Supplementary materials for

A millennium-long '*Blue Ring*' chronology from the Spanish Pyrenees reveals severe ephemeral summer cooling after volcanic eruptions

Running title: Blue Rings and volcanic eruptions

Alma Piermattei[1], Alan Crivellaro[1,2], Paul J. Krusic[1,3], Jan Esper[4], Petr Vítek[5], Clive Oppenheimer[1], Martin Felhofer[6], Notburga Gierlinger[6], Frederick Reinig[4], Otmar Urban[5], Anne Verstege[7], Hannah Lobo[1], Ulf Büntgen[1,5,7,8,*]

*[1]Department of Geography, University of Cambridge, United Kingdom. [2] "Stefan cel Mare" University of Suceava, Romania. [3]Department of Physical Geography, Stockholm University, Sweden. [4]Department of Geography, Johannes Gutenberg University, Mainz, Germany. [5]Global Change Research Institute of the Czech Academy of Sciences, Brno, Czech Republic. [6]Department of Nanobiotechnology (DNBT), University of Natural Resources and Life Sciences (BOKU), Vienna, Austria. [7]Swiss Federal Research Institute, WSL, Birmensdorf, Switzerland. [7]Department of Geography, Masaryk University, Brno, Czech Republic. *Corresponding author: ulf.buentgen@geog.cam.ac.uk*

Revised as an original research article to ***Environmental Research Letters***, 19th September 2020

Raman imaging analyses were performed on a wood blocks with a Renishaw InVia spectrometer (Renishaw, Wotton-under-Edge, UK) equipped with a confocal microscope (Leica, Wetzlar, Germany). A point-to-point imaging mode was applied using a 20x magnification objective with NA=0.40 was used (Leica, Wetzlar, Germany). The Raman scattering signal was collected by the same objective and detected by Peltier-cooled CCD. A diode laser line at 785 nm was employed with a 100 mW source power, 1 s exposure time, 1 accumulation at each point (50 mW power, 1s, and 3 accumulations for analysis of the extracted wood). The Raman signal was recorded in the 650–1750 cm^{-1} spectral range. Step-size of the piezo motorized scan stage XY movement was set to 6x6 μm. The imaging acquisition was provided using the Wire 3.4 software interface (Renishaw, Wotton-under-Edge, UK). The Raman image dataset processing was provided by the ImageLab software, version 2.93 (Epina, Retz, Austria). Spikes (due to cosmic rays) were detected and removed using the following parameters: spike half-width - 3; threshold - 1. Next, the spectra were smoothed out using the Savitzky-Golay polynomial function, window: 7. Then, the baseline was corrected using the Eilers algorithm using the following parameters: smoothness - 10000; asymmetry - 0.002; iterations - 7. The images were created as intensity or ratio of intensities at particular wavenumber positions.

Microtome thin-sections were prepared for this purpose. The block was tightly clamped in a rotary microtome (RM2235, Leica Biosystems Nussloch, Wetzlar, Germany) with an orientation perpendicular to the main fibre axis. Disposable microtome blades (N35HR Blade 35°, Feather, Osaka, Japan) were used to perform 10-20 μm thick transverse sections. During the cutting process, only D_2O was used to avoid drying of the specimen. The thin sections were placed on a standard glass slide with a drop of D_2O, covered with a glass coverslip (0.17 mm thick) and sealed with nail polish. The BRS were marked on the bottom of the slide and measured immediately or kept frozen until the analysis. Raman spectra were acquired with a Confocal Raman Microscope (alpha 300RA, WITec, Ulm, Germany) equipped with a piezo motorized scan stage (x-y-z). The excitation light source was a linear polarized (0°) coherent compass sapphire green laser at 532 nm (WITec, Ulm, Germany) focused through a coverslip-corrected 100x oil objective (NA 1.4, Carl Zeiss, Jena, Germany). The

Raman scattering signal was collected by the same objective, delivered by an optic multifibre (diameter = 50 μm) to the spectrometer (600 g mm^{-1} grating, UHTS 300 WITec) and finally recorded by a CCD camera (Andor DU401ABV, Belfast, UK). The orientation of the sample with respect to the laser polarization (the radial direction within the y-axis of the table) was kept constant during all measurements. All Raman scans were taken with a lateral resolution of 0.3 μm by acquiring at every pixel one spectrum with an integration time of 0.08 s and laser power of 35 mW. The control Four (WITec) acquisition software was used to set experimental parameters for hyperspectral image acquisition. Raman data analysis was performed with Project FOUR (WITec, Ulm, Germany) software. The extracted spectra were analysed with Opus 7.5 software TM (Bruker, Rheinstetten, Germany). Before the Raman images were generated based on integration of specific bands, a cosmic ray removal filter was applied. Based on the integrated images, average spectra of distinct areas of the samples (cell corner, cell wall, deposits) were obtained by drawing areas of interest or using an intensity threshold.

Figures S1–S7 and Table S1

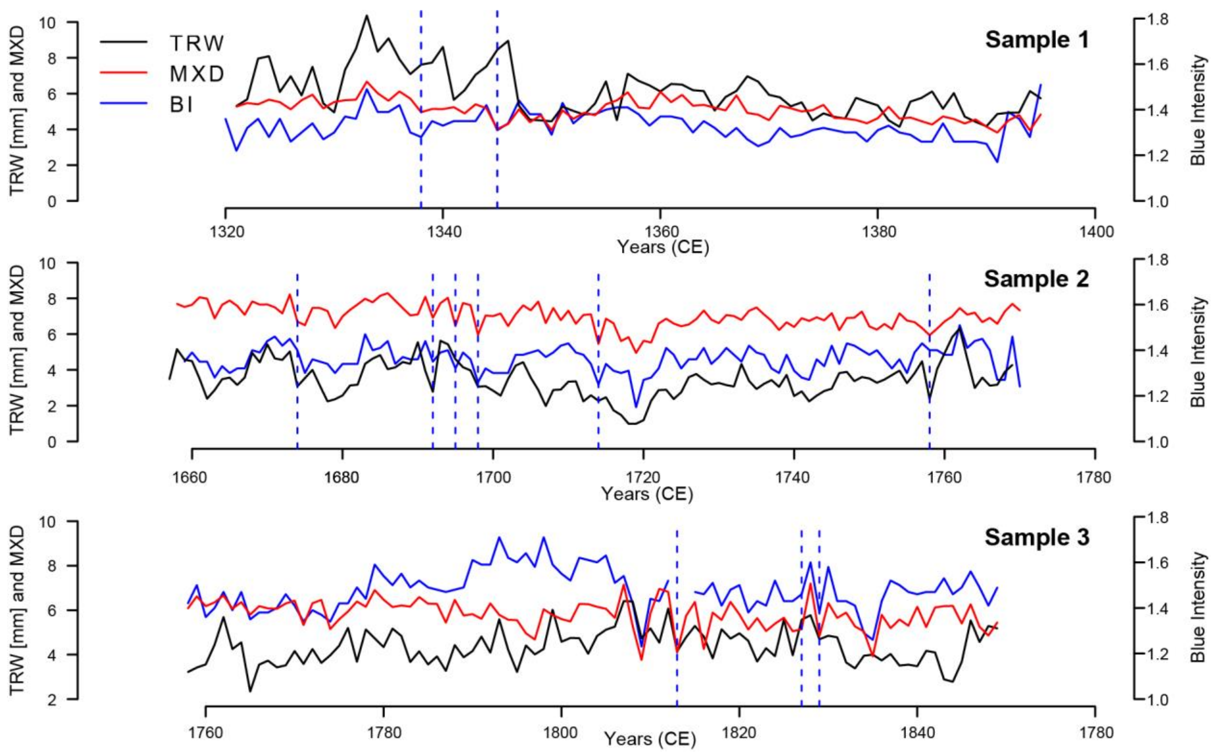

Figure S1. Tree-ring width and maximum latewood density chronologies of three relict wood samples. Trend of tree-ring width (TRW; mm; black line), maximum latewood density (MXD; g cm^{-3}; red line), and Blue Intensity (BI; blue line) of the three historical samples spanning from 1320–1850 CE. The vertical dashed blue line shows the occurrence of BRs.

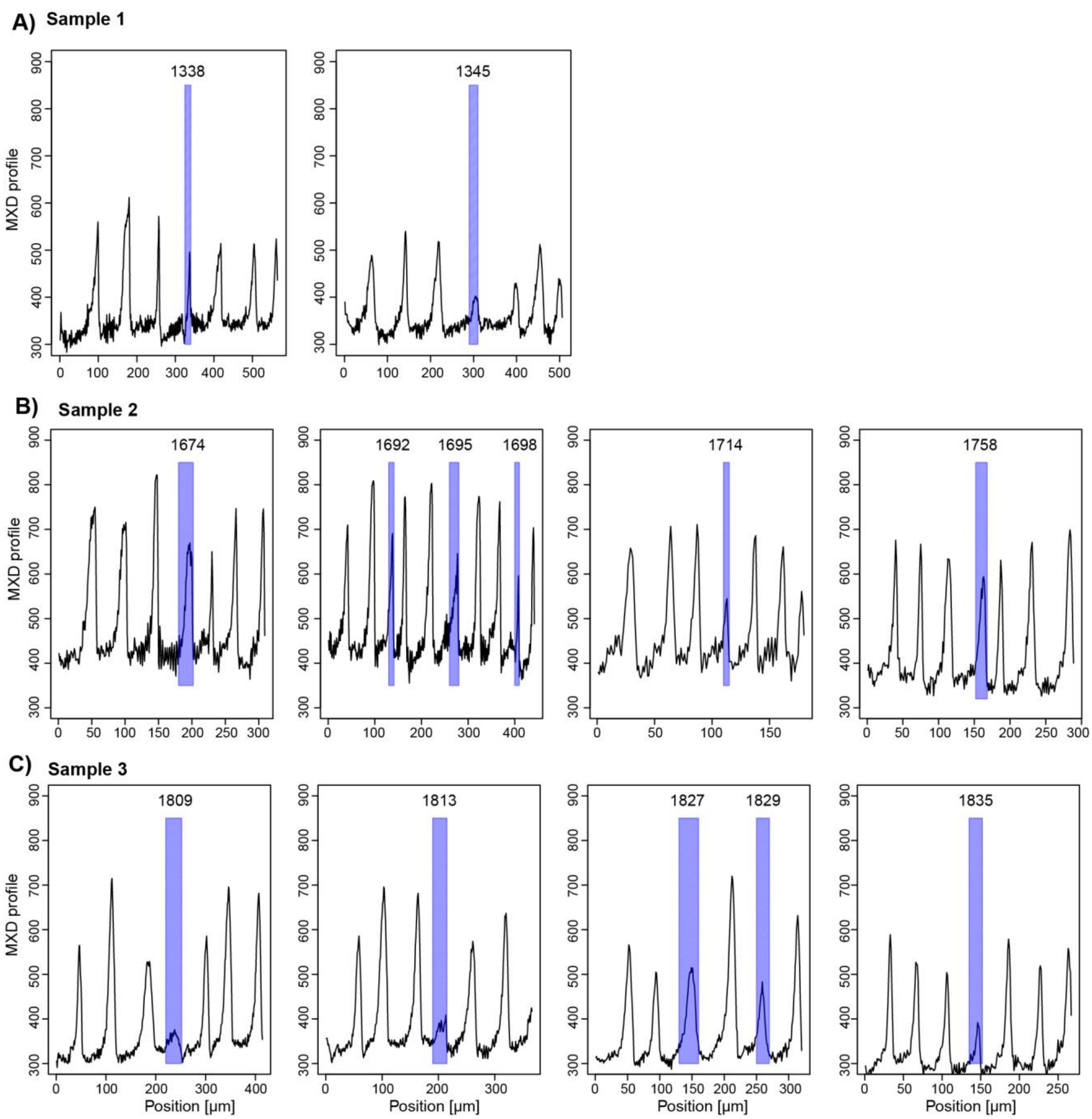

Figure S2. X-ray density profile of three relict wood samples. (**A-C**) X-Ray density profile (black line) of three years before and three years after BR occurrence from three relict samples spanning from 1320–1850 CE. The vertical blue rectangles show the latewood portion of the Blue Ring and the date of each Blue Ring is written in each rectangle.

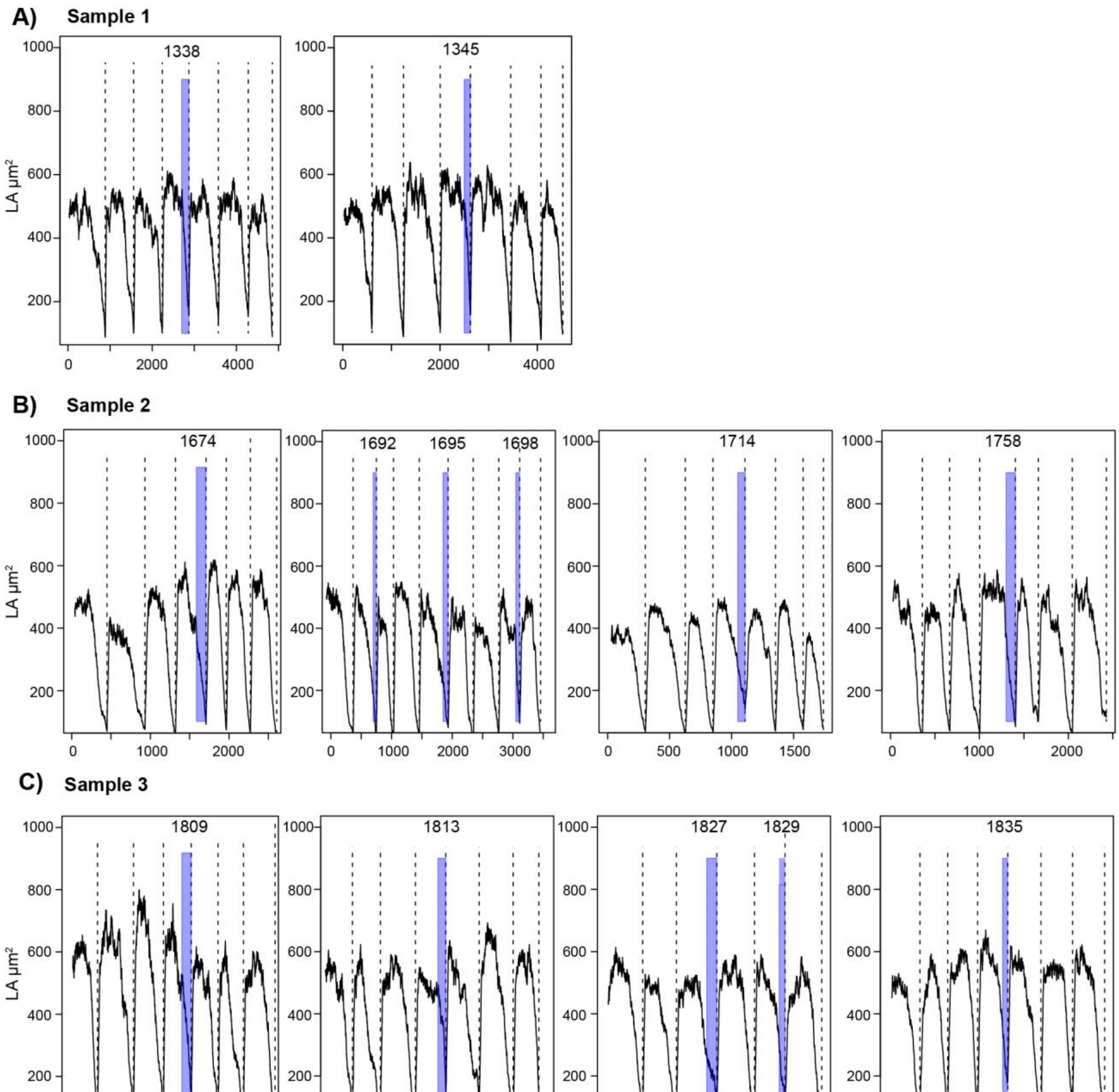

Figure S3. Lumen area profile of three relict wood samples. (**A-C**) Lumen area (LA) profile (black line) of three years before and three years after a Blue Ring occurrence from the three relict samples spanning from 1320–1850 CE. The vertical blue rectangles show the latewood portion of the Blue

Ring and the date of each Blue Ring is written in each rectangle. The vertical dashed lines represent the boundary of each ring.

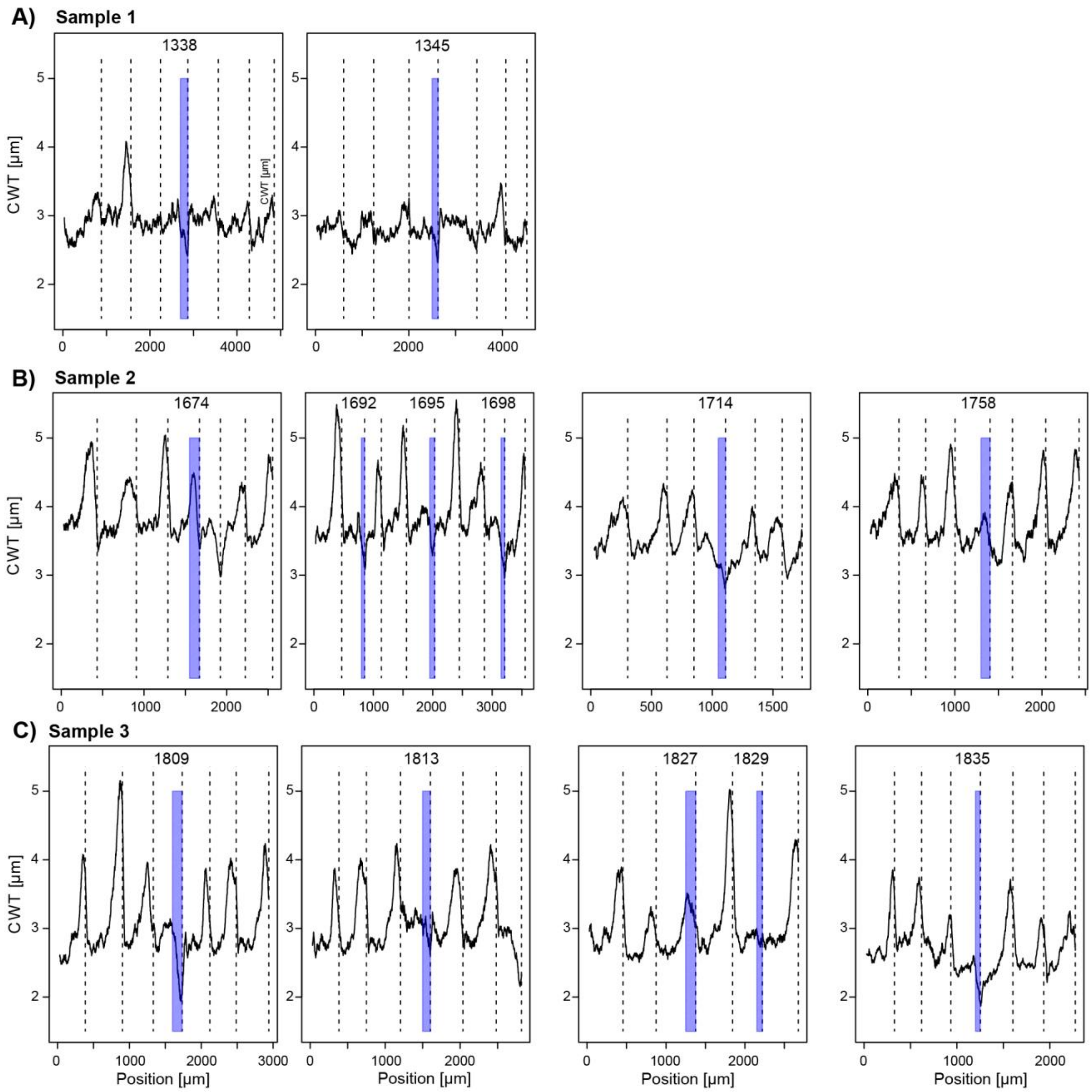

Figure S4. Cell Wall Thickness profile of three relict wood samples. (**A-C**) Cell Wall Thickness (CWT) profile (black line) of three years before and three years after a Blue Ring occurrence from the three relict samples spanning from 1320–1850 CE. The vertical blue rectangles show the latewood portion of the Blue Ring and the date of each Blue Ring is written in each rectangle. The vertical dashed lines represent the boundary of each ring.

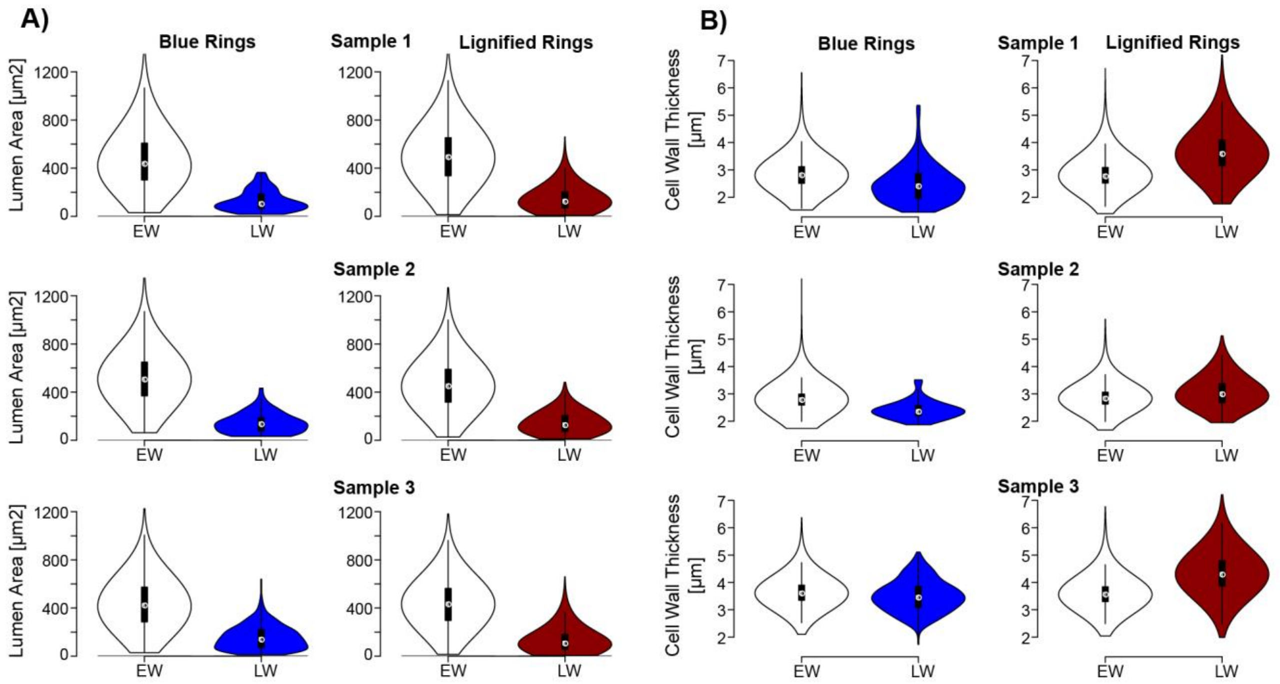

Figure S5. Lumen area and cell wall thickness of three relict wood samples. Violin plot of the lumen area (LA) (**A**) and cell wall thickness (CWT) (**B**) of earlywood and latewood of BRs (no fully lignified rings) and fully-lignified rings of the three relict samples spanning from 1320–1850 CE. Only the latewood violin plots are in blue and in red for Blue Rings and for fully lignified rings, respectively. The asterisk shows the statistical difference (p <0.001) between BRs and fully lignified ring in the latewood portion. Latewood CWT of Blue Rings in the three samples is statistically different to latewood CWT of fully lignified rings (p <0.001).

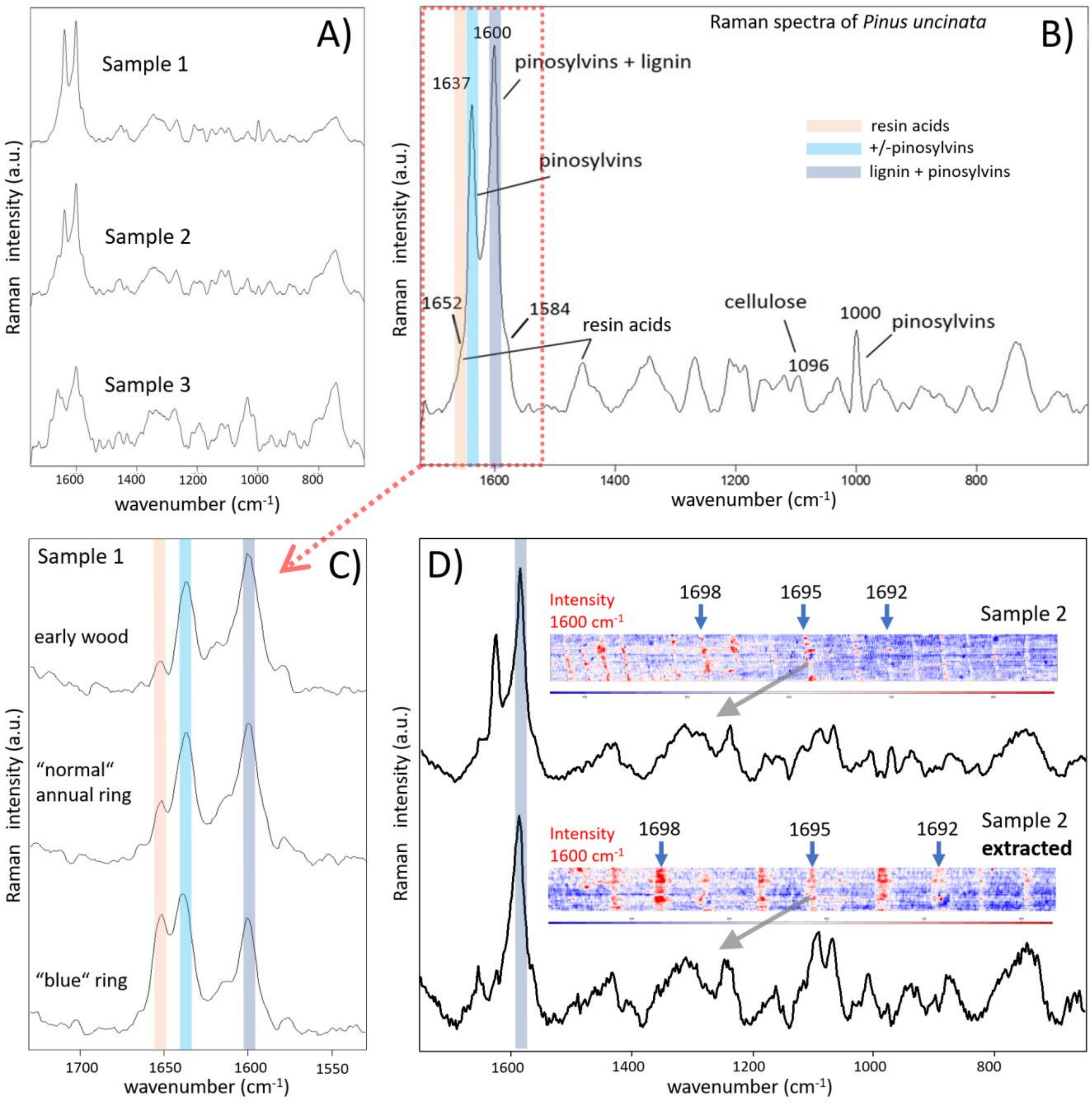

Figure S6. Raman spectroscopy and imaging of pine samples. (**A**) Averaged Raman signal from the three measured samples. (**B**) Description of the most important bands of the typical Raman record from *Pinus uncinata*. (**C**) Magnified region of phenolics showing differences between "normal" annual ring, Blue Ring and early wood obtained on Sample 1. (**D**) Raman images (Sample 2) representing signal intensity at 1600 cm^{-1}, where lignin and pinosylvin features occur. The bottom image represents a dataset obtained on the sample after extraction of extractive phenolics, with lignin and cellulose dominating the spectra. Examples of spectra are averaged from multiple points from annual ring 1695. These Raman data were obtained using 785 nm excitation.

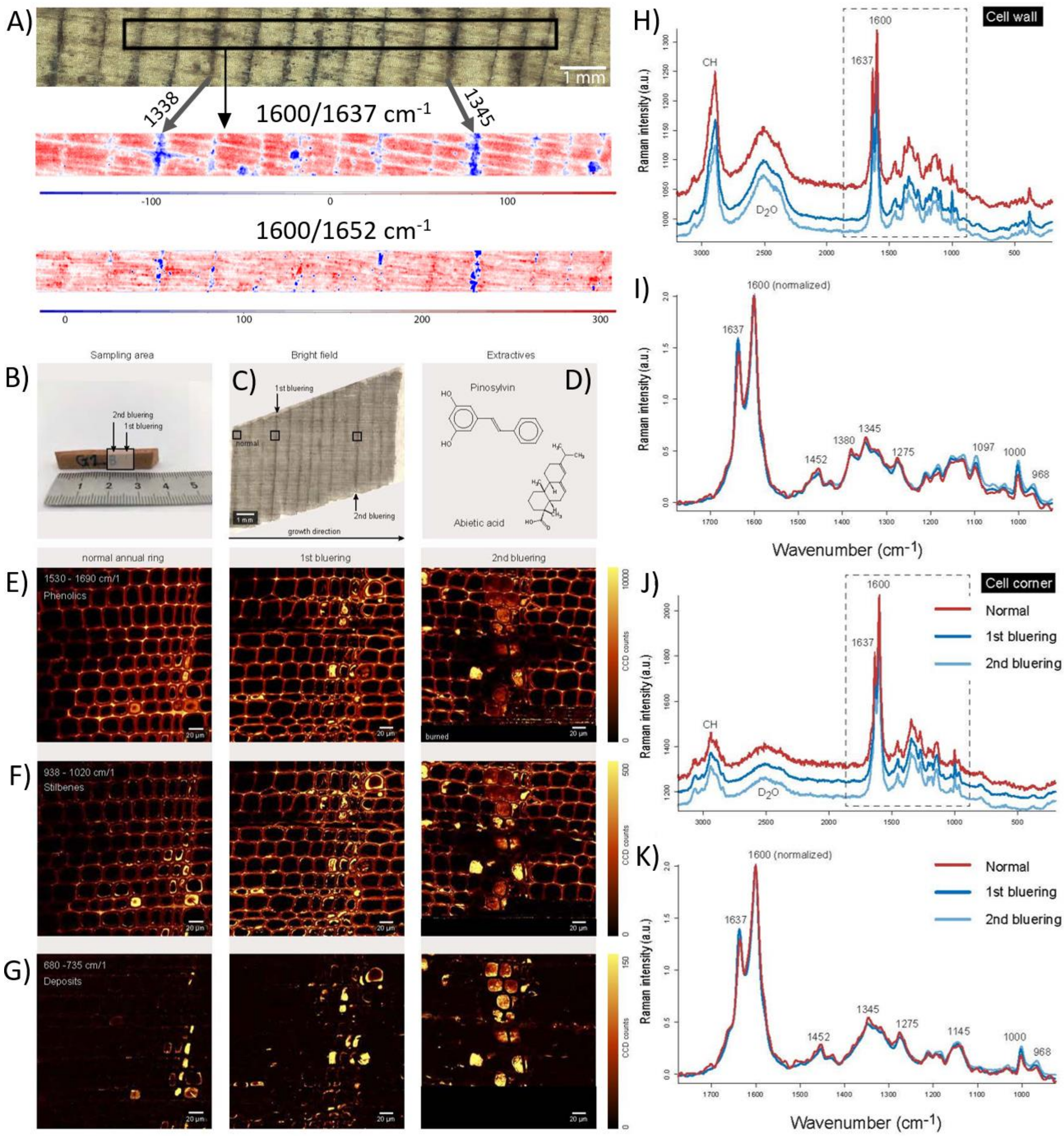

Figure S7. In-situ Raman imaging of selected Blue Rings. (**A**) Raman images obtained using 785 nm excitation depicting distribution of 1600/1637 cm^{-1} and 1600/1652 cm^{-1} Raman band ratios. Enhanced signal at 1637 and 1652 cm^{-1}, represented by blue colour is due to pinosylvins and resin acids, respectively. (**B**) Pine wood block with the sampling area (rectangle). (**C**) Bright field image of a transverse microsection including two Blue Rings and the measurement areas. (**D**) Chemical formulae of the two extractive compounds. (**E-G**) Raman images at cellular level obtained using 532 nm excitation (normal, BRs in years 1338 and 1345) based on bands for **E)** phenolic compounds (1530–1690 cm^{-1}), (**F**) stilbenes (938–1020 cm^{-1}) and (**G**) abietic acid (680–735 cm^{-1}). (**H-K**) Extracted spectra averaged from the zones of interest (from images **E-G**), including cell wall and cell corner; magnified spectral region is shown in (**K** and **I**).

Table S1. Blue Ring Inventory. Each row represents the occurrence year of ≥20% BRs and pBRs. For each BR and pBR the number of samples, TRW, MXD, and the reconstructed summer temperature (June–August) from 1186–2014 for Maximum Latewood Density expressed as temperature anomalies from the instrumental reference period 1961–1990 (Büntgen *et al* 2017) is listed in Wood Anatomy, and Dendro - Climate columns. The sum of the stratospheric aerosol optical depth (SAOD) for the North Hemisphere (NH) (Toohey and Sigl 2017), the name, location and estimated age of known volcanic eruption is listed in the volcanic forcing column. The value of SAOD represents the highest value in a window of ±3 years from the occurrence of a BR or pBR. BR and pBR years that coincide with high SAOD values are marked in bold

	Wood Anatomy					Dendro - Climate				Volcanic Forcing		
Year	**No. Sample**	**No. BRs**	**No. pBRs**	**% BRs (≥20%)**	**%pBRs (≥20%)**	**TRW (mm)**	**MXD (g/cm^3)**	**Temp. Anomalies**	**sum SAOD - NH (±3 years)**	**Volcanic Eruption**	**Location**	**Estimated Eruption Date**
1178	2	0	1	0	50	0.600	0.620	0.000	0.106 (1175)			
1180	2	0	1	0	50	0.530	0.570	0.000	3.018 (1182)			
1224	2	2	0	100	0	0.670	0.638	0.867	0.297 (1222)			
1233	2	1	0	50	0	0.750	0.618	-0.819	2.423 (1231)			
1258	**5**	**4**	**1**	**80**	**20**	**0.810**	**0.506**	**-4.394**	**5.722 (1258)**	**Samalas**	**Indonesia**	**1257**
1260	**5**	**0**	**1**	**0**	**20**	**0.620**	**0.601**	**-1.791**	**1.284 (1260)**			
1283	**6**	**2**	**3**	**33**	**50**	**1.270**	**0.530**	**-2.924**	**0.041 (1283)**			
1286	**7**	**1**	**2**	**14**	**29**	**1.460**	**0.645**	**0.661**	**1.159 (1286)**			
1288	7	3	4	43	57	0.970	0.490	-4.108	1.447 (1287)			
1290	8	4	0	50	0	0.810	0.562	-2.291	0.211 (1289)			
1298	**8**	**4**	**0**	**50**	**0**	**0.830**	**0.615**	**-1.089**	**0.039 (1298)**			
1305	8	0	2	0	25	0.980	0.591	-1.643	0.068 (1307)			
1331	11	0	3	0	27	1.020	0.664	0.906	1.131 (1329)			

1338	11	4	1	36	9	1.080	0.605	-1.138	0.386 (1341)			
1345	**11**	**2**	**7**	**18**	**64**	**0.970**	**0.561**	**-2.425**	**1.193 (1345)**			
1346	**11**	**2**	**3**	**18**	**27**	**1.000**	**0.581**	**-1.833**	**1.487 (1346)**			
1359	**12**	**0**	**4**	**0**	**33**	**1.070**	**0.565**	**-2.033**	**0.039 (1359)**			
1387	13	1	4	8	31	1.000	0.664	1.278	0.344 (1390)			
1394	13	3	1	23	8	0.870	0.641	0.062	0.052 (1393)			
1431	**14**	**0**	**3**	**0**	**21**	**0.780**	**0.602**	**-1.564**	**0.039 (1431)**			
1456	13	1	5	8	38	0.710	0.580	-1.690	1.092 (1454)			
1463	14	2	4	14	29	0.540	0.551	-2.338	1.048 (1460)			
1465	14	1	5	7	36	0.500	0.578	-1.576	0.260 (1463)			
1470	**15**	**0**	**3**	**0**	**20**	**0.600**	**0.624**	**-0.028**	**0.451 (1470)**			
1480	14	3	1	21	7	0.640	0.610	-0.554	1.455 (1477)			
1496	**13**	**5**	**1**	**38**	**8**	**0.600**	**0.539**	**-3.748**	**0.039 (1496)**			
1519	**12**	**2**	**3**	**17**	**25**	**0.730**	**0.635**	**0.408**	**0.039 (1519)**			
1544	12	4	2	33	17	0.550	0.564	-2.154	0.077 (1542)			
1574	**12**	**4**	**1**	**33**	**8**	**0.640**	**0.587**	**-0.964**	**0.040 (1574)**			
1576	12	3	3	25	25	0.600	0.553	-2.226	0.055 (1577)			
1587	12	5	1	42	8	0.600	0.528	-2.683	0.955 (1586)	Kelut	Indonesia	1586
1593	10	2	2	20	20	0.580	0.560	-1.881	0.061 (1591)			
1598	10	0	2	0	20	0.440	0.596	-0.708	0.899 (1596)			
1601	**10**	**1**	**3**	**10**	**30**	**0.420**	**0.544**	**-2.435**	**2.090 (1601)**	**Huaynaputina**	**Peru**	**19.2.1600**
1612	**10**	**3**	**2**	**30**	**20**	**0.520**	**0.577**	**-1.287**	**0.039 (1612)**			
1629	**11**	**1**	**6**	**9**	**55**	**0.540**	**0.554**	**-2.351**	**0.039 (1629)**			
1638	12	2	3	17	25	0.550	0.580	-1.720	0.424 (1637)			
1640	13	4	2	31	15	0.550	0.567	-2.021	1.938 (1641)			
1665	13	0	3	0	23	0.590	0.601	-0.938	0.968 (1668)			
1674	**14**	**4**	**3**	**29**	**21**	**0.590**	**0.588**	**-1.172**	**0.509 (1674)**			
1675	**14**	**6**	**4**	**43**	**29**	**0.460**	**0.533**	**-3.616**	**0.251 (1675)**			
1690	**14**	**3**	**4**	**21**	**29**	**0.530**	**0.584**	**-0.806**	**0.039 (1690)**			
1692	14	9	1	64	7	0.570	0.541	-2.448	0.276 (1694)			
1695	**14**	**3**	**3**	**21**	**21**	**0.530**	**0.564**	**-1.634**	**1.287 (1695)**			
1698	13	11	0	85	0	0.420	0.509	-3.894	1.516 (1696)			
1714	**13**	**8**	**1**	**62**	**8**	**0.540**	**0.495**	**-4.006**	**0.039 (1714)**			
1757	13	0	3	0	23	0.670	0.589	-1.147	0.378 (1756)			
1758	13	3	2	23	15	0.630	0.584	-1.969	0.163 (1757)			
1787	13	1	3	8	23	0.710	0.585	-1.920	4.282 (1784)			
1789	13	0	4	0	31	0.690	0.593	-1.619	0.683 (1787)			
1808	13	0	4	0	31	0.710	0.645	1.142	1.489 (1809)			

1809	13	4	5	31	38	0.620	0.570	-2.250	1.862 (1810)			
1813	13	4	1	31	8	0.600	0.591	-1.199	0.258 (1812)			
1816	**13**	**0**	**4**	**0**	**31**	**0.550**	**0.562**	**-2.510**	**3.019 (1816)**	**Tambora**	**Indonesia**	**10.4.1815**
1829	12	6	3	50	25	0.740	0.582	-1.421	1.155 (1831)			
1835	12	4	4	33	33	0.760	0.545	-2.916	0.998 (1836)	Cosigüina	Nicaragua	20.1.1835
1884	**10**	**1**	**2**	**10**	**20**	**0.930**	**0.621**	**0.009**	**1.343 (1884)**	**Krakatau**	**Indonesia**	**26.8.1883**
1885	**10**	**2**	**1**	**20**	**10**	**0.980**	**0.645**	**1.140**	**0.689 (1885)**			
1894	10	2	0	20	0	0.860	0.604	-0.713	0.786 (1891)			
1896	**10**	**0**	**4**	**0**	**40**	**0.780**	**0.575**	**-1.831**	**0.048 (1896)**			
1903	**10**	**0**	**2**	**0**	**20**	**0.860**	**0.640**	**0.647**	**1.292 (1903)**			
1905	9	0	2	0	22	0.960	0.627	0.277	0.621 (1904)			
1910	9	1	2	11	22	0.820	0.557	-2.357	1.239 (1912)			
1932	8	0	3	0	38	0.920	0.606	-0.409	0.199 (1929)			
1939	**8**	**2**	**1**	**25**	**13**	**0.920**	**0.578**	**-1.750**	**0.057 (1939)**			
1944	**8**	**2**	**0**	**25**	**0**	**0.860**	**0.650**	**0.884**	**0.059 (1944)**			
1974	6	2	0	33	0	0.850	0.605	-0.141	0.291 (1975)			
1993	5	1	0	20	0	0.900	0.664	2.048	1.237 (1992)			
1996	5	0	1	0	20	0.780	0.613	-0.035	0.226 (1994)			

135