

3 **Testing annual tree-ring chemistry by X-ray fluorescence for**
4 **dendroclimatic studies in high-elevation forests from the Spanish**
5 **Pyrenees**

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32 **Abstract**

33 Climate reconstructions based on tree-ring features rely on the assumption that growth
34 drivers are mainly meteorological variables. Consequently, annually resolved and
35 absolutely dated temperature reconstructions in high-elevation conifer forests are
36 mainly based on tree-ring width (TRW) and maximum wood density (MXD). However,
37 long-term and annually resolved chronologies of chemical elements are scarce despite
38 they could complement TRW- and MXD-based reconstructions. Here, we used a novel
39 non-destructive method by applying Micro X-ray fluorescence (μXRF) to wood samples
40 of old mountain pine (*Pinus uncinata*) trees from two Pyrenean high-elevation forests.
41 We tested if Calcium (Ca) relative concentration, as estimated through μXRF , is a
42 valuable proxy of long-term climatic and environmental changes in two sites with basic
43 and acid soils, respectively. We compared the climate sensitivity of TRW, MXD and Ca
44 by using calculating correlations with monthly climate data (mean temperature and total
45 precipitation). Then, we built linear regressions to predict temperatures for the period
46 1900-2009. Prior-autumn and spring temperatures were positively related to TRW,
47 MXD and Ca. However, Ca series revealed a high sensitivity to temperatures during the
48 prior winter and spring but also to summer precipitation. We confirm the potential of
49 long-term wood-chemistry studies based on the μXRF non-destructive technique to
50 reconstruct environmental changes.

51

52 **Keywords** dendrochemistry; *Pinus uncinata*; dendroclimatology; Itrax; tree-rings;
53 paleoclimate.

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81 **1. Introduction**

82 Climate warming is expected to enhance forest productivity and tree growth,
83 particularly in cold-limited environments such as high-elevation areas (Körner, 2012;
84 Camarero et al., 2017). In those mountain forests, the observed rise in temperatures has
85 been shown to increase productivity by augmenting radial-growth rates and maximum
86 wood density as have illustrated several tree-ring studies (e.g., Tardif et al., 2003; Salzer
87 et al., 2009; Büntgen et al., 2017). However, warmer conditions leading to a longer
88 growing season and thus enhancing radial-growth rates could also accelerate the
89 nutrient use by trees (e.g., Silva et al., 2016). Such enhanced nutrient-use efficiency will
90 depend on the trees' capacity to uptake soil nutrients under warmer conditions but also
91 on the soil chemical features since acid and nutrient-poor soils will be less prone to
92 show this enhancement than basic and nutrient-rich soils (Augustin et al., 2005).
93 Dendrochemistry, i.e., the analysis of chemical features of annually resolved tree-rings,
94 can help to solve this question because it allows analyzing the coupling between
95 climatic variables and nutrient proxies as element concentrations in stem wood (Smith
96 et al., 2014; Witt et al., 2017).

97 Since many essential nutrients as Calcium (Ca) play a relevant role in tree
98 functioning (water uptake, stomatal conductance rates, cell division, cell-wall synthesis)
99 and contribute to the structural chemistry and function of wood (Lautner and Fromm,
100 2010), novel approaches to quantify long-term changes in nutrients fixed in wood and
101 its relation to climate are needed (Gessler et al., 2017). Calcium is a critical element
102 leading to cascade effects on tree-soil interactions (e.g., Panyushkina et al., 2016), since
103 it is involved in the ion exchange processes through cell walls (Peterson and Anderson,
104 1990). Furthermore, some authors have argued that increasing Ca concentrations in the

105 heartwood and towards the tree pith are indicative of: (i) low element mobility
106 (Helmisaari and Siltala, 1989), (ii) limited translocation in tree-rings (Hanger, 2008),
107 and (iii) lower availability of Ca in the soil over time (Andrews and Siccama, 1995).
108 These three characteristics make Ca a valuable proxy for dendrochemical and
109 dendroclimatic analyses.

110 In addition, Ca concentrations in tree-ring wood could differ from the classical
111 dendrochronological variables (e.g., tree-ring width and density) as the former may
112 reflect physical conditions and tree responses to soil Ca availability rather than
113 reflecting ontogenetic changes in tree growth. Therefore, Ca concentrations may
114 provide complementary climatic information in areas where the relationships between
115 climate variables and classical tree-ring features as width (TRW) or maximum density
116 (MXD) are complex, unstable or weak (e.g., Galván et al., 2015; Büntgen et al., 2017).

117 Studies focused on the interaction between temperature and long-term plant
118 nutrition are strikingly scarce (but see Penninckx et al., 2001; Meerts, 2002), and little is
119 known about the multi-centennial changes in wood chemical elements of old trees (but
120 see Pearson et al., 2006, 2009; Vaganov et al., 2013). Moreover, determining the nature
121 of the responses of nutrient cycling to climate changes is essential to forecast the
122 impacts of rising temperatures on soil-nutrient availability, and hence their potential
123 influences on forest functioning. For instance, the increase in temperature and air CO₂
124 concentration could not stimulate tree growth and forest productivity if essential
125 nutrients (N, P, K, Ca) lack or are not readily available (Yuan et al., 2017). Thus, the
126 comparison of chemical element profiles from several individuals under similar soil
127 conditions allows evaluating if tree-ring variables reflect changes in nutrient availability
128 and climate (Hevia et al., 2018).

129 Since several factors control tree-ring chemical elemental variability under most
130 conditions, reconstructions of a single climate variable based on TRW or MXD series
131 are prone to oversimplification (McCarroll et al., 2003; Neukon et al., 2010); thus, using
132 inter-annual variation of nutrients' concentrations may provide further consistency to
133 paleoclimate reconstructions (Reynolds-Henne et al., 2007). European temperature
134 reconstructions covering the last few centuries are mainly based on TRW and MXD
135 data from northern Europe sites (Briffa et al., 2002; Esper et al., 2014). In southern
136 Europe, the longest temperature reconstructions come from mountain regions and are
137 based on MXD measurements from high-elevation forests such as those from the
138 Spanish Pyrenees (Büntgen et al. 2008, 2010, 2017; Dorado-Liñán et al., 2012; Galván
139 et al., 2015). In this range, the variability of climate and soils makes it important to test
140 new dendrochemical proxies since the Pyrenees lie in the transition between cold-
141 temperate conditions northwards and drought-prone Mediterranean conditions
142 southwards (Ceballos and Ruiz de la Torre, 1979). In fact, recent dendrochemical
143 studies in this area have demonstrated a strong coherence between wood Ca content and
144 growing-season temperatures as the primary driving factor (see Hevia et al., 2018). In
145 this sense, disentangling the role of the main controls of chemical elements and their
146 associated climate variables is highly desirable to obtain reliable climate reconstructions
147 based on dendrochemical proxies.

148 In this study, we evaluated the potential of annual tree-ring Ca concentration in
149 wood measured by using micro X-ray fluorescence (μXRF) non-destructive techniques
150 (Smith et al., 2008, 2014). We tested if Ca is a reliable proxy to improve dendroclimatic
151 reconstructions in two mountain pine (*Pinus uncinata*) forests with different soil
152 properties (basic vs. acid soils), and thus contrasting soil Ca availability, located in the
153 Spanish Pyrenees. Using long-term and multi-proxy tree-ring series (TRW, MXD and

154 Ca relative concentration) we carry out a detailed sensitivity study of these proxies as
155 related to regional climate. Our objectives are: (1) to provide new insights regarding the
156 climate signal contained in Ca series depending on climate and soil properties, (2) to
157 compare which tree-ring variable (TRW, MXD or Ca) is most sensitive to climate, and
158 (3) to present the potential element-based temperature reconstruction for the Spanish
159 Pyrenees, a well-studied area for developing long-term dendroclimatic reconstructions
160 (e.g. Büntgen et al., 2008).

161

162 **2. Material and Methods**

163 *2.1. Study tree species and field sites*

164 The mountain pine (*Pinus uncinata* Ram.) is a long-lived, slow-growing and shade-
165 intolerant conifer which exhibits large ecological amplitudes regarding topography
166 (slope, exposure, altitude) and soil type (Ceballos and Ruiz de la Torre, 1979; Bosch et
167 al., 1992). This species dominates high-elevation forests in the Pyrenees, and form the
168 alpine treeline (Camarero and Gutiérrez, 1999). In this species, warm previous autumn
169 and current spring enhance radial growth (Tardif et al., 2003). Based on studies of
170 xylogenesis, it has been shown that cambial resumption starts in May and ca. 80% of
171 the annual tree-ring is formed in July (Camarero et al., 1998).

172 We sampled two high-elevation *P. uncinata* forests located in protected areas of
173 the Spanish Pyrenees that have not been disturbed by logging since the establishment of
174 the conservation policies at the beginning of the 20th century (Table 1): ‘Ordesa y
175 Monte Perdido’ National Park (hereafter, Ordesa site) and ‘Aigüestortes i Estany de
176 Sant Maurici’ National Park area (hereafter Aigüestortes site) (see Galván et al., 2015
177 for further details). In Ordesa, the soil of the study site is basic, whereas soil in
178 Aigüestortes is acid (Table 1). Mean annual temperature in the studied sites range from

179 2.0 to 4.9 °C, respectively (Table 1), with the coldest and warmest months being
180 January (mean -2.0 °C) and July (12.5 °C), respectively (see Camarero, 1999) (Figure 1
181 and Table 1).

182

183 *2.2. Field sampling and dendrochronological methods*

184 A total of 10 and 37 old trees (ages over 400 years) were sampled between 2008 and
185 2009 in Ordesa and Aigüestortes sites, respectively, of which 10 and 17 trees were used
186 for wood μXRF measurements. Among them, five trees per site (those with the longest
187 time spans, 412–731 years; see Table 2) were selected for dendrochemistry analyses
188 (see below). Topographic (altitude, slope, aspect) and biometric variables (DBH:
189 diameter at breast height –measured at 1.3 m-; tree height) were recorded for each tree
190 (Table 1).

191 Two to three cores per tree were collected at 1.3 m height with a Pressler
192 increment borer (5-mm diameter) along the perpendicular direction to maximum slope.
193 Then, the cores were mounted and sanded with sandpapers of progressively fine grain
194 until tree rings were clearly visible to obtain growth data (Stokes and Smiley, 1968).
195 These samples were visually cross-dated and measured to a precision of 0.01 mm using
196 a LINTAB measuring device (Rinntech, Heidelberg, Germany). Cross-dating and TRW
197 measurements were evaluated using COFECHA, which calculates cross-correlations
198 between individual series of each core and a master (mean) site chronology obtained
199 averaging all measured series in each site (Holmes, 1983). In the cases of cores without
200 pith, the distance to the pith was estimated by fitting a template of concentric circles
201 with known radii to the curve of the innermost rings (Norton et al., 1987). This allowed
202 the estimation of the missing radius length to transform it into the number of missing
203 rings. In those trees in which the central core section could not be estimated because the

204 innermost rings did not curve, we used the DBH of each tree to estimate the tree radius
205 without bark (see Camarero et al., 2015).

206

207 *2.3. Wood density and chemistry*

208 The trees selected for density and chemistry analysis fulfilled several conditions: (i)
209 they corresponded to the oldest trees with the highest correlation with the mean TRW
210 site chronology, (ii) they showed a low frequency of missing rings and contained no
211 TRW sequences with widths narrower than 0.1 mm to ensure enough material to resolve
212 chemical compounds at annual resolution, and (iii) they did not present significant
213 growth changes (suppressions and releases) or reaction wood (Granda et al., 2017;
214 Hevia et al., 2018).

215 Two 12-mm diameter cores from each selected tree were used for chemistry and
216 after resin extraction for density measurements (Table 2) (Galván et al., 2012, 2014,
217 2015). The cores were air-dried and mounted (Balouet and Chalot, 2015). Then, thin
218 laths (1.0 ± 0.02 mm thick) were cut transversely using a high-precision twin-bladed
219 saw (AFUME, France). Wooden laths were kept under constant temperature and
220 humidity before and while being X-rayed in an Itrax Multiscanner (Cox Analytical
221 Systems, Sweden) at the laboratory of CETEMAS (Siero, Asturias, Spain). The Itrax
222 was operated at 30 kV and 30 mA with a Cr-tube, and samples were exposed to the X-
223 ray beam for 10 s at each measurement point in radial direction (50- μ m step size).
224 Simultaneously, count rates of fluorescent photons of elements (for chemical
225 characterization) and a radiographic greyscale image (for wood density) in each
226 measurement point of each sample were obtained. Peaks in the micro μ XRF spectrum
227 were assigned to specific elements using the Q-spec software (Cox Analytical systems,
228 Sweden), producing relative concentrations (count rates of fluorescent photons) (e.g.,

229 Croudace et al., 2006) of those elements detected and pre-defined within the wood
230 structure for each analysed point.

231 The radiographic images were cross-dated with previously built TRW mean site
232 series or chronologies (see Galván et al., 2012), and then used to assign the element
233 specific count rates of every measurement point to annual rings. This process enabled a
234 comparison of the different samples by changing the ordination from a spatial (position
235 on the sample) to a common temporal (year of wood formation) scale. For this purpose,
236 tree-ring boundaries were defined on each radiographic image using WinDendro™
237 (Regent Instruments, Canada) and the resulting pixel-wise data output was used to relate
238 boundaries with the elemental count rates (e.g., Croudace et al., 2006). In addition,
239 MXD data were obtained from the radiographic images by calibrating the greyscale
240 intensities to wood densities using a light calibration curve derived from a calibration
241 wedge (Grudd, 2008). Average annual time series of relative concentrations of Ca (i.e.,
242 the count rates for the whole tree-ring; cf. Smith et al., 2014), were also obtained for
243 each annual tree-ring as it is considered an important nutrient in cold environments
244 (Lautner and Fromm, 2010).

245

246 *2.3. Chronology building*

247 To emphasize the climatic signal and to maximize the relationship between climatic
248 variables and tree-ring variables we built chronologies or mean series of indexed data
249 (Fritts, 2001). First, we applied detrending methods using the ARSTAN v.44 program
250 (Cook and Holmes, 1986) to remove tree-age and size related, non-climatic growth
251 trends from the raw TRW, MXD and Ca measurement series (Cook and Kairiukstis,
252 1990). Second, we preserved variability at inter-annual to centennial-scales detrending
253 each TRW, MXD and Ca individual series by fitting a negative exponential function

254 followed by a 150-year long spline to retain high-frequency variability. Detrending
255 allowed transforming tree-ring individual series to dimensionless growth indexes by
256 dividing observed ring values by fitted values. The resulting series were pre-whitened
257 by autoregressive models to remove temporal autocorrelation. We performed a variance
258 stabilization technique to every single series for minimizing the effects of changing
259 sample size throughout time (Frank et al., 2007). Mean site chronologies were then
260 calculated using a bi-weight robust mean (Cook, 1985). Finally, we presented mean site
261 residual chronologies without first-order autocorrelation.

262 The statistical quality of the chronologies was assessed by means of the
263 following parameters calculated over the period 1900–2009: mean and standard
264 deviation (SD); AC, first-order autocorrelation, a measure of the year-to-year growth
265 similarity; MS, mean sensitivity, a measure of the year-to year variability in width or
266 other variables over consecutive tree rings; rbt, mean correlation between trees, a
267 measure of the similarity in growth among trees; EPS, the Expressed Population Signal,
268 a measure of the statistical quality of the mean site chronology as compared with a
269 perfect infinitely replicated chronology; and PC1, the percent of variance accounted by
270 the first principal component which accounts for the common variability in growth
271 among all trees at each site. The chronology segment with $EPS > 0.85$ was considered
272 as the common interval that was statistically reliable (Wigley et al., 1984).

273

274 *2.3. Relationships between tree-ring variables and climate*

275 We used monthly temperature (maximum and minimum) and precipitation data (CRU
276 TS 4.01; Harris and Jones, 2017) for analysing the responses of tree-ring variables to
277 climate. We considered 0.5° resolution grid-box data covering the sampled sites
278 (Ordesa, 0.5°-0.0° W, 42.5°-43.0° N grid; Aigüestortes, 0.5°-1.0° E, 42.5°-43.0° N grid).

279 The detrended TRW, MXD and Ca chronologies were correlated with monthly and
280 seasonal means of maximum and minimum temperatures and total precipitation.
281 Climate data and chronologies indices were related by calculating bootstrapped Pearson
282 correlation coefficients for the common period 1950-2009 which is the most reliable
283 timespan for this region (Brunet et al., 2006). These correlations were calculated from
284 previous October to current September, i.e., prior and during the year of tree-ring
285 formation, based on previous analyses (Galván et al., 2015). To assess whether these
286 relationships were stable through time, we calculated 30-year long moving correlations
287 lagged by one year, taking into consideration only those climate variables strongly and
288 significantly ($P < 0.01$) correlated with TRW, MXD or Ca series. Bootstrapped and
289 moving correlations were calculated using the software *treeclim* (Zang and Biondi,
290 2015). On the other hand, we quantified spatial correlation fields between selected tree-
291 ring series and monthly climate variables using the web-based Climate Explorer
292 (<http://climexp.knmi.nl>).

293

294 *2.4. Testing climate reconstructions*

295 Correlation analyses identified those monthly climatic factors most strongly and
296 significantly ($P < 0.01$) related to TRW, MXD and Ca indexed series. Then, we built a
297 linear regression (transfer-function model) based on those climatic variables which
298 showed strong and temporally stable associations with the Ca regional chronologies.
299 Such transfer-function analysis is a linear regression model in which the tree-ring
300 indexed variables (TRW, MXD or Ca) are the predictors and the temperatures are the
301 predictands. This model was evaluated using regression statistics such as R^2 (Meko
302 1997). Then, the skill of Ca proxy for reconstructing past climate was tested by
303 performing a split-sample procedure for the most significant temperature correlation and

304 was also used to verify model stability (Touchan et al., 2008). This procedure divides
305 the full period into two sub-periods (1901–1950 and 1950–2009), which were used as
306 calibration and verification periods, respectively. The reduction of error statistic (RE),
307 the coefficient of efficiency (CE), and the Durbin–Watson test (DW) were calculated to
308 test for skill beyond that possible simply by using the calibration period mean of
309 observed temperature as the reconstruction (Biondi et al., 2008). The value of RE
310 theoretically ranges from minus infinity to +1.0, and an RE value greater than 0 is
311 considered positive skill of the model (Fritts, 2001). The calculated transfer function
312 was then applied to the site chronology to obtain the time series of reconstructed
313 seasonal temperature. All stages of chronology building and data analyses were
314 performed using the package ‘*dplR*’ (Bunn et al., 2018) in ‘R’ (R Development Core
315 Team, 2018).

316

317 **3. Results**

318 Trees from the Ordesa site formed wider rings (0.72 mm) and were younger (464 years)
319 but taller (9.9 m) than those from Aigüestortes site (0.59 mm, 565 years, 7.9 m) (Tables
320 1 and 2). The year-to-year tree-ring width persistence (AC) and inter-annual variability
321 (MS) of growth were similar at these two sites which suggest that climatic constraints
322 on growth are important for these high-elevation forests (Table 2). Ca chronologies
323 presented similar or lower series inter-correlation and mean sensitivity compared to
324 MXD and TRW, respectively, albeit these comparisons must be made with caution
325 since the number of trees uses for each variable differed. The remaining
326 dendrochronological statistics confirm the coherence among TRW, MXD and Ca series
327 (Table 2, Figure 2). TRW, MXD and Ca chronologies showed a very similar shape but
328 TRW presented a higher year-to-year variability (Figure 2). MXD was more strongly

329 correlated with TRW than with Ca series in both study sites. Indeed, the indexed TRW
330 and Ca series were positively associated in the Ordesa site but not in the Aigüestortes
331 site (Figure 3).

332 Mean maximum (TMx) and minimum (TMn) temperatures displayed the higher
333 positive correlations with TRW, MXD and Ca. TRW and MXD were positively
334 influenced by previous-year November and current May and September temperatures in
335 both study sites (Figure 4). Mean temperatures showed positive correlations with Ca
336 during late winter to early spring, mainly in March (TMx, Ordesa) and February (TMn,
337 Aigüestortes), and also in August (TMx) in Ordesa (Figure 4). Aigüestortes site showed
338 the highest correlations with TMn in the case of MXD and Ca. Contrary to TRW and
339 MXD, Ca displayed positive correlations with winter TMn (December and February) in
340 Aigüestortes. There was also a positive effect of previous December TMn on Ca in
341 Aigüestortes (Figure 4).

342 The relationships between TRW, MXD and precipitation were less important
343 compared with those found for temperature (Figure 4). However, Ca was related to
344 previous autumn-winter (October to December) precipitation in both sites, and also with
345 June precipitation in Aigüestortes (Figure 4). Moving correlations between TRW, MXD
346 and Ca and selected climate variables indicated relatively stable climate-tree-ring
347 associations along the 20th century (Figure 5).

348 The strongest responses of the Ca to any climatic variable were observed in
349 response to March mean maximum temperature in Ordesa and previous December mean
350 minimum temperature in Aigüestortes, explaining 26% and 13% of Ca variability,
351 respectively (Figure 6, Table 3). The TRW chronology in Ordesa showed a much
352 stronger association with previous November TMx (Figure 4 and Table 3), whereas
353 MXD showed higher values with May TMx. In contrast, Aigüestortes TRW and MXD

354 showed the highest correlations with May temperatures (Figure 4 and Table 3). Ca
355 series emerged as a potential proxy for reconstructing past March TMx in Ordesa and
356 December TMn in Aigüestortes (Table 3). The regression models between Ca and
357 temperatures explained the 33% (TMx) and 27% (TMn) variability during the period
358 1901–2009, in Ordesa and Aigüestortes, respectively. The calibration/verification
359 statistics indicated that there is reconstructive capacity in the models (Table 3), even if
360 developed using a limited number of samples for wood chemistry (Table 1). Observed
361 and modelled temperatures values for the two sub-periods in the Ca models showed
362 significant correlations (Ordesa, 1901-1950: $r = 0.45$, $P < 0.05$; 1951-2009: $r = 0.67$, P
363 < 0.001 ; Aigüestortes, 1901-1950: $r = 0.46$, $P < 0.05$; 1951-2009: $r = 0.56$, $P < 0.01$).

364 Significant field correlations ($P < 0.01$) were also found for TRW, MXD and Ca
365 series and selected climate variables. Some showed clear spatial patterns as the
366 association between Ca and March TMx in Ordesa which portrayed significant
367 correlations across the Mediterranean eastern Spain (Figure 7).

368

369 **4. Discussion**

370 We carried out a first attempt at developing new potential proxies based on tree-ring Ca
371 relative concentrations. We generated long-term climate reconstructions based on Ca
372 obtained using a novel μXRF non-destructive technique in old and temperature-
373 sensitivity trees from cold high-elevation forests. Our findings are coherent with studies
374 demonstrating similar long-time series in wood-Ca relationships (Fromm, 2010, Lautner
375 and Fromm, 2010), stable isotopes series (Krass et al., 2010; Andreu-Hayles et al.,
376 2017), wood-anatomical parameters (Pellizari et al., 2016; Ziaco et al., 2016) and
377 classical tree-ring variables as TRW and MXD (Büntgen et al., 2008; Esper et al., 2010;
378 Dorado-Liñan et al., 2012; Galván et al., 2015). To our knowledge such long

379 chronologies on nutrients based on old trees are scarce (but see Pearson et al., 2006,
380 2009; Vaganov et al., 2013; Panyushkina et al., 2016), despite the presence of long-
381 living conifers in Eurasia and also in the Spanish Pyrenees (Dorado-Liñan et al., 2012,
382 2015). Furthermore, our results evidence that wood Ca concentration can be considered
383 as a marker of local site conditions on the tree nutrition and the influence of
384 environmental changes during centuries (Figures 3 and 4) (*sensu* Hevia et al., 2018).
385 The developed annual tree-ring Ca chronologies represent a valuable and robust proxy
386 of temperature and water availability, two constraints which may be amplified by
387 climate warming and drying trends over high-elevation areas from southern Europe
388 under the influence of Mediterranean climate conditions (Galván et al., 2015).

389 The dendrochronological statistics reflected common coherency among and
390 within variables (TRW, MXD and Ca), and the variability in growth-climate responses
391 (Figures 4 and 5) were similar to those reported for previous dendroclimatic studies at
392 Spanish Pyrenean high-elevation forests (e.g., Gutiérrez, 1991; Rathgeber and Roche,
393 2003; Tardif et al., 2003; Andreu et al., 2007; Büntgen et al., 2008, 2017; Esper et al.,
394 2010; Dorado-Liñan et al., 2012; Hevia et al., 2018). The relationships between Ca and
395 temperatures showed in general more stable signals as compared with MXD or TRW
396 (Figure 5). Overall, our study constitutes a step forward in this topic, because (i) we
397 found a strong Ca-temperature relationship possibly connected with the relevant role
398 played by Ca on water transport and ion exchange through cell walls before the cambial
399 onset (Lautner and Fromm, 2010), and (ii) this finding suggested water limitations of
400 growth on these cold and moist environments, at least during the late growing season
401 (Figure 4). This last result could explain why no ‘divergence’ phenomenon between
402 growth and rising temperatures has been found in these forests (cf. Büntgen et al., 2008,
403 2017; Galván et al., 2015) due to the additional influence of water shortage on tree

404 growth in this cold area. According to our results, temperature and precipitation
405 reconstructions performed in the Spanish Pyrenees using MXD or TRW may take into
406 account the role of additional tree-ring chemical elements as Ca that could potentially
407 reveal new dendroclimatic proxies (Poussart et al., 2006; Hevia et al., 2018).

408 Previous dendroclimatic studies in the Pyrenees found that TRW was reduced by
409 cold temperatures during previous-autumn and current spring (Tardif et al. 2003;
410 Buntgen et al., 2008, 2017; Galván et al., 2015). Prior-autumn cold conditions may limit
411 the formation of metabolic reserves (carbohydrates) prior to the growing season or delay
412 the cambial onset in May, respectively (Rolland and Schueller, 1994). Increased growth
413 of high-elevation conifer forests has been observed in other mountain ranges than the
414 Pyrenees and it has been usually attributed to increased temperatures and extended
415 growing season enhancing radial growth in these cold-limited ecosystems (Rolland et
416 al., 1998; Corona et al., 2015). Those studies, however, assumed the dominant effect of
417 temperature over precipitation (Creus and Puigdefábregas 1976; Rolland and Schueller,
418 1994, 1998; Tardif et al., 2003), but using Ca as a new climate proxy could improve the
419 interpretation of climate constraints and growth at these high-elevation forests by
420 disentangling the relative contributions of temperature and precipitation (Sánchez-
421 Salguero et al., 2015) (Figure 4).

422 Several studies have showed that MXD of conifers growing in cold biomes
423 (high-elevation and boreal forests) is highly sensitive to temperatures during spring and
424 summer when cell walls thicken and lignify (e.g., Buntgen et al., 2008, 2017). However,
425 Ca is sensitive to the early growing-season temperatures and water availability during
426 summer (August in Aigüestortes), integrating influences from weather conditions over
427 much of the growing season by assimilates deposition in cell walls during latewood
428 production in the late-growing season (Lautner and Fromm, 2010). The positive

429 relationships between TRW and Ca in the Ordesa site could also reflect the effects of
430 multiple drivers such as temperature and summer precipitation rather than a causal
431 relationship (Figures 3 and 4) (cf. Galván et al., 2015). On the contrary, non-significant
432 correlations were found between Ca and TRW in Aigüestortes, which points to a
433 dependence between soil conditions and Ca wood concentrations (Hevia et al., 2018)
434 (Figure 3).

435 Our results agree with previous research showing that warmer spring conditions
436 directly affect cambial activity and may trigger earlier growth resumption after winter
437 dormancy and enhance growth (Camarero et al., 1998, 2010). Regarding the Ca
438 response to climate, we also observed differences between sites in response to thermal
439 and rainfall climate conditions (Figure 4). This can be explained because Ca
440 incorporates previous-year climatic and ecological conditions (e.g., local climate
441 conditions, soil pH), together with ecological carryover effects and temperature forcing
442 over long-time windows (Fromm, 2010) (Figure 5). In fact, our results show a
443 significant correlation between Ca and previous December temperature and
444 precipitation, and also with current August precipitation in Aigüestortes, which
445 indicates that warmer prior-winter conditions may enhance carbohydrates' synthesis and
446 Ca uptake, and these reserves may be used the following year to build the tree ring,
447 whilst wet summer conditions improve Ca storage in wood (von Felten et al., 2007;
448 Hanger, 2008).

449 Different time scales of physiological processes driven by temperature and water
450 availability might therefore explain the seasonal responses of Ca identified in this study
451 on mountain pine, particularly in the Aigüestortes site where soils are acid (Granda et
452 al., 2017; Hevia et al., 2018). On the other hand, at high elevations, daily temperature
453 variability is regulated by incoming solar radiation (Sánchez-Lorenzo et al., 2017).

454 Therefore, cloudy skies are associated with a lower chance of high maximum
455 temperature, although these conditions also limit evapotranspiration (Biondi and Rossi,
456 2015). Since mean temperatures during the growing season have increased during the
457 last century and precipitation regimes have not significantly changed on the study area
458 (see Galván et al., 2015), our results suggest that warming-induced thermal stress in
459 spring-summer could increasingly influence Ca concentrations in the 21st century by
460 enhancing evapotranspiration rates; which agrees with observations from other Iberian
461 mountain conifer forests (Andreu et al., 2007; Camarero et al., 2015; Sánchez-Salguero
462 et al., 2015, 2017). In this sense, the Ca relative concentrations could be regarded as a
463 potential proxy of drought stress in these high-elevation forests (Figure 7).

464 A dense wood (higher MXD) is formed because of the production of tracheids
465 with narrower lumens and thicker cell walls (Jyske et al., 2009). This thickening and
466 lignification of the cell walls is directly related to the Ca concentration in the cambium
467 (Brett and Waldron 1996; Fromm, 2010). Thus, a close relationship between Ca and
468 lignin concentrations and mechanical properties of wood seems obvious (Wimmer et al.,
469 1997). During the first part of the growing season, when the earlywood is formed,
470 climatic variations affect radial tracheid enlargement, whereas during the latter part of
471 the growing season climate mainly affects the cell-wall thickening and lignification
472 processes of tracheids (Camarero et al., 1998). In this sense, several studies have
473 observed that Ca has a stiffening effect on the developing xylem walls (Follet-Gueye et
474 al., 1998; Brett and Waldron, 1996, Guglielmino et al., 1997; Lautner and Fromm,
475 2010). In addition, Ca seems to be essential for the onset of cambial reactivation and
476 cell division in spring (Fromm, 2010). In agreement with this, our results show that
477 warmer spring conditions could enhance earlywood formation and anticipate the onset
478 of cell production, reducing earlywood density (Figures 3 and 4). This could potentially

479 lead to wider and tracheids with thinner cell walls and a subsequent delayed summer
480 lignification producing a less dense wood (Galván et al., 2015, Bügtem et al., 2017).
481 The strongest positive correlation of Ca with TMx was found for March in Ordesa
482 suggesting an important role on cambial dynamics prior to the onset of cambial
483 resumption in May (Fromm and Latman, 2010) (Figures 6 and 7). On the other hand, Ca
484 in Aigüestortes showed negative associations with previous minimum December TMn
485 but positive relationships with current August precipitation (Poussart et al., 2006). This
486 means that wet and cool summer conditions could enhance the cell expansion and
487 xylem differentiation in this site leading to increased Ca concentrations in the cell walls
488 (Brett and Waldron, 1996). We further acknowledge that other local factors such as the
489 contrasting nutrient availability in the soil (basic soils in Ordesa vs. acid soils in
490 Aigüestortes) or site differences (wider rings and higher MXD values in the drier
491 Ordesa site compared with the cool-wet Aigüestortes site) could also be responsible for
492 contrasting responses between sites given that mountain pine photosynthesis can be
493 constrained in sites with poorly developed soils having low N concentrations
494 (Fernández-Martínez and Fleck, 2016). Therefore, growth fertilization could also be
495 expected if N loads increase in the future, albeit current N deposition rates are relatively
496 low in most Pyrenean high-elevation areas (Badeau et al., 1996; but see Boutin et al.,
497 2015). On the other hand, several studies found interrelated processes between soil and
498 aboveground chemistry confirming the key role of Ca in the productivity of high-
499 elevation forests (Smith et al., 2014; Hevia et al., 2018).

500 Note than the indirect influences of previous winter conditions on TRW and
501 MXD may be related to direct effects of Ca on xylogenesis (From, 2010), and that we
502 detected these associations in the Aigüestortes site where acid soils may limit Ca uptake
503 by trees. While tracheids undergo the autumnal transition from an active to a resting

504 state, their cell walls thicken thanks to biochemical processes by Ca bridges (Lautner
505 and Fromm, 2010); hence for the cambium to resume its radial expansion in spring,
506 these calcium bridges must be degraded (Funada and Catesson, 1991). Since Ca is
507 immobile in the phloem and it is mainly deposited in the cell walls after leaving the
508 xylem cells (Brett and Waldron 1996), it acts as an important regulator in many
509 processes related to both radial growth and xylogenesis responses to environmental
510 stresses (Lautner and Fromm, 2010). The ecological meaning of climate-Ca
511 relationships can be better understood if further studies integrate Ca analyses on the
512 characterization of xylogenesis by repeated micro-coring (Ziaco et al., 2014; Pacheco et
513 al., 2016).

514 To conclude, our study shows that climate reconstructions based on tree-ring Ca
515 concentrations derived from μXRF analyses could be feasible and provide
516 complementary information to other dendrochronological proxies (TRW, MXD). Since
517 Ca variations reflect processes related to wood formation processes we argue that Ca
518 concentrations can be integrated in long-term reconstructions of past climatic variability
519 in high-elevation forests. Our findings indicate that intra-annual Ca concentrations
520 incorporate a strong seasonal signal linked to temperature, but also to precipitation in
521 these cool-wet environments, and these signals change as a function of soil acidity and
522 Ca availability.

523

524 **Data statement**

525 The research data are confidential.

526

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803 **Tables**

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805 **Table 1.** Site features of the two study sites located in the Spanish Pyrenees (Ordesa

806 and Aigüestortes).

Variable	Ordesa	Aigüestortes
Latitude N	42° 38'	42° 34'
Longitude	0° 03' W	0° 59' E
Elevation (m a.s.l.)	2247	2315
Aspect	N	SE
Slope (%)	39 ± 12	34 ± 18
Diameter at breast height (cm)	80.4 ± 24.1	83.3 ± 26.1
Tree height (m)	9.9 ± 1.5	7.9 ± 1.6
Age at 1.3 m (years)	464 ± 69	565 ± 112
Mean annual temperature (°C)	4.8 ± 0.8	3.1 ± 0.8
Mean maximum temperature (°C)	10.9 ± 0.8	9.3 ± 0.7
Mean minimum temperature (°C)	0.7 ± 0.7	-1.3 ± 0.8
Annual precipitation (mm)	1221 ± 146	1728 ± 135
Bedrock type	Limestones	Granodiorites
Soil type	Entisols	Umbric leptosols
Soil pH	7.8	5.1

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810 **Table 2.** Statistical characteristics of selected tree-ring chronologies: tree-ring width
 811 (TRW), tree-ring maximum wood density (MXD) and Ca relative concentration (Ca) for
 812 the common period 1900-2009. SD is the standard deviation. All dendrochronological
 813 statistics were calculated for residual chronologies excepting AC which was obtained
 814 from raw tree-ring width data.

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Raw data	Ordesa			Aigüestortes		
TRW ± SD (mm)	0.72 ± 0.41			0.59 ± 0.32		
MXD ± SD (g cm ⁻³)	0.65 ± 0.07			0.56 ± 0.05		
Ca ± SD (unitless)	0.14 ± 0.03			0.10 ± 0.02		
Time span	1421-2010			1390-2009		
Mean length (years)	486 ± 31			557 ± 22		
Residual chronologies	Ordesa			Aigüestortes		
	TRW	MXD	Ca	TRW	MXD	Ca
No. trees	37	10	5	27	17	5
Reliable time span (EPS > 0.85)	1571-2010	1664-2010	1775-2010	1475-2009	1613-2009	1675-2009
AC*	0.85	0.72	0.83	0.83	0.54	0.82
rbt	0.28	0.28	0.23	0.34	0.31	0.22
MS	0.14	0.12	0.11	0.17	0.12	0.10
PC1 (%)	29.51	32.25	28.95	41.88	38.66	29.12

816 *Abbreviations: AC, first-order autocorrelation for raw tree-ring series; rbt, mean between-trees
 817 correlation of indexed values; MS, mean sensitivity; PC1, variance accounted for by the first principal
 818 component; EPS, Expressed Population Signal.

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830 **Table 3.** Calibration-verification statistics for the dendroclimatic reconstructions of
 831 climatic variables using tree-ring width (TRW), tree-ring maximum wood density
 832 (MXD) and Ca series for the period 1901-2009. Climate variables are: mean maximum
 833 temperatures of the previous November (TMx11_{t-1}), mean maximum and minimum
 834 temperatures of the current May (TMx5_t and TMn5_t, respectively), mean maximum
 835 temperatures of the current March (TMx3_t), and previous-December mean minimum
 836 temperatures (TMn12_{t-1}). Statistics' abbreviations: RE, reduction of error; CE,
 837 coefficient of efficiency; and DW, Durbin–Watson test.

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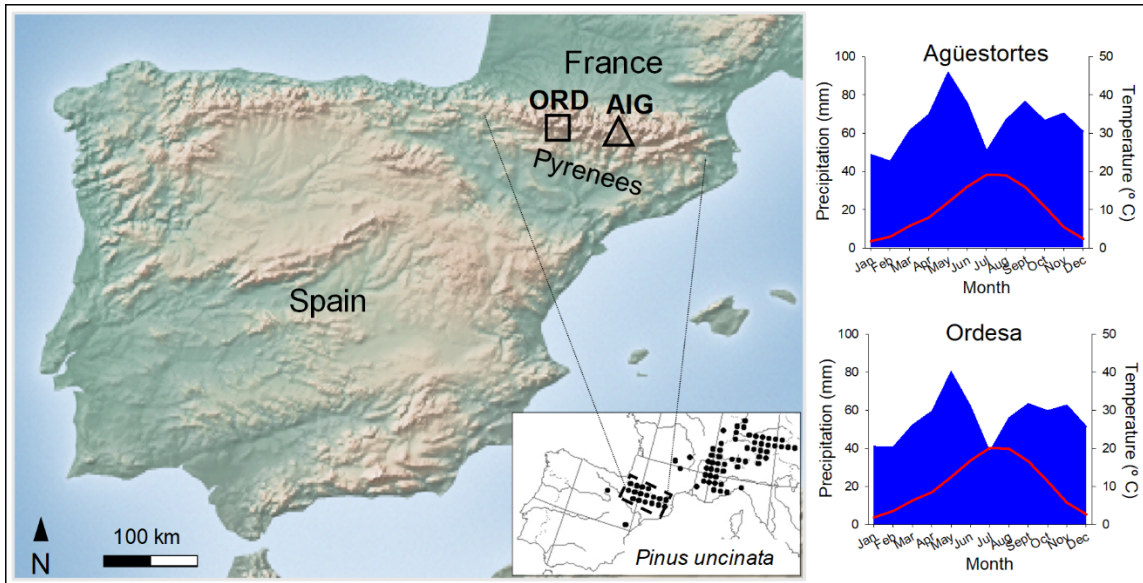
	1901-1950			1951-2009			1901-2009		
	RE	CE	DW	RE	CE	DW	Intercept	Slope	R ²
Ordessa									
TRW vs. TMx11 _{t-1}	0.010	-0.098	1.441	0.294	0.543	1.916	5.312	6.164	0.39
MXD vs. TMx5 _t	0.097	0.311	1.660	0.189	0.435	1.553	7.709	12.402	0.45
Ca vs. TMx3 _t	0.099	0.062	1.739	0.219	0.468	1.642	3.239	10.230	0.33
Aigüestortes									
TRW vs. TMx5 _t	0.094	0.307	1.588	0.130	0.361	2.085	6.060	3.821	0.41
MXD vs. TMn5 _t	0.121	0.348	1.641	0.117	0.341	1.372	0.472	6.289	0.44
Ca vs. TMn12 _{t-1}	0.037	-0.193	2.077	0.133	0.364	1.698	-17.539	16.889	0.27

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842 **Figures**

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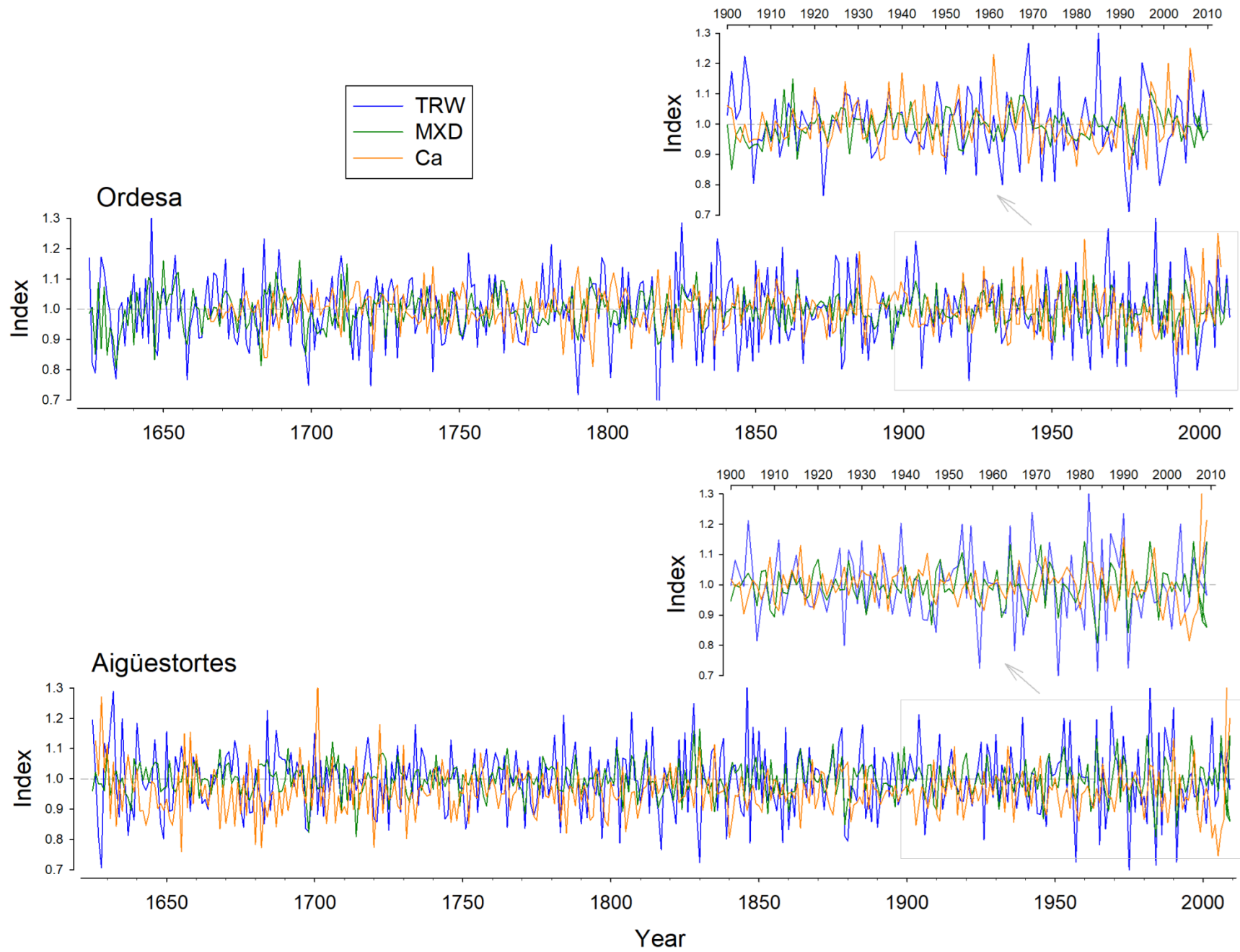


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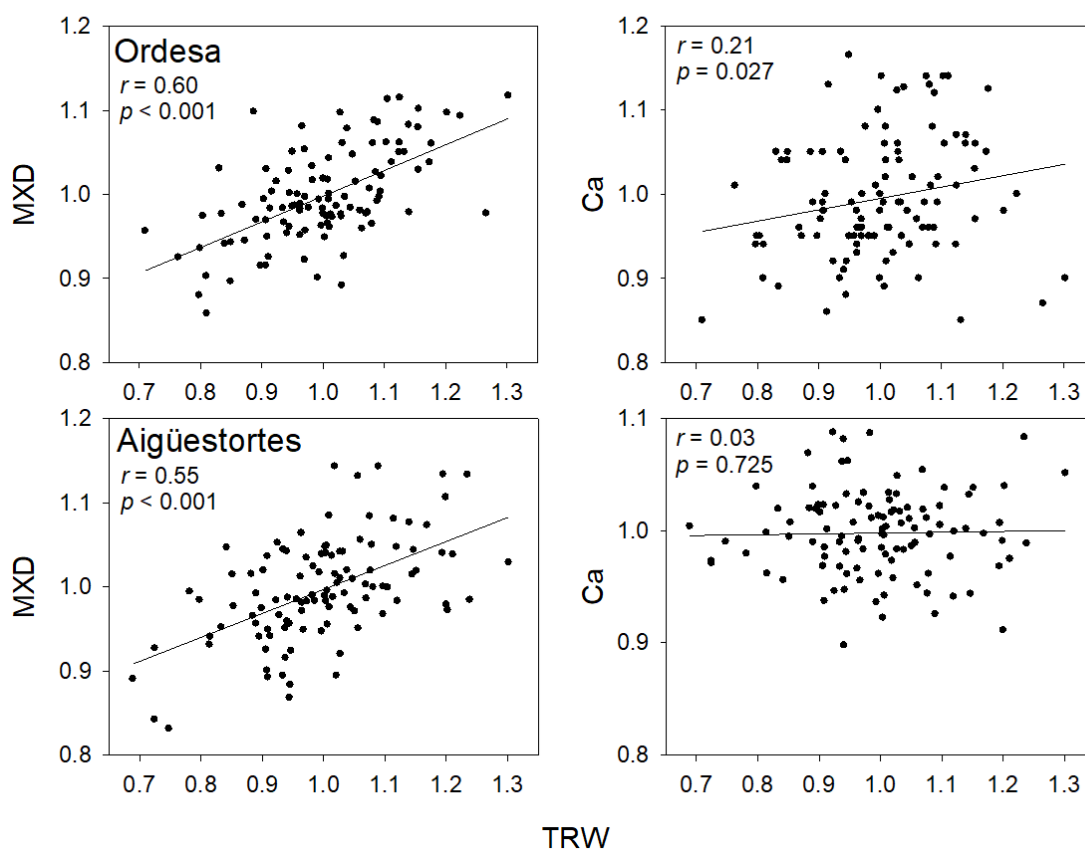
845 **Figure 1.** Map showing the geographic location of the two study sites in the Spanish
846 Pyrenees (ORD, Ordesa; AIG, Agüestortes) and distribution of *Pinus uncinata* in SW
847 Europe (lower inset). Right panels show the climatic diagrams of the study sites (mean
848 temperature, red lines; precipitation, blue areas).

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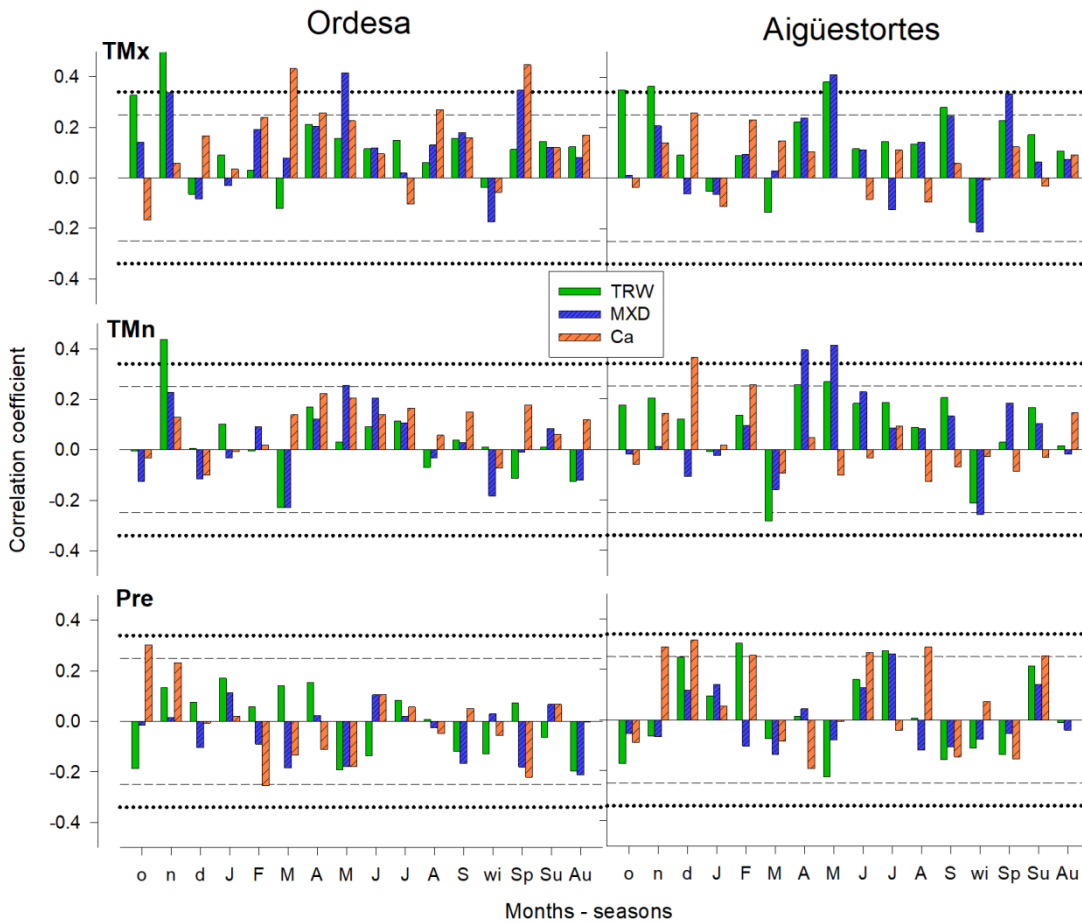


852 **Figure 2.** Comparison between the standardized residual chronologies of tree-ring
853 width (TRW, blue lines), tree-ring maximum wood density (MXD, green lines) and
854 calcium series (Ca, orange lines) for each study site. Insets show the chronologies
855 comparison for the period 1900-2009 for TRW, MXD and Ca series.



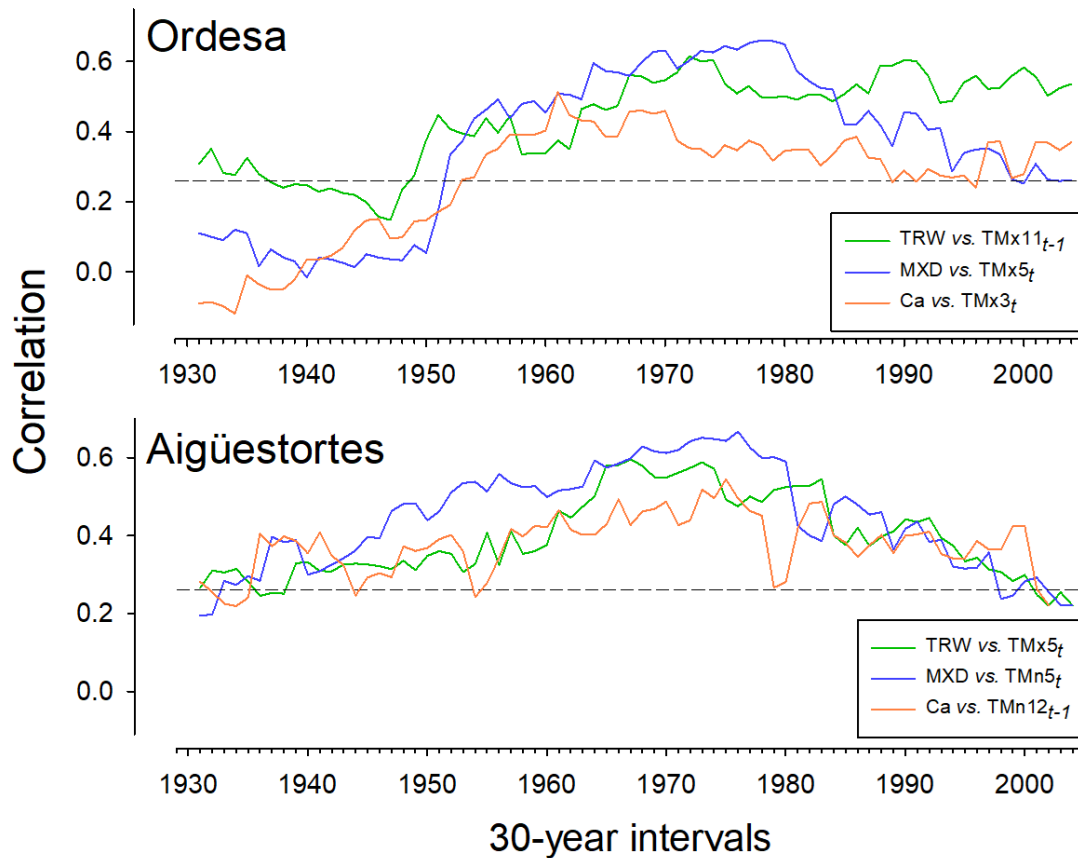
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858 **Figure 3.** Relationships observed between residual chronologies of the two study sites
 859 considering tree-ring variables (TRW, tree-ring width; MXD, maximum wood density;
 860 Ca, relative Ca concentrations). Data correspond to mean site chronology for the
 861 common 1900–2009 period. Linear regressions and correlation statistics are also shown.



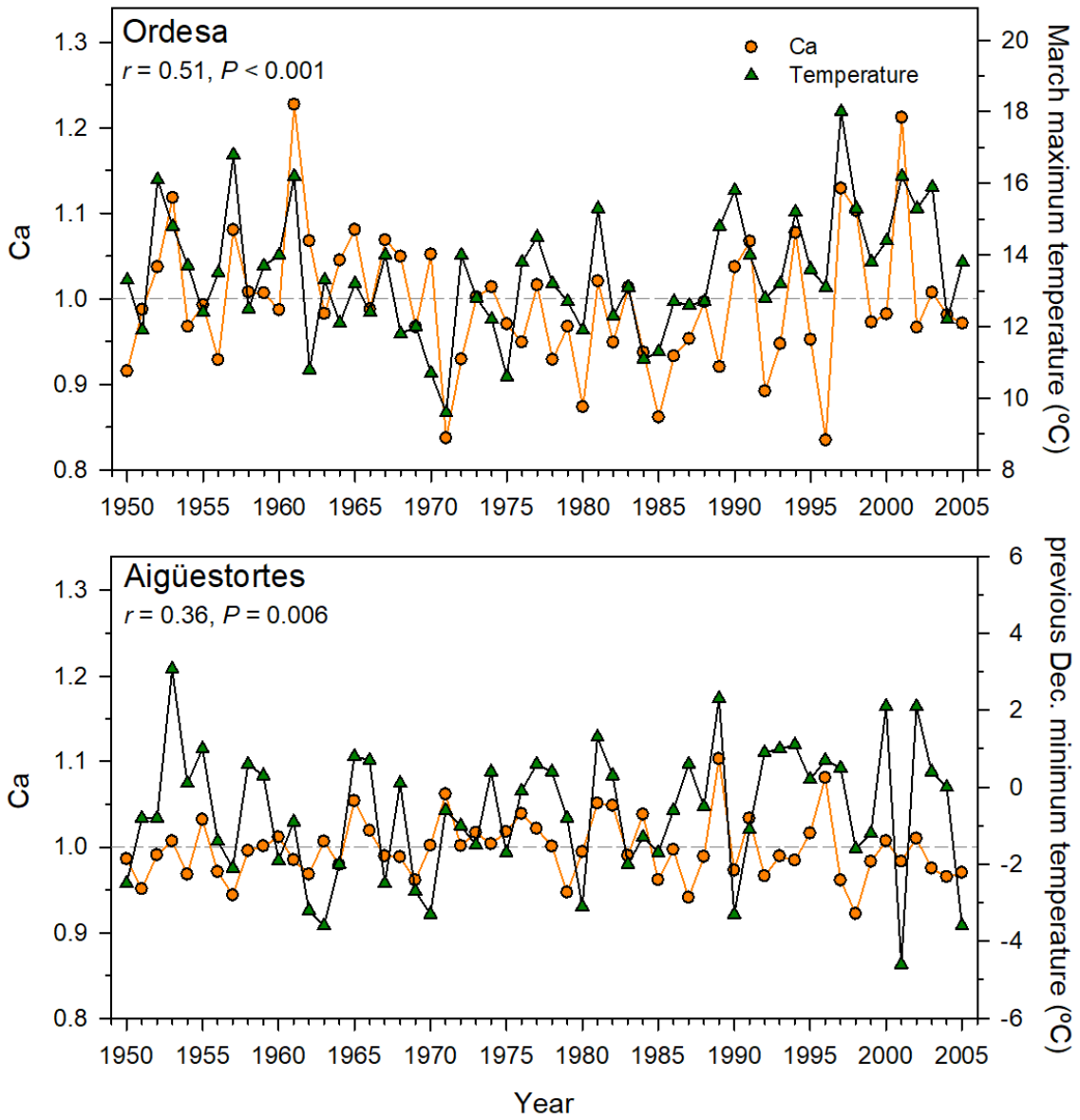
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864 **Figure 4.** Correlation coefficients calculated between mean maximum (TMx) and
 865 minimum (TMn) temperatures and precipitation (Pre) with indexed chronologies of
 866 tree-ring width (TRW), maximum wood density (MXD) and Ca relative concentrations
 867 (Ca). Monthly correlations are computed from previous October (months abbreviated by
 868 lowercase letters) up to current September (months abbreviated by uppercase letters
 869 corresponding to the year of tree-ring formation). Seasonal values (means in the case of
 870 TMx and TMn; sums in the case of precipitation, Pre) are computed for prior winter
 871 (wi; DJF), current spring (Sp; MAM), summer (Su; JJA) and autumn (Au; SON).
 872 Correlations were calculated over the common period 1950-2009. Dashed and dotted
 873 horizontal lines indicate the significance thresholds at $P < 0.05$ and $P < 0.01$,
 874 respectively.



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877 **Figure 5.** Temporal changes in bootstrapped Pearson correlation coefficients using 30-
 878 year long intervals lagged by one year for indexed chronologies of tree-ring width
 879 (TRW), maximum wood density (MXD) and Ca relative concentrations (Ca) with
 880 climate variables: mean maximum temperatures of the previous November ($TMx11_{t-1}$),
 881 mean maximum and minimum temperatures of the current May ($TMx5_t$ and $TMn5_t$,
 882 respectively), mean maximum temperatures of the current March ($TMx3_t$), and
 883 previous-December mean minimum temperature ($TMn12_{t-1}$). Dashed horizontal line
 884 indicate the significance thresholds at $P < 0.05$.

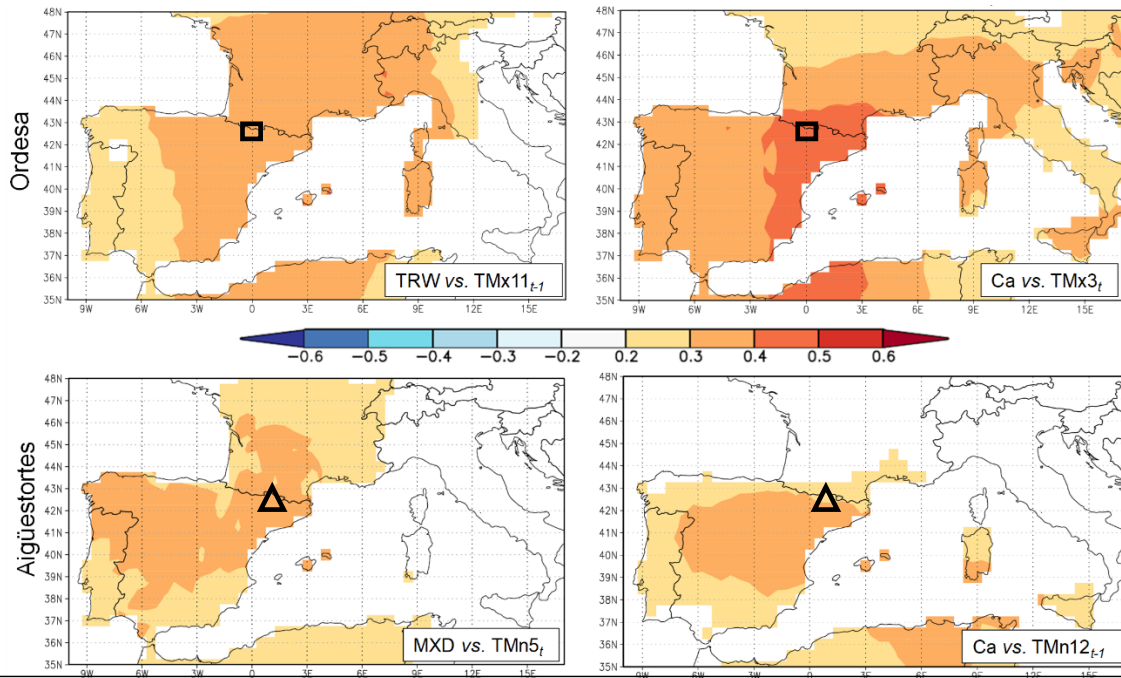


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888 **Figure 6.** March maximum and previous December minimum temperature (green
 889 triangles) compared with indexed chronologies of Ca relative concentrations (orange
 890 dots) in Ordesa and Aigüestortes study sites for the 1950-2005 period.

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896 **Figure 7.** Field correlations calculated between monthly climate variables (TMx11_{t-1},
897 previous November maximum temperature; TMx3_t, mean maximum temperatures of the
898 current March; TMn5_t, mean minimum temperatures of the current May; TMn12_{t-1}, and
899 previous-December mean minimum temperature) and tree-ring elements indexed
900 chronologies (TRW, tree-ring width; MXD, maximum wood density; and Ca ,Ca
901 relative concentrations) in the Ordesa (upper plots, square symbol) and Aigüestortes
902 study sites (lower plots, triangle symbol).

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