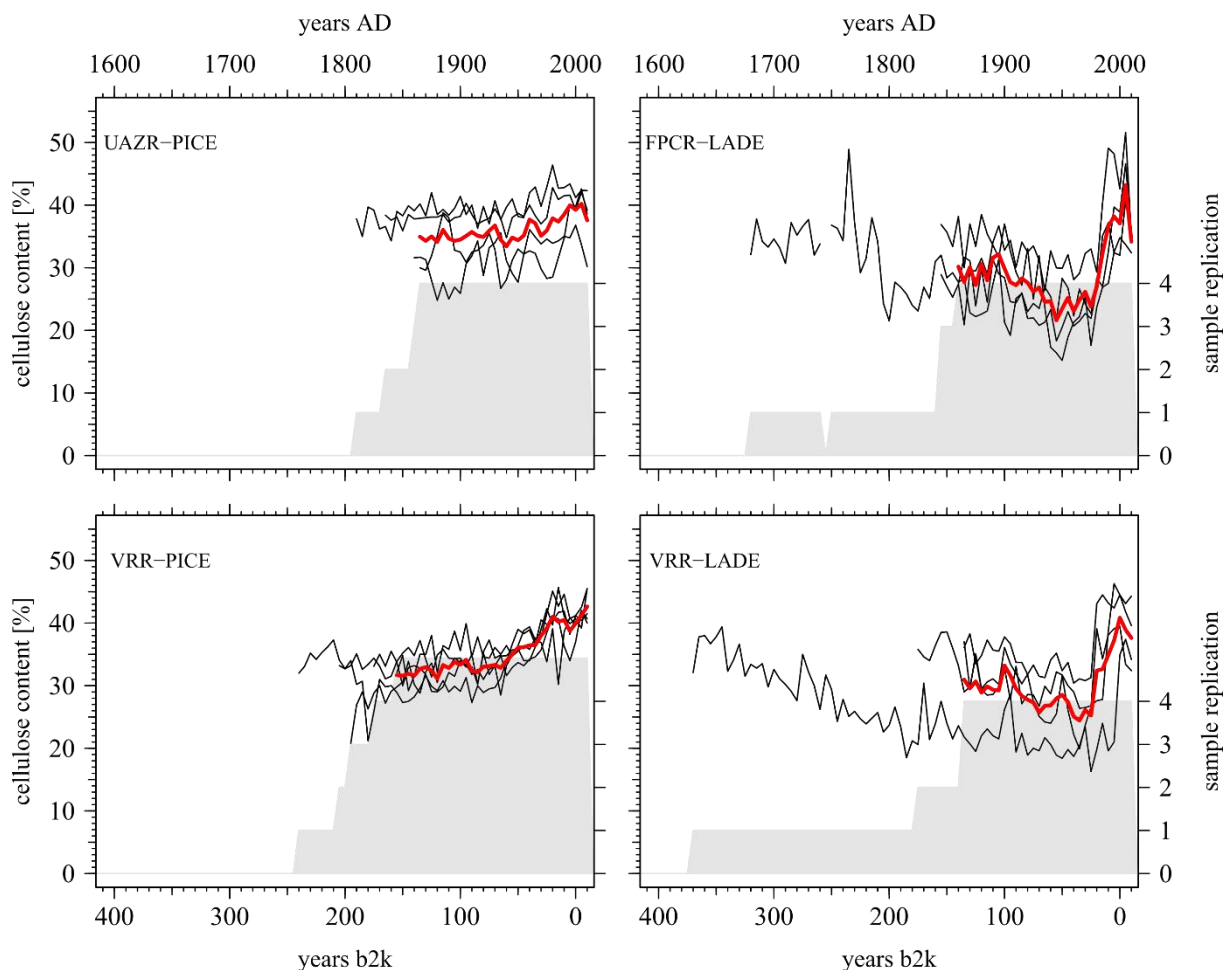
Fig. 3. Individual cellulose content series for two coniferous species (LADE, PICE) at the modern sites. The individual cellulose content time series (black lines) are supplemented by the mean cellulose content per species and site (red line), calculated as the arithmetic mean of the individual trees for the common growth period (sample replication ≥ 4). The sample replication (grey area) displays the temporal coverage per site.
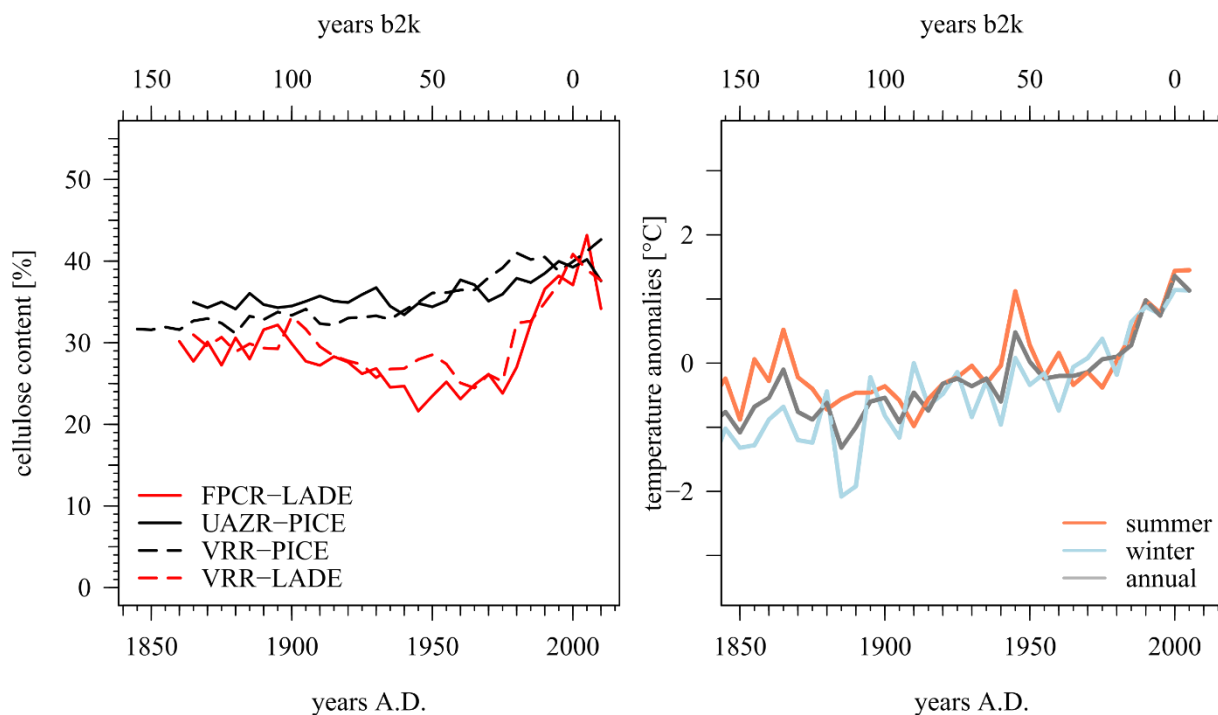
Fig. 4. Modern cellulose content chronologies per site and species (left) and temperature anomalies shown for the NW and SW subregions within the Greater Alpine Region from HISTALP for summer (April-September) and winter (October-March) half-year and per year (right). The CC [%] chronologies are calculated as arithmetic means from individual series for their common period (sample replication ≥ 4). HISTALP data is given for summer season (April-September), winter season (October-March) and annual anomalies calculated as 5-year averages with respect to the sample resolution of CC [%] series. Temperature anomalies for SW and NW subregion in the displayed season means are equal.

Temperature
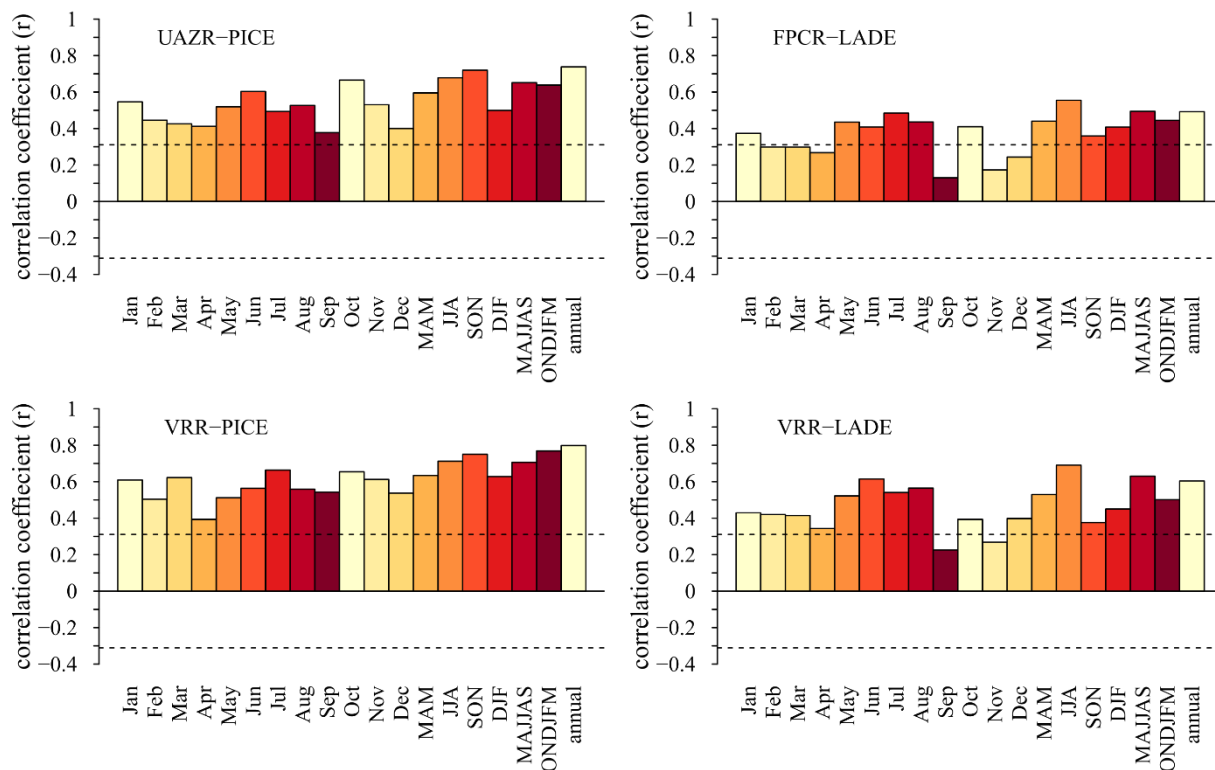
Fig. 5. Pearson's correlation coefficient for mean CC [%] and temperature anomalies (w.r.t. 1961-1990) for the period 1865 – 2005 A.D. UAZR and FPCR are correlated with HISTALP NW region data, whereas VRR species are correlated with the SW region dataset. The dashed horizontal lines indicate the level of significance ($p < 0.05$).

Precipitation
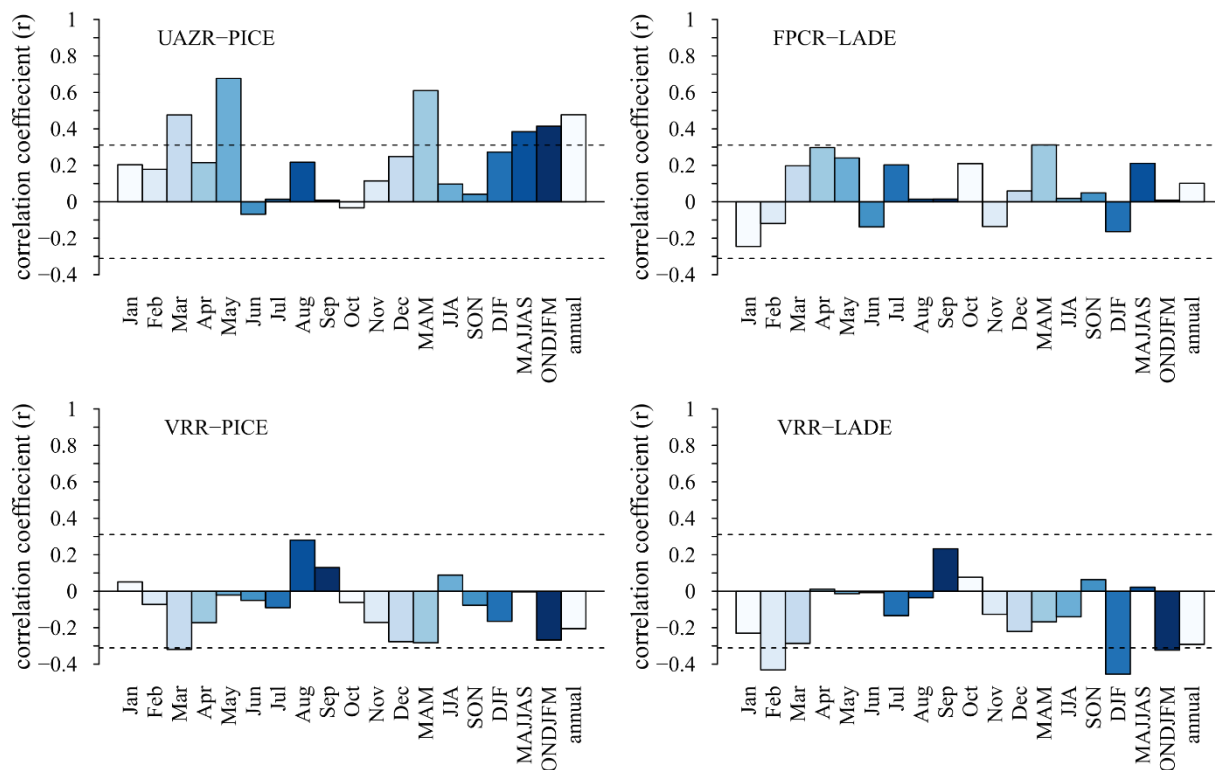
Fig. 6. Pearson's correlation coefficient for mean CC [%] and temperature anomalies (w.r.t. 1961-1990) for the period 1865 – 2005 A.D. UAZR and FPCR are correlated with HISTALP NW region data, whereas VRR species are correlated with the SW region dataset. The dashed horizontal lines indicate the level of significance ($p < 0.05$).
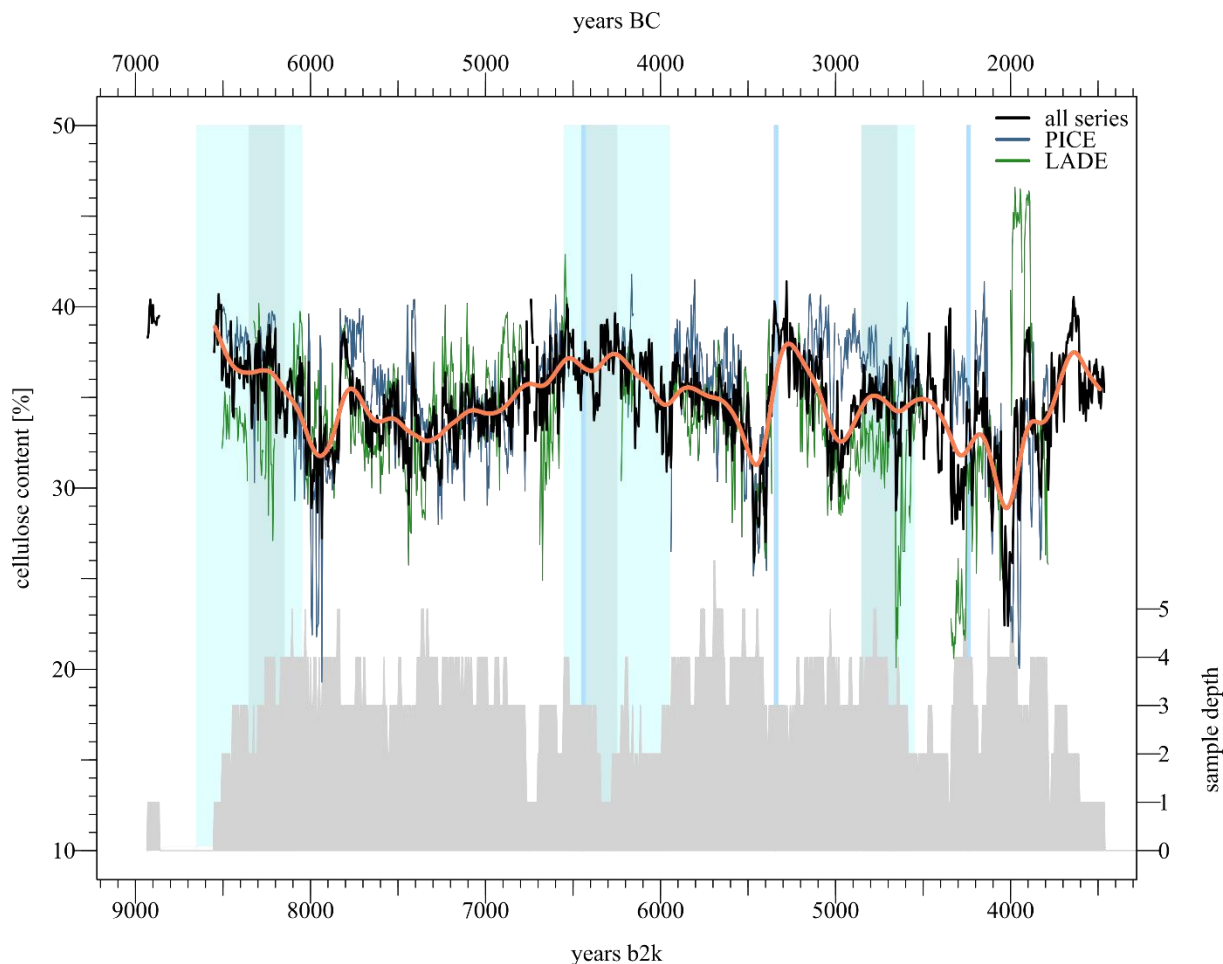
Fig. 7. Progression of mean cellulose content during the time period 9000 to 3500 years b2k. Mean cellulose content is calculated for the two species *Larix decidua* Mill. (LADE, green line) and *Pinus cembra* L. (PICE, grey line); the overall mean series is shown in black and its smoothed values in orange. The sample depth is on the right side (grey polygon). Major cold phases are indicated as cyan rectangles, where darker colors implicit the cold events and the lighter colors their entire duration (Wanner et al., 2011). Additional cold events are marked as blue vertical lines (Wanner et al., 2015)