Highlights

- 110-year long series of wood-chemistry to understand drought-induced dieback
- Declining trees showed lower growth and higher minimum wood density than non-declining trees
- Alterations in wood nutrient concentrations are coherent with hydraulic failure hypothesis
- Mn, Ca:Mn and P:Mn rings content may serve as early-warning signals of dieback

Science of the Total Environment

Research Article

Long-term nutrient imbalances linked to drought-triggered forest dieback

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Abstract

Drought-induced forest dieback is causing reductions in productivity, increasing tree mortality and impairing terrestrial carbon uptake worlwide. However, the role played by long-term nutrient imbalances during drought-induced dieback is still unknown. To improve our knowledge on the relationships between dieback and nutrient imbalances, we analysed wood anatomical traits (tree-ring width and wood density), soil properties and long-term chemical information in tree-ring wood (1900-2010) by non-destructive Micro X-ray fluorescence (μXRF) and destructive (ICP-OES) techniques. We studied two major European conifers with ongoing drought-induced dieback in mesic (*Abies alba*, silver fir) and xeric (*Pinus sylvestris*, Scots pine) sites. In each site we compared coexisting declining (D) and non-declining (ND) trees. We used dendrochronology and generalized additive and linear mixed models to analyse trends in tree-ring nutrients and their relationships with wood traits. The D trees presented lower growth and higher minimum wood density than ND trees, corresponding to a smaller lumen area of earlywood tracheids and thus a lower theoretical hydraulic conductivity. These differences in growth and wood-anatomy were more marked in silver fir than in Scots pine. Moreover, most of the chemical elements showed contrasting concentrations in D than in ND trees during the last two-five decades (e.g., Mn, K and Mg), whilst Ca and Na increased in the sapwood of ND trees. The Mn concentrations, and related ratios (Ca:Mn, Mn:Al and P:Mn) showed the highest differences between D and ND trees for both tree species. These findings suggest that a reduced hydraulic conductivity, consistent with hydraulic impairment, is affecting the use of P in D trees, making them more prone to drought-induced damage. The retrospective quantifications of Mn ratios may be used as early-warning signals of impending dieback.

Keywords: *Abies alba*, *Pinus sylvestris*, dendrochemistry, wood density, X-ray fluorescence, Itrax, ICP-OES

1. Introduction

Forest dieback episodes in response to severe droughts have been observed worldwide in diverse biome types (Allen et al., 2010). Since droughts are expected to last longer and be more severe as climate warms, forest ecologists and managers are increasingly concerned about the prevalence of such dieback episodes under more arid conditions (Anderegg et al., 2015). Dieback often precedes high tree mortality and both processes are characterized by increased crown transparency and leaf shedding, decreased forest productivity, reduced radial-growth rates and increased tree mortality rates, among other symptoms (e.g., Bréda et al., 2006). Forest dieback is a complex phenomenon where several drivers may interact in addition to drought, including nutrient availability and biotic stress factors (Camarero et al., 2015; Gazol et al., 2018; Manion, 1991; Sangüesa-Barreda et al., 2015).

Two main physiological mechanisms (non-mutually exclusive) have been proposed to explain drought-induced dieback: hydraulic failure and carbon starvation (e.g., McDowell et al., 2008). Retrospective analyses based on tree-ring variables (width, anatomy and wood density, C and O isotope discrimination) used as surrogates of tree growth and hydraulic conductivity and water use, allow inferring the relative importance of these two major mechanisms involved in dieback processes (e.g., Colangelo et al., 2017; Dalla-Salda et al., 2009; Pellizzari et al., 2016; Sevanto et al., 2014). Both mechanisms are linked but, nowadays, hydraulic failure seems to be involved in most cases concerning gymnosperms subjected to severe and lasting droughts (Adams et al., 2017). However, other potential mechanisms driving forest dieback, such as diminished nutrient availability and uptake remain understudied (Gessler et al., 2017; Salazar-Tortosa et al., 2018).

Drought reduces growth and sap flow, decreases C uptake by restricting gas exchange through stomata and increasing relative respiration costs and, as a result, may uncouple growth from photosynthesis (Hsiao, 1973; Muller et al., 2011). Drought also contrains the availability of nutrients by reducing nutrient diffusion and mass flow in soil, and by impairing root hydraulic conductivity and nutrients uptake from the soil. Thus, such decrease in nutrient availability may reduce photosynthesis rates and contribute to forest dieback (St. Clair et al., 2008), particularly in species following a conservative water-use strategy such as gymnosperns (Irvine et al., 1998; Salazar-Tortosa et al., 2018). In gymnosperns, where dieback is a long-term process characterized by lasting and low radial-growth rates of declining trees prior to tree death (Cailleret et al., 2017), nutrient imbalances (i.e., the inability of trees to absorb and retain certain nutrients) could contribute to explain dieback (Gessler et al., 2017; Szczepkowski and Nicewicz, 2008).

In forests where conspecific individuals show different growth and vigor responses to drought stress, the most vulnerable trees would be expected to show lower growth rates due to lower soil nutrient uptake rates (Gessler et al., 2017; McDowell et al., 2008; Timofeeva et al., 2017). However, the opposite is also possible since trees acclimated to high nutrient supply could grow more and produce wider xylem conduits making them prone to drought-induced cavitation (Levanic et al., 2011; Voltas et al., 2013). A retrospective approach could help to elucidate the role of nutrient imbalances during drought-induced dieback in relation to the two major dieback mechanisms proposed. Therefore, novel approaches to retrospectively quantify long-term changes of nutrients in wood rings are needed.

Nutrient imbalances in tree tissues (wood, foliage) have been previously related to forest dieback (e.g., Duchesne et al., 2002; Rozas and Sampedro, 2013). Such

115 imbalances are known to increase the long-term tree sensitivity to drought (Helama et
116 al., 2009; Horsley et al., 2000). Essential nutrients (Ca, K, P, Mn, N) play relevant roles
117 in tree functioning (water uptake, stomatal conductance, cell division and cell-wall
118 synthesis), so trees may modify their use and allocation in response to drought (Lautner
119 and Fromm, 2010; Schlesinger et al., 2016; Yoshimura et al., 2016). Nonetheless,
120 studies on the interactions between drought-induced dieback and long-term nutrition
121 imbalances are still scarce (but see Gessler et al., 2004, 2017).

122 Here, we used dendrochemistry, i.e., the study of nutrients in annually dated
123 tree-rings (cf. Smith et al., 2014), to assess how nutrient imbalances may contribute to
124 drought-induced dieback. We compared the long-term wood nutrient relative
125 concentrations in coexisting declining (hereafter D trees) *vs.* non-declining (hereafter
126 ND trees) individuals of two gymnosperms subjected to recent drought-induced
127 dieback. These two conifers are very important European tree species and inhabit
128 climatically contrasting sites: silver fir (*Abies alba* Mill.) was studied in a wet-cool or
129 mesic site, and Scots pine (*Pinus sylvestris* L.) was studied in a dry-continental or xeric
130 site (see more details in Camarero et al., 2011, 2015; Gazol and Camarero, 2016). We
131 also looked for early signals in the wood nutrient annual series since similar analyses
132 based on tree-ring width (Cailleret et al., 2017; Camarero et al., 2015) or tracheid lumen
133 area (Pellizzari et al., 2016) found differences between coexisting D and ND trees
134 several decades before the dieback started.

135 We hypothesize that D trees were more vulnerable to drought due to long-term
136 nutrient imbalances. Specifically, we compare wood anatomical traits (tree-ring width
137 and wood density) and nutrient concentrations (e.g., Ca, Mn, K, Mg, Na and Zn) in
138 coexisting D and ND trees to determine if drought-related dieback is linked by long-
139 term nutrient imbalances. We expect that: (i) D trees will show lower growth rates than

ND trees; (ii) D trees will have a denser earlywood (higher minimum wood density) than ND trees because the former will produce tracheids with smaller lumen diameter, i.e. having a lower theoretical hydraulic conductivity, than the latter (cf. Pellizzari et al., 2016); and (iii) D trees will show decreases in hydraulic conductivity and growth linked to long-term nutrient imbalances. Investigating the role of nutrient imbalances in drought-induced dieback processes will improve our understanding of which forests, tree species or individual trees will be the most vulnerable to drought damage and reduction in carbon uptake and productivity (William et al., 2015).

2. Material and methods

2.1. Study sites and tree species

We selected two sites in northeastern Spain subjected to wet and cool conditions (silver fir, *A. alba*) and continental and dry conditions (Scots pine, *P. sylvestris*) (see Table A1 in Appendix A). These sites were characterized by presenting abundant defoliated, dying or recently dead trees affected by different severe droughts. The percentage of radial growth reduction relative to background levels was applied to identify drought-induced abrupt decreases of growth following Camarero et al. (2018). The most abrupt growth reductions occurred in: 1986, 1994-1995 and 2012 in the case of silver fir; early 1980s, 1994-1995, 1999, 2005 and 2012 in the case of Scots pine (see Camarero et al., 2011, 2015, 2018). Climatic data showed temperature rises in the region during the 1940s and 1980s (Camarero et al., 2011). Annual precipitation increased during the 1970s, whereas it decreased in the 1980s and 1990s (Camarero et al., 2011). The two study conifers show isohydric regulation of their water status by avoiding hydraulic failure through rapid stomatal closure (Bréda et al., 2006). The soil in both sites was

neutral to slightly basic in pH, although soils are deeper and more developed at the silver fir than at the Scots pine site (Gazol et al., 2018; see also Tables A1 and A5).

2.2. Field sampling and dendrochronological data

At each study site, 38 trees of each species were selected in an area between 0.5 and 2.0 ha, with target trees located at least 10 m apart from each other (Camarero et al., 2015; Gazol et al., 2018). We measured size variables (DBH, diameter at breast height measured at 1.3 m; H, total tree height) and defoliation status in the selected trees to characterize the dieback pattern. Tree defoliation was estimated as the percentage of crown defoliation using binoculars with a reference tree with the maximum amount of foliage at each site (Dobbertin, 2005), and two vigor classes were differentiated: ND trees (crown defoliation < 50%) and D trees (crown defoliation ≥ 50%). This has been shown as a robust criterion to differentiate ND from D trees (see Camarero et al., 2015) (Table A1).

We used dendrochronology to retrospectively characterize growth and wood density of trees (Fritts, 2001). Radial growth was measured by extracting two radial cores per tree at 1.3 m using a 5-mm Pressler increment borer. Sampling was done in late 2012. We measured the length of the sapwood in the collected cores in the field; and for silver fir we applied stain (bromocresol green) to distinguish the sapwood (Table A2). Wood samples were sanded until tree-rings were visible and then visually cross-dated. Tree-ring width (hereafter RW) was measured to the nearest 0.01 mm using a binocular microscope and a LINTAB measuring device (Rinntech, Heidelberg, Germany). The accuracy of visual cross-dating and ring-width measurements was checked using the COFECHA program, which calculates moving correlations between each individual tree-ring series and the mean site series (Holmes, 1983).

189

190 *2.3. Tree-ring traits and dendrochemical non-destructive analyses measured at annual*
191 *scale*

192 Since dendrochemical studies require fewer sampled trees than classical
193 dendrochronological studies (cf. Balouet and Chalot, 2015), we randomly selected five
194 trees per vigor class (D *vs.* ND trees) in each species (Table A1). We selected trees with
195 high correlation with the mean ring-width site chronology, a low frequency of missing
196 rings, no tree-ring sequences with widths narrower than 0.1 mm to ensure enough
197 material to resolve chemical compounds at annual resolution, no significant growth
198 suppressions and releases and no scars or reaction wood (see Hevia et al., 2018). These
199 selected trees were cored at 1.3 m using a 10-mm Pressler increment borer. The cores
200 were mounted and thin laths of around 1.0 ± 0.02 mm thickness were cut transversely
201 using a high-precision twin-bladed saw. These thin laths were kept under controlled
202 conditions (temperature and humidity) before X-ray analysis in an Itrax Multiscanner
203 (Cox Analytical Systems, Sweden) at the laboratory of CETEMAS (Siero, Asturias,
204 Spain). Itrax was operated at 30 KV and 50 mA with a Cu-tube, and samples were
205 exposed to the X-ray beam for 10 seconds at each measurement point in radial direction
206 (50-μm step size). Count rates of fluorescent photons of elements (for chemical
207 characterization) and a radiographic greyscale image (for wood density) in each
208 measurement point of each sample were obtained. Peaks in the micro X-ray
209 Fluorescence (μXRF) spectrum were assigned to specific elements using the Q-spec
210 software (Cox Analytical Systems, Sweden), producing relative concentrations (count
211 rates of fluorescent photons) (e.g., Croudace et al., 2006) of those elements detected
212 within the wood structure for every analyzed point. The same chemical elements were
213 detected for both species. The radiographic images were cross-dated with previously
214 built chronologies in each site to assign the element specific count rates of each annual

ring. For this purpose, tree-ring-boundaries were defined on each radiographic image using WinDendro^{TM} (Regent Instruments, Canada). In addition, tree-ring width (RW), earlywood width (EW), latewood width (LW), earlywood proportion (PEW) , latewood proportion (PLW) , mean ring density (RD), earlywood density (ED), latewood density (LD), maximum (MXD) and minimum wood densities (MND) were extracted (see Table A2) from the radiographic images after calibrating the greyscale intensities to wood densities using a light calibration curve derived from a calibration wedge (Schweingruber, 1996). Average annual time series for each tree were produced considering the following detected elements: magnesium (Mg), calcium (Ca), potassium (K), phosphorus (P), sulphur (S), strontium (Sr), aluminium (Al), manganese (Mn) and zinc (Zn). Lastly, seven meaningful molar ratios were also explored: Al:Ca, Ca:Mn, K:Ca, Mg:Mn, Mn:Al, P:Mn and Sr:Ca (e.g., Hevia et al., 2018; Houle et al., 2007; Kuang et al., 2008; Scharnweber et al., 2016; Silkin and Ekimova, 2012).

2.4. Destructive wood nutrient analyses measured at decadal scale

To compare the changes in tree-ring nutrients by non-destructive μXRF and destructive direct methods, individual cores were also analyzed for element (Al, Ca, K, Mg, Mn, Na, P, S, Sr, Zn) concentrations by inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Elemental Iris Intrepid II XDL, Franklin, MA, USA) after a microwave-assisted digestion with HNO_3:H_2O_2 (4:1, v:v) in the Ionomics laboratory at CEBAS-CSIC (Murcia, Spain). The remaining wood from laths of the same trees selected for dendrochemistry were carefully separated in decadal wood segments for the period 1900-2010. Decadal pools were obtained for ND and D trees of each species. Then, 10-year bulk samples of coarse wood were ground and homogenized to a fine powder using a ball mill (Retsch MM301, Haan, Germany).

Wood N mass-based concentration (%) was measured with an elemental analyser (Elementar VarioMAX N/CM, Hanau, Germany). We also calculated N:P, N:K and P:K ratios to determine (i) whether the D and ND trees show different long-term trends and (ii) whether each of the two studied species presented significant effects on long-term nutrient imbalances by analyzing the data of all elements and elements' ratios together.

2.5. Soil traits and nutrient availability

For each sampled tree, we collected three top soil samples from the uppermost 25 cm close to the trunk (< 50 cm in distance) below the canopy of D and ND trees. Prior to collection of the soil samples the litter layer was removed carefully. Soil C and N concentrations and the C:N ratio were determined with an elemental analyzer (Elementar VarioMAX N:CM, Hanau, Germany) (Table A1). Soil samples were then sieved at 2 mm and air-dried to measure soil pH in distilled water. Soil nutrients Al, Ca, K, Mg, Na, P, S and Zn) were measured by ICP-OES (see Table A5).

2.6. Data analyses

To constrain high variance and differences of concentration (element specific count rates), tree-ring μXRF series were normalized to their mean and standard deviation (Z-scores, i.e., the mean was subtracted from each value and divided by the standard deviation). Z-scores were calculated for each series of element measurements per sampled tree, and then averaged into a mean series of each element. Each mean series included at least 5 trees for any given year for the 1900-2010 period (years after 2010 were deleted to eliminate potential edge effects due to diffraction at the sample edges in Itrax (cf. Scharnweber et al., 2016)). Decadal values of elements concentrations were obtained by destructive methods (ICP-OES, pooled tree-rings) (see Figs. 3 and A1).

In order to assess the common variance among time series of element concentrations (annual resolution, μXRF), and possible inter-correlations between the elements and wood traits (RW, MXD and MND), we used Spearman correlation analysis because some variables (e.g., MXD) did not follow normal distributions. Values of wood traits and element concentrations were compared between D and ND trees using the non-parametric Mann-Whitney *U*-test. To test within- and between- year variability in tree-ring elements between D and ND trees, Generalized Additive Mixed Models (GAMM; Wood, 2006) were fitted at tree level since these models allow characterizing non-linear trends that vary at different time scales. To assess if the chemical elements presented different trends between D and ND trees and within each species we proposed five different models: (i) containing only the intercept; (ii) considering only tree vigor; (iii) considering only calendar year; (iv) considering calendar year and tree vigor; and (v) considering calendar year, tree vigor and their interaction. The last model indicates the presence of different temporal trajectories in chemical elements between individuals. Tree identity was considered random factor since each tree represents repeated measures over the same individual. We also included the first-order autocorrelation structure (AR1()) to account of the dependency of chemical composition in year *t* of year *t-1*. We ranked the five potential models according to the second-order Akaike information criterion (AICc). The model showing the lowest AICc value and largest Akaike weight (Wi, relative probability quantifying if the selected model is the best one) was selected as the best model.

To study the influence of vigor class and wood traits on annual tree-ring elements (μXRF), we used Linear Mixed-Effects models (LME; Pinheiro and Bates, 2000) at the tree scale. Specifically, we proposed a model in which the vigor class (D *vs.* ND) and individual wood traits (tree-ring widths and wood densities) were used as

fixed factors, whereas tree identity was regarded as random factor. Separate models were constructed for each element based on annual Z-scores data. To identify the set of predictors that better explained the observed patterns for each element, we used a multi-model inference approach based on information theory (Burnham and Anderson, 2002). The model with the lowest AICc value and largest Akaike weight (Wi) was considered as the best (most parsimonious) model. We also calculated the ΔAICc (AICc differences between each model and the best model) to rank competing models. For each explanatory variable, we calculated its relative importance across all the models. To quantify the strength of the model, we calculated a pseudo-R^2 (Nakagawa and Schielzeth, 2013). Residuals of the models were checked for normality, homoscedasticity and temporal autocorrelation. Lastly, to elucidate potential influences of outliers, we evaluated the fit of the model by graphical examination of the residuals and the fitted values.

All statistical analyses were performed in the R environment (R Development Core Team, 2018). Tree-ring chronology building and related analyses were done using the package *dplR* (Bunn et al., 2018). The *gamm* function of the *mgcv* package was used to fit the GAMM (Wood, 2006), and the *lme* function of the *nlme* package was used to fit the LMEs (Pinheiro et al., 2014) models. The *MuMIn* package was used to perform the multi-model selection (Barton, 2012).

3. Results

3.1. Radial growth and wood density

The D trees showed significantly ($P < 0.05$) lower DBH and sapwood proportion (PSW) than ND trees for both species (Tables A1 and A2). D trees also presented narrower rings than ND trees (Figs. 1 and A2; Table A2) afer 1950s. This reduction in growth of

D trees as compared to ND trees corresponded to lower EW values and this difference was amplified after the 1950s in silver fir and after the 1980s in Scots pine (Fig. 1; Table A2).

Wood density traits significantly differed between vigor classes in both tree species, with higher values (RD, ED and MND) in D than in ND trees after the 1980s (Figs. 1 and A2; Table A2). In silver fir a clear divergence in MND was observed with higher values for D than for ND trees since the 1990s (Fig. A2).

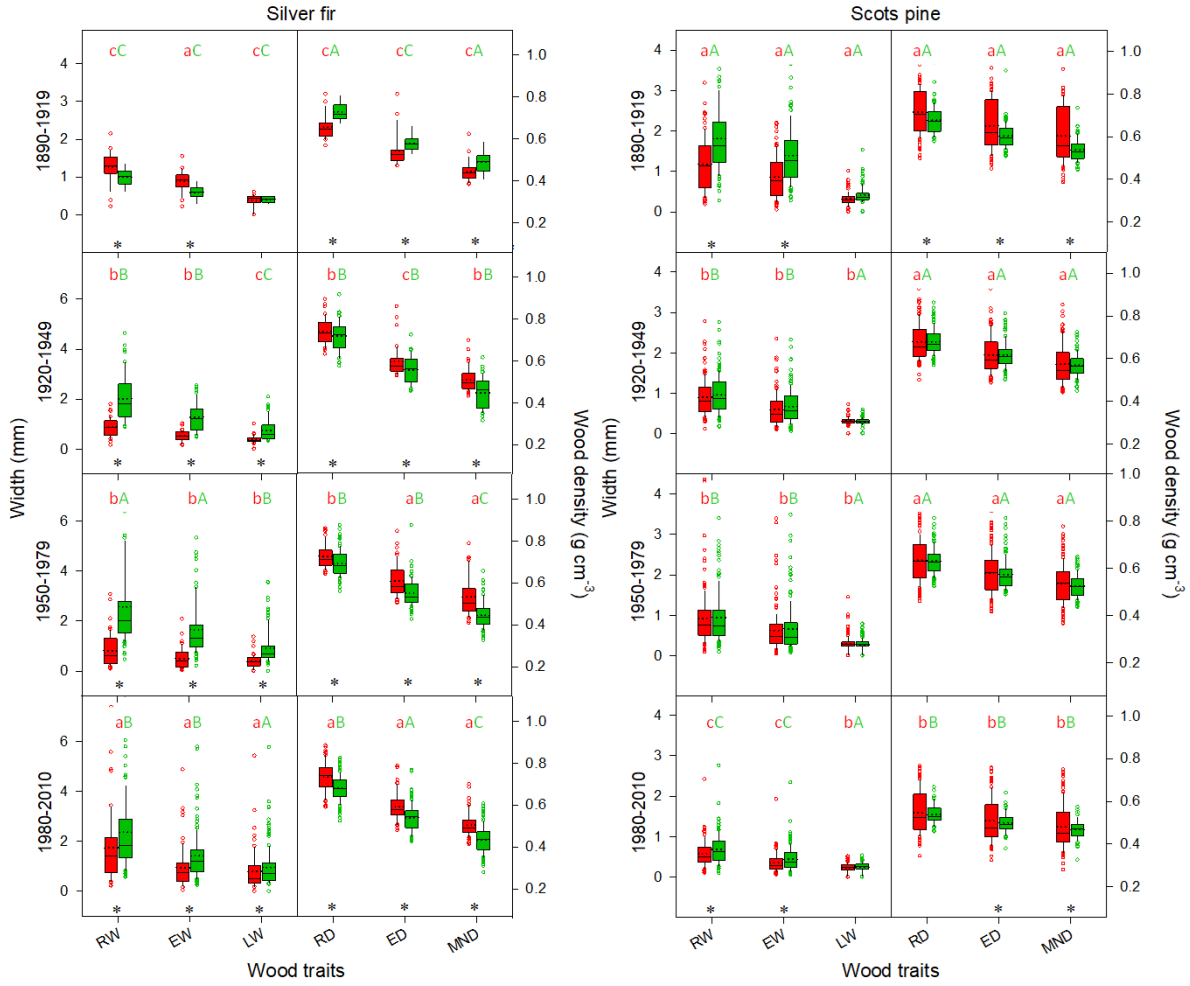

Figure 1. Temporal variability in the wood traits (RW, tree-ring width; EW, earlywood width; LW, latewood width; RD, ring density; ED, earlywood density; MND, minimum wood density) for the periods 1890-1919, 1920-1949, 1950-1979 and 1980-2010 in D (declining, red colour) and ND (non-declining, green colour) silver fir (*Abies alba*, left column) and Scots pine (*Pinus sylvestris*, right column) trees. Box-plots present median (horizontal black line), mean values (horizontal dotted black line), outliers (dots) and

standard deviation (black bars). Asterisks indicate significant ($P < 0.05$) differences in wood traits between vigor status for each period; and uppercase and capital letters mean differences within status among the four periods according to Mann-Whitney *U*-test.

3.2. Long-term wood chemistry patterns

D and ND trees showed similar long-term trends and high-frequency patterns of tree-ring chemical elements within each species (Figs. 2, 3 and A3). According to the fitted GAMMs, the explained variability for chemical elements in silver fir and Scots pine was strongly variable depending on the element considered (Tables A3 and A4). Negative long-term trends were observed for Ca and Mn in both tree species, with differences between D and ND trees during the last decades (Figs. 2, 3 and 4). In silver fir, the Ca concentration started to decrease in the 1960s for D trees, and in the 1990s for ND trees (Fig. 3). However, Scots pine presented a marked decreasing trend for Ca after the dry 1980s regardless the vigor class. In contrast, the Mn trend decreased before in ND trees than D trees in both species, starting in the 1970s in silver fir and in the 1950s in Scots pine, with a lightly increasing trend in the D trees after 1980s for both tree species (Figs. 2 and 4).

Conversely, Na presented a positive trend for both species and tree classes in the long-term (Fig. 3; Tables 1 and A3). K also presented sharp long-term changes, decreasing in silver fir but increasing in Scots pine at the heartwood-sapwood boundary for both D and ND trees (Fig. 3). In this respect, mean heartwood/sapwood transitions were dated approximately in 1975 and 1945 for silver fir and Scots pine, respectively.

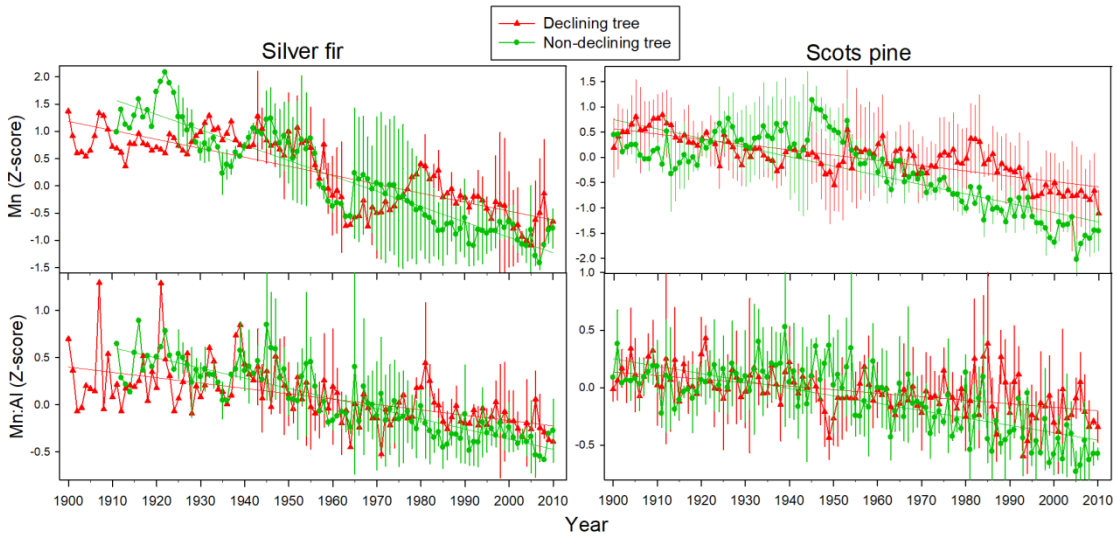

Figure 2. Annual values of element relative concentration of selected elements (Mn and molar ratio Mn:Al) obtained through μXRF of declining (D, red dots and lines) and non-declining (ND, green dots and lines) trees in silver fir (left column) and Scots pine (right column). Values are means Z-scores ± SD.

Mg, Zn and Al did not show clear differences between D and ND trees for silver fir, in contrast to Scots pine which presented differences for P and those elements during the 20th century (Table A3; Fig. 3). During the last decades, K, Mg, Mn and Zn increased in D Scots pine trees, whereas Ca and Na increased in ND trees (Fig. 3). Lastly, P and S showed different trends between species after 1980s (increasing values in ND trees in silver fir and D trees in Scots pine, respectively; see Table A3 and Fig. 3).

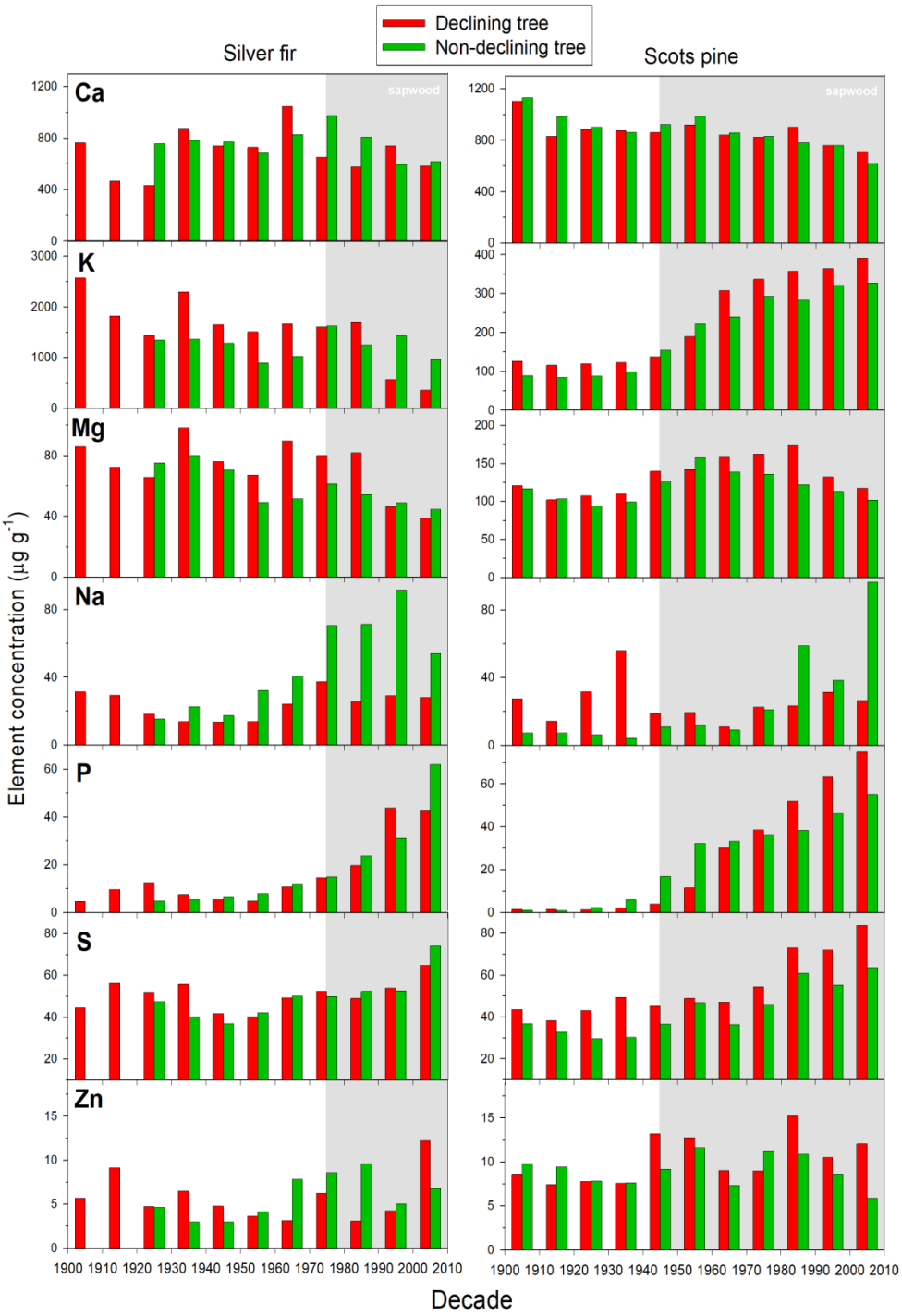

Figure 3. Decadal values of elements concentrations in wood (ICP-OES, µg g^{-1}). Data are presented for declining (D, red bars) and non-declining (ND, green bars) silver fir (left column) and Scots pine (right column) trees. The grey boxes include the decades corresponding to the mean sapwood for the sampled trees (35 years for silver fir and 45 years for Scots pine).

The GAMMs showed that Mn in Scots pine and its molar ratios (Ca:Mn, Mn:Al, Mg:Mn and P:Mn) and also P for both species were the best indicators of long-term nutrient divergences between D and ND trees in both species (Table A3; Figs.4, A3 and A4). The most clear difference between D and ND trees was observed in Ca:Mn and P:Mn (decrease in D) and Mn:Al (increase in D) (Figs. 4 and A4; Table A3).

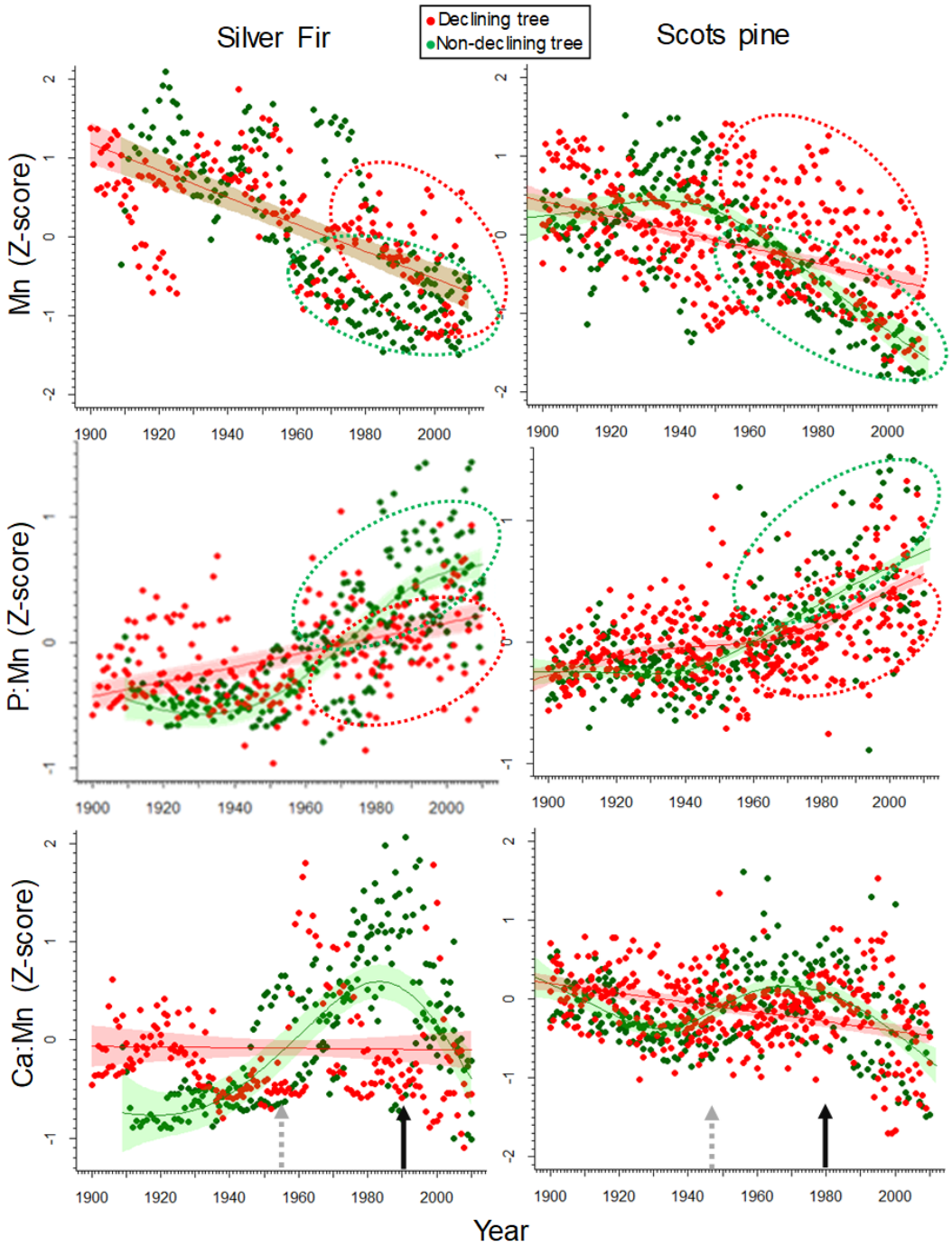

Figure 4. Observed (points) and fitted (lines with confidence intervals) long-term trends in Z-scores of selected elements obtained through μXRF of declining (D, red dots and

lines) and non-declining (ND, green dots and lines) silver fir (left column) and Scots pine (right column) trees. Generalized Additive Mixed Models (GAMM) were used to characterize the changes in analysed elements (see Table A3). Vertical grey and black arrows indicate nutrient (i.e., molar ratio Ca:Mn) and tree growth (see Fig. A2, cf. Camarero et al., 2015) divergences in trends between D and ND trees, respectively. Ellipses (dashed lines) include the 95% data for each tree status after nutrient (Mn and P:Mn) divergences (see vertical grey arrow).

The N:P and N:K ratios showed similar trends in D than in ND trees, but the N:K and P:K ratios started diverging between D and ND trees in the 1980s, markedly for silver fir, and slightly for Scots pine (Fig. 5).

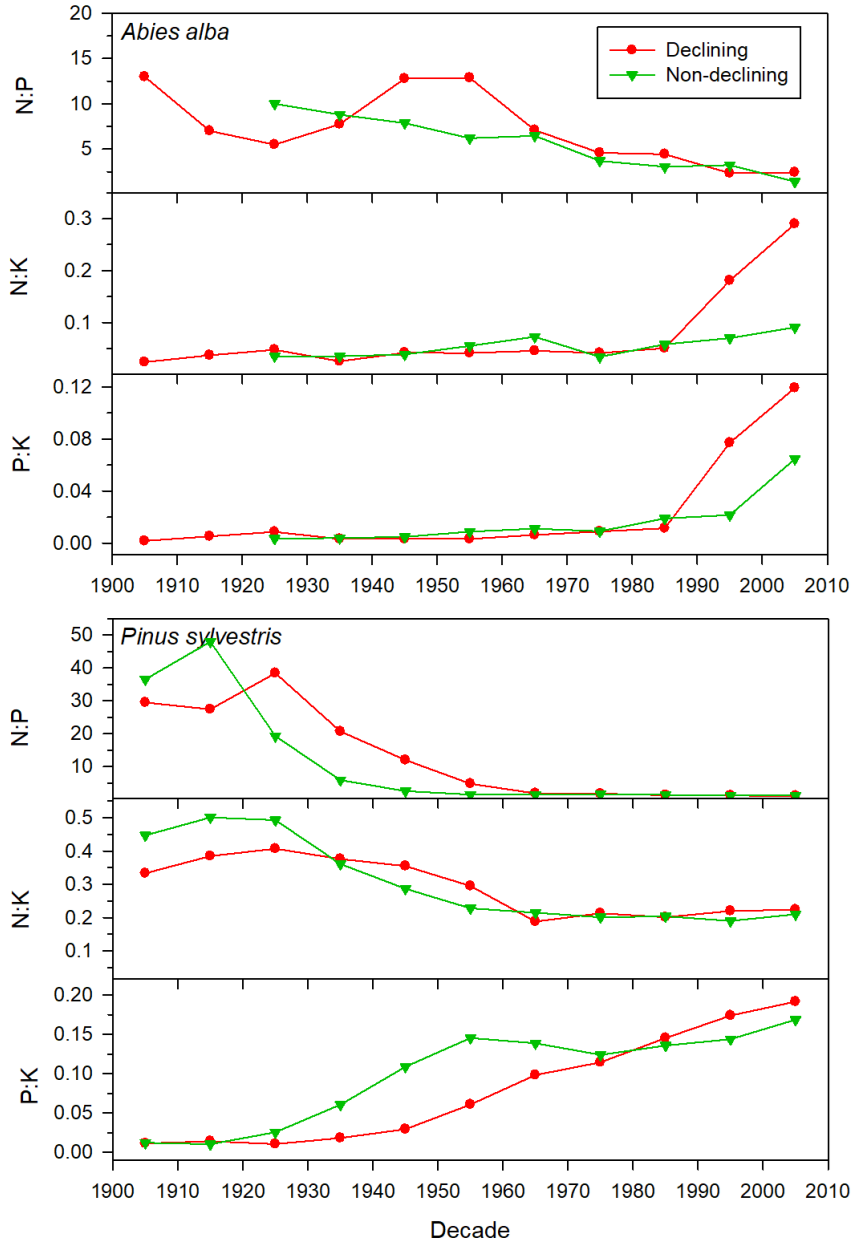

Figure 5. Decadal values of stoichiometric ratios (N:P, N:K and P:K) in wood obtained from ICP-OES analyses. Data are presented for declining (D, red lines) and non-declining (ND, green lines) silver fir (*Abies alba*, upper plots) and Scots pine (*Pinus sylvestris*, lower plots) trees.

Table 1. Model selection table of Linear Mixed-Effects models (LME) fitted to wood nutrients data and wood traits trends in silver fir (*Abies alba*) and Scots pine (*Pinus sylvestris*) for the 1900-2010 period. The model showing the lowest Akaike Information Criteria (AICc) was selected as the best model. For each model, we show the selected variables, the most parsimonious model, which reduced ΔAICc, and the model Akaike weight (Wi) or probability that the selected model is the best one, and coefficients associated to each variable and their significance levels (*$P < 0.05$; **$P < 0.01$).

Tree	Variable	ED†	ED*S	EW	EW*S	LD	LD*S	LW	LW*S	S	Wi	ΔAICc	R^2
Abies alba	Mg	-1.01	2.95**			-2.60*				-2.98*	0.21	1.26	0.04
	Al			1.41	-3.06**	0.99	2.82**	-1.71		-2.26	0.16	0.51	0.08
	P					1.68					0.06	0.02	0.01
	S			-1.63							0.05	0.22	0.01
	K										0.11	0.75	0
	Ca			-3.33**	1.55	-4.14**	2.41*	-4.25**	1.95	-2.83*	0.14	0.20	0.06
	Mn	2.90**	-1.62	-2.65**				-2.62**	2.34*		0.10	0.56	0.06
	Zn										0.11	1.33	0
	Sr	-4.52**		2.71**		-5.35**		-2.94**	2.51*	-1.30	0.20	1.73	0.16
	Al:Ca	-2.87**								-1.89	0.07	0.57	0.05
	Ca:Mn	-4.74**	-4.20**	-2.18*	3.36**	-1.50	1.55			-4.42*	0.17	0.13	0.06
	K:Ca			1.95		1.74		1.65			0.06	0.66	0.01
	Mg:Mn			2.20*		-2.42*					0.08	0.74	0.03
	Mn:Al	3.23**	-2.10*	-2.05*						1.94	0.09	0.47	0.07
	P:Mn	-2.40**		2.11*							0.08	0.90	0.03
	Sr:Ca	-1.12	-3.71**	2.74**				2.45*	-1.71	3.68*	0.13	0.35	0.06
Pinus sylvestris	Mg										0.15	1.68	0
	Al					-2.38*					0.13	1.21	0.01
	P					-0.93	-2.27*	1.73		2.57*	0.09	0.73	0.04
	S					-2.89**					0.05	0.26	0.02
	K	-2.37*				-5.82**					0.19	1.51	0.03
	Ca	-4.15**	2.43*			-5.71**	-3.34**	-2.70**		0.28	0.36	1.81	0.05
	Mn	6.24**									0.11	0.73	0.04
	Zn					1.79					0.13	1.16	0.01
	Sr	-4.87**		1.63		-3.13**					0.16	0.61	0.23
	Al:Ca	1.66	2.66**			-2.95**					0.14	0.14	0.05
	Ca:Mn	-5.32**				-3.75**		-1.85			0.11	0.45	0.21
	K:Ca	-2.14*				2.63**	2.13*			-0.30	0.06	0.03	0
	Mg:Mn	-4.40**	3.70*							-3.59*	0.21	1.42	0.04
	Mn:Al	5.29**	-4.32**			3.28**				4.31**	0.16	1.03	0.14
	P:Mn	-5.30**	2.47*	-2.84**						-2.33	0.14	1.52	0.12
	Sr:Ca	-2.43*				3.56**		2.64**			0.20	1.68	0.01

†Abbreviations: EW – earlywood width; LW – latewood width; ED – earlywood density; LD – latewood density; S – status: declining (D trees, status = 1) and non-declining (ND trees, status = 0) trees.

406 *3.3. Associations between wood traits and tree-rings nutrients imbalances*

407 We found contrasting relationships between wood traits (RW, MXD, MND) and elements
408 (Ca, K, Mg, Mn; molar ratios Ca:Mn, P:Mn, K:Ca and Sr:Ca) depending on the vigor status in
409 both species (Figs.6, A5 and A6; Tables 1 and A6). In silver fir, positive correlations between
410 elements such as K, Mn and P, and variables related to maximum wood density (MXD or LD)
411 were observed for D trees, while RW showed negative correlations with Mn, P:Mn and K:Ca
412 (Table 1 and Fig. A5). In contrast, Scots pine showed positive correlations between
413 earlywood density (MND or ED) and Mn, with higher values in ND than in D trees (Table 1;
414 Figs. 6 and A5).

415 According to the LMEs, the earlywood density (ED) and width (EW) were the wood
416 traits which accounted for most nutrient variability in silver fir and Scots pine, respectively
417 (Table 1; Figs. 6 and A6). These models suggested that the ED, a proxy of the lumen area of
418 earlywood tracheids and conductivity, had diverged between D and ND trees at least since the
419 1950s in both species, and that change was linked to changes in chemical elements (Tables 1
420 and A6; Figs. 1 and A2). The LMEs showed that Mn, Ca:Mn and Mg:Mn were suitable
421 indicators of long-term nutrient imbalances and divergences between D and ND trees in
422 relation to wood traits (i.e., Mn for both species, Ca:Mn for silver fir and Mg:Mn for Scots
423 pine) (Table 1; Fig. 6). We found that P, S and K:Ca showed the highest divergences between
424 D and ND trees in relation to latewood width (LW) in both species (Table 1). In silver fir,
425 Mn, Al:Ca, Ca:Mn and P:Mn showed that earlywood density (ED) was the most important
426 wood trait related to nutrient trends, with significant differences since 1980s between D and
427 ND trees (F= 18.21, P = 0.007; Fig. 6 and Table 1).

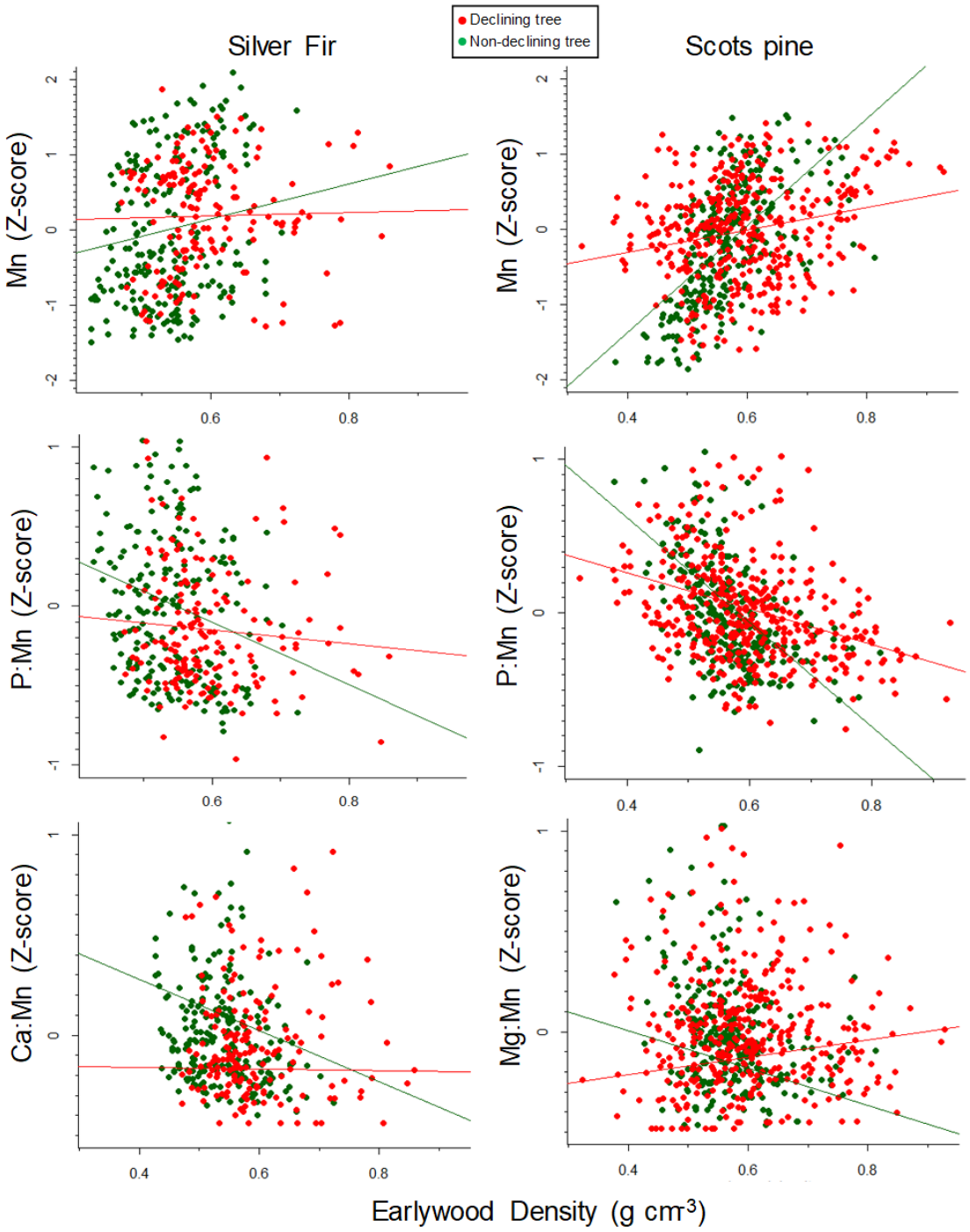

Figure 6. Relationships between observed elements concentration (Z-scores of selected elements obtained through μXRF) and earlywood density of declining (D, red dots and lines) and non-declining (ND, green dots and lines) in silver fir (left column) and Scots pine (right column) trees. Linear mixed-effects models were used to characterize the trends in the relationships for the analysed elements (see Table 1).

Only D silver fir trees presented a sustained increase in ED and MND, particularly since the late 1980s (Fig. A2), in agreement with the changes in Ca, Mn and Mg concentrations (Fig. 6). Overall, the nutrient variability responded more to year-to-year variability of wood density in Scots pine than in silver fir, with minor differences between the two vigor classes (Table 1; Figs. 6 and A6).

3.4. Soil chemical properties

Soil nutrient concentrations presented some differences between vigor classes (Table A5). In the case of silver fir, soil contents of Al, Ca, K, Mg, Na and Zn were higher for soils sampled below D trees than below ND trees (Table A5). In the case of Scots pine, soil content of the same nutrients, except Zn, but also P and S where higher below D than below ND trees.

4. Discussion

Tree-ring variables are important sources of retrospective, long-term information on tree growth and vigor (e.g., Camarero et al., 2015; Carrer et al., 2018), but also an invaluable source of data about environmental changes and tree nutritional status (e.g., Hevia et al., 2018; Smith et al., 2014). Our findings provide insights into the role of long-term nutrients imbalances in the physiological mechanisms underlying the contrasting responses of tree species to forest dieback processes. We showed that long-term nutrient imbalances could contribute to dieback and growth decline. Therefore, such nutrient imbalances could be used as early-warning signals of impending tree mortality. Specifically, the reduction in growth rates and the increase of earlywood density, which indicates a loss in hydraulic conductivity, of declining trees were consistent with hydraulic impairment. This was reflected in D trees with a decrease in Na and increase in Mn concentratrions relative to ND trees, as well as steeper rises in N:K and P:K ratios in younger sapwood (Fig. 5). Our findings revealed that

459 the combination of wood traits, surrogates of growth and hydraulic properties (Lachenbruch
460 and McCulloh, 2014), and nutrient contents in wood, proxies of basic physiological functions
461 (e.g., Fromm, 2010; Lautner and Fromm, 2010; Oddo et al., 2014), allows uncovering and
462 monitoring dieback processes decades prior to the onset of tree growth decline, supporting the
463 role of wood nutrients as potential early-warning signals of forest dieback.
464
465 *4.1. Tree-ring growth and wood density patterns in relation to droughts*
466 Our findings agree with previous studies in which tree-ring width (Cailleret et al., 2017) or
467 tracheid lumen area (Pellizzari et al., 2016) showed differences between D and ND trees
468 several decades prior to tree death (Figs. 1 and A2). We found larger differences in growth
469 rates and earlywood density between D and ND trees in silver fir than in Scots pine (see Figs.
470 1 and A2), which could be related with the limiting xeric climate conditions of the Scots pine
471 site, which severely constrains growth in both D and ND trees, compared to the mesic silver
472 fir site, where decline symptoms became conspicuous in the 1980s (Camarero et al., 2015;
473 Gazol and Camarero, 2016). Nevertheless, both species were sensitive to drought at the two
474 studied rear-edge populations (Sánchez-Salguero et al., 2017).
475 Silver fir presented a clear divergence in growth between D and ND trees after the
476 1980s due to the intensification of the warming trend and drought severity in the 1990s and
477 the legacy effects of the 1985-1986 extreme drought (Camarero et al., 2011, 2015; Sangüesa-
478 Barreda et al., 2015). Attending to a recent study showing that ND trees presented higher
479 growth rates than D trees (Camarero et al., 2018), our results highlight the capacity of silver
480 fir to recover after extreme droughts at long-term scales (Fig. A2; Table A2). In contrast, the
481 reduction in growth for D trees of Scots pine in relation to ND trees started in the 1980s,
482 which was a warm and dry decade (Pellizzari et al., 2016). Futhermore, radial growth of this
483 species was severely impacted by successive droughts in the 1990s, 2000s and 2010s

484 suggesting a chronic drought-induced dieback in this xeric site (Camarero et al., 2015, 2018).
485 This could explain the small differences observed between D and ND trees regarding MND
486 (Figs. 1 and A2; Table A2).

487 Several studies have also revealed that radial growth is closely linked to intrinsic tree
488 features such as sapwood area in conifers which affects hydraulic conductivity and stem water
489 storage (e.g., Galván et al., 2012). Trees which produce a greater proportion of sapwood are
490 expected to show greater radial growth (Galván et al., 2014), which concurs with our results
491 in ND trees for both species (Table A2; Camarero et al., 2015). A hydraulic deterioration
492 prior to the dieback onset has been associated to a chronic process for both conifers (Pellizzari
493 et al., 2016). Probably, the decline started as a consequence of the drier conditions from the
494 1950s onwards in both study sites, which concurs with our findings for minimum wood
495 density (Figs. 1 and A2). During the 1980s, MND of D and ND trees started to diverge in
496 silver fir (Fig. A2), suggesting that D silver firs were more prone to drought-induced dieback
497 due to their lower growth rate and production of smaller diameter tracheids (higher MND) as
498 compared with ND trees (e.g., Camarero et al., 2017). A denser earlywood in the D trees is
499 characterized by tracheids with narrow lumens and a decreased theoretical hydraulic
500 conductivity which leads to low growth rates (Domec et al., 2009).

501

502 *4.2. Changes in elements as early-warning signals of dieback*

503 Wood nutrient concentrations are element- and species specific (e.g., Hietz et al.,
504 2015, Meerts, 2002, Penninckx et al. 2001), although decreasing cations concentrations from
505 pith to outer heartwood (or to cambium) have been commonly observed in many different tree
506 species. For example, this was the case of: Ca and Mn in *Fagus sylvatica* L. (Penninckx et al.
507 2001); Ca in *Pinus uncinata* Ram. (Hevia et al., 2018), and other *Pinus* spp. (Peterson and
508 Anderson, 1990), or Mn in *P.sylvestris* (Scharnweber et al., 2016)). Here, a declining trend

was also observed for Ca and Mn (Figs. 2, 3 and 4). The steady decline of cation stemwood contents from older to younger wood can be explained by the decreasing moisture content, non-structural carbohydrates concentrations and cation binding capacity with increasing distance from the pith to the bark (e.g., Bondietti et al., 1990; Momoshima and Bondietti, 1990). Changes in concentration at the boundary between the heartwood and sapwood can be also sharp for some elements, as seen in our study for K (Fig. 3), in line with previous research (e.g., Hevia et al., 2018; Penninckx et al., 2001; Smith et al., 2014). Instead, our results for the concentration of most other elements did not change much between sapwood and heartwood in agreement with other species (e.g., Hevia et al., 2018; Hietz et al., 2015). Ultimately, the reduction of hydraulic conductivity and growth negatively affects the plant's nutrient status given the importance of active water flux along the soil-plant continuum for nutrient uptake (Salazar-Tortosa et al., 2018; Sardans et al., 2008, 2012). Moreover, the hydraulic properties of trees are modulated by changes in xylem sap cation concentrations (Nardini et al., 2011; Oddo et al., 2014), particularly K (see Fig. 3). Ca also plays a relevant role during cell division (White and Broadley, 2003) and the synthesis of cell walls (Eklund and Eliasson 1990), especially during latewood production (Lautner and Fromm, 2010; Sánchez-Salguero et al., 2019). Therefore, Ca availability may affect cambium dynamics and the rate of wood production and tracheid expansion (Fromm, 2010). Since Ca is an essential nutrient for tree metabolism and various physiological processes related to growth (e.g., St. Clair et al., 2005), hydraulic conductivity and tree vigor (Cronan and Grigal, 1995; Drohan et al., 2002; Houle et al., 2007 and others), low Ca concentrations are associated to growth decline (Bal et al., 2015). Along this, Mn is also essential for cell division and elongation (Seresinhe, 1996), and it is involved in many enzymatic activities and the structural stabilization of tissues (Guo et al., 2016).

When trees are subjected to drought stress, sapwood cation concentrations can increase to modulate hydraulic conductivity (Nardini et al., 2011). This is the case of K in silver fir wood (Oddo et al., 2014), with an increasing concentration in D trees as compared with ND trees after the 1980s (Fig. 3). This pattern matches with the increase in ED and the inferred decrease in tracheid lumen area and theoretical hydraulic conductivity (Pellizzari et al., 2016). An increase in xylem sap K content will compensate the loss of hydraulic conductivity (Trifilò et al., 2011), but prolonged drought stress can lead to K deficit and imbalance (Figs. 3 and 5) that might exacerbate hydraulic failure, and cause phloem dysfunction and carbon starvation (Salazar-Tortosa et al., 2018). Active upregulation of sapwood ion concentration under drought stress may enhance the hydraulic efficiency of viable xylem conduits, thus allowing plants to avoid catastrophic runaway embolism (Nardini et al., 2010). Plant Ca concentrations will also respond to drought (Bush, 1995), which reduces the uptake rates of this nutrient (Rouphael et al., 2012). Decreases in Ca concentrations can be also linked to a reduction in tracheid size due to a loss of turgor during xylogenesis (Pellizzari et al., 2016). This could explain the lower Ca concentrations we detected after 1980s droughts in the D silver fir trees (Fig. 3). In contrast, Mn can increase its accumulation in cells under water stress (Man et al., 2011), which would explain the higher Mn concentrations found in D trees up to five decades prior to the onset of the growth decline, suggesting that this element may serve as early-warning signals of impending dieback (see Figs. 2 and 4). In addition, under stress conditions plants deploy a strong P-mobilising strategy that increases the Mn concentrations of plant tissues (see Lambers et al., 2015), which explains the changes in Mn and P concentrations during the 1980s and 1990s droughts found in the D trees in both species (Fig. 4). These results are also consistent with dieback impacts on P uptake which may decrease as a consequence of the reduction in root activity (Avila et al., 2016; Schneider et al., 2001). Mn deficiency is a widespread problem, but more

558 pronounced in cool and wet environments (Alloway, 2008). High foliar Mn concentrations
559 have been also related to low rates of photosynthesis (St. Clair et al., 2005) and impaired
560 growth through metabolic interferences with other nutrient (El-Jaoual and Cox, 1998). The
561 excess of Mn in the soil is toxic to many plants, and can inhibit the uptake of essential
562 elements, such as Ca, K, Mg, Fe and P (Marschner, 2012; Millaleo et al., 2013). Mn
563 interference with the uptake of other essential nutrients could explain the lower Mg:Mn, P:Mn
564 or Ca:Mn ratios in wood of D trees for both species (Figs. 4, A3 and A4). High Mn
565 concentrations in sapwood and leaves have also been considered as predisposing or
566 contributing factors in forest dieback (e.g., Houle et al., 2007; Kogelman and Sharpe, 2006).
567 Our findings agree with previous studies in which sugar maple (*Acer saccharum*) trees were
568 predisposed to decline due to imbalances in Ca and Mn (cf. Hallett et al., 2006; Houle et al.,
569 2007; Kogelmann and Sharpe, 2006). Divergent trends between D and ND trees were also
570 found for Mg, Zn (annual) and Na (decadal) concentrations during the last decades, which
571 agrees with previous studies on sugar maple in Canada (Côte and Camire, 1995; Houle et al.,
572 2007). In particular, the sharp decline in wood Na concentration relative to ND trees further
573 supports that D trees had lower hydraulic conductivity after the 1980s droughts in agreement
574 with their reduction in tracheid size and growth rates.

575 All in all, divergence between vigor classes was more remarkable in the long-term for
576 annual Mn, alone and combined with other elements in molar ratios (Ca:Mn, Mn:Al and
577 P:Mn) (Figs. 2, 4 and A4). Several studies have recommended the use of molar ratios as better
578 diagnostic of environmental changes (e.g., DeWalle et al., 1991; Hevia et al., 2018;
579 Panyushkina et al., 2016); our results indicate that tree responses related to decline are
580 stronger for Mn and its molar ratios, and can be traced back to several decades before the
581 onset of the growth decline.

582

583 *4.3. Associations between wood traits and changes in tree-ring nutrient concentrations*

584 The concentration of elements in wood was strongly related to wood anatomy traits (see Table
585 1 and Figs.6 and A6), given that secondary cell walls contain few ion exchange sites, which
586 means that tissue with thick cell walls will have low concentrations of most elements (Hietz et
587 al., 2015). In our study sites, xylem nutrient concentrations differed between D *vs.* ND trees,
588 especially in the sapwood (Fig. 3). Our results confirmed that wood trais relationships with
589 several key nutrients (e.g., Mn, P, Sr, K, Ca, Mg, Na) showed divergences between D and ND
590 trees in both species (Figs. 3, 6 and A6). The models showed that Mn, Ca:Mn, Mg:Mn and
591 P:Mn are strongly related to earlywood density (ED) in both species, conforming good
592 indicators of long-term nutrient imbalances in relation to tracheid lumen size and hydraulic
593 conductivity (Table A2; Figs. 6, A5 and A6). Our results showed also a strong relationship
594 between Sr:Ca ratio and wood density as previously observed in other conifers (Silkin and
595 Ekimova, 2012). The concentrations of these ions change with the development of different
596 cell wall layers; Ca ion binding occurs more effectively in earlywood, whereas Sr is more
597 effectively bound in latewood tracheids (Silkin and Ekimova, 2012) (Fig. A6). The behavior
598 of Ca and Sr is almost identical for many forest biogeochemical processes (Drouet and
599 Herbauts, 2008).

600 Improved nutrient status and plant N uptake could support the regrowth of tissue lost
601 during drought (e.g., roots, foliage) resulting in improved resilience of trees to droughts
602 (Gessler et al., 2017). Interestingly, N did not seem to be involved in these nutrient
603 imbalances in D trees, since the high wood N:K and P:K ratios found in the D trees for the
604 drought-sensitive silver fir, are largely indicative of strong K limitation of growth under low
605 soil moisture conditions (Salazar-Tortosa et al., 2018; Urbina et al., 2015; see Fig. 5). The
606 nutrient deficit and imbalance observed in D trees for both trees species could be related to
607 20^{th} century climate trends towards warmer and drier conditions (Urbina et al., 2015). The

608 N:P, N:K and P:K ratios were much higher at the xeric Scots pine site than at the mesic silver
609 fir site (Fig. 5). This suggests that the severe nutrient imbalance in Scots pine was largely the
610 result of chronic drought-induced stress, rather than of low soil nutrient availability (Salazar-
611 Tortosa et al., 2018). This stoichiometric imbalance can impair photosynthesis, transpiration,
612 water-use efficiency and growth (Sardans and Peñuelas, 2012, 2015). Moreover, our results
613 highlight the need to further explore nutrient dynamics in the drought-induced tree mortality
614 framework, particularly under forecasted hotter droughts (Gessler et al., 2017).
615
616 *4.4. Soil chemical properties and differences between tree vigor classes*
617 Soil drying can also alter nutrient availability to trees (Sardans and Peñuelas, 2007), in
618 addition to the observed site differences in nutrients (Table A5). Although soil nutrient
619 availability is highly heterogeneous at multiple spatial and temporal scales (Ettema and
620 Wardle, 2002), our results showed a strong variation between the two vigor classes (Table
621 A5), with higher values of several elements below D trees. This could be explained if D trees
622 are located in particularly dry microsites (e.g., Lloret et al., 2015; Matías et al., 2011) where
623 root and microbial activity are partially constrained by low soil moisture (Bal et al., 2015).
624 Soil physical and chemical properties such as low water-holding capacity largely affected by
625 soil hydrophobicity (water repellence), and nutrient deficiency can exacerbate the negative
626 consequences of drought stress on tree vigour leading to forest die-off (Pinto and Peñuelas,
627 2007; Gazol et al., 2018). This makes therefore difficult to know if differences in soil
628 nutrients could also explain the forest dieback or they arise as a consequence of this process
629 (Gazol et al., 2018). On one hand, soil nutrients like Ca, Mg, K, and Na are mainly cycled
630 through mineral weathering (Gallardo, 2003), but also by biological pumping through leaf
631 litter decomposition (Lucas, 2001). For this reason, an increased needle shedding of D trees
632 could be asociated to an increased nutrient input if fine-root and mycorrhiza activity has not

diminished. On the other hand, the higher nutrient concentration in soils for D trees could predispose to dieback due to maladaptive drought survival in relation to tree nutrient uptake (Gessler et al., 2017). In addition, the uptake of nutrients with limited mobility and diffusion rates in soil (for example, P, Zn, Cu) may be particularly vulnerable to severe decreases in transpiration and mass flow to roots during consecutive droughts, compared to mobile nutrients as N (Rengel and Marschner, 2005; Salazar-Tortosa et al., 2018).

4.5. Dendrochemistry improves the understanding of drought-induced forest dieback

Soil chemistry data and leaf nutrient measurements have been considered good predictors of tree vulnerability to forest decline (Bailey et al., 2004; Vitousek, 2004). Our novel tree-ring nutrients approach provides a more dynamic assessment of nutritional conditions across multiple growing seasons (e.g., Hevia et al., 2018; Sánchez-Salguero et al., 2019). Future research should investigate if the relationships between long-term wood traits, genetic predisposition and soil features can determine the contrasting susceptibility of neighboring trees to dieback, since tree-rings can reflect the behavior of individual trees and underlying soil during the year of ring formation but also capture historical legacy effects (Bondietti and McLaughlin, 1992).

As expected, pooling chemical data (ICP-OES) showed significant relationships with averaged decadal values by μXRF (Fig. A1), but revealed differences in the long-term trends and did not capture clear divergences (Figs. 2 and 4; Table A5). In this sense, pooling wood material from decadal blocks of several trees excludes the statistical uncertainty associated with inter-tree variability (c.f. Dorado-Liñán et al., 2011).

5. Conclusions

We found that D trees presented lower radial growth rates and higher earlywood wood density than ND trees, but these differences were more pronounced and started before in silver fir than in Scots pine. Silver fir and Scots pine D trees showed Mn and Ca imbalances (along with K starvation and imbalance in Scots pine), and such alterations in wood nutrient concentrations are coherent with hydraulic failure due to severe drought stress. We propose the use of Mn and related ratios (Ca:Mn, Mn:Al or P:Mn) as early-warning signals of forest dieback since they diverged between D and ND trees several decades before the growth decline onset. This divergence was very clear in Silver fir, a species with a good potential for dendrochemistry studies related to dieback. This study highlights the potential of μXRF methods to reveal long-term macro- and micro-nutrients changes and imbalances in trees at seasonal to annual scales.

Acknowledgements

This study was supported by projects FunDiver (CGL2015-69186-C2-1-R), CoMo-ReAdapt (CGL2013-48843-C2-1-R), AMB95-0160, CGL2011-26654 (Spanish Ministry of Economy, Industry and Competitiveness) and EU projects: CANOPEE (Interreg V-A POCTEFA 2014-2020-FEDER funds). The authors are grateful to Juan Majada for providing support for this study, Laura González Sánchez and Elena Lahoz who assisted in the laboratory. RSS and GSB were supported by a Postdoctoral grant (IJCI-2015-25845 and FJCI 2016-30121, respectively, FEDER funds). We thank the editor E. Paoletti and two anonymous reviewers for improving the manuscript.

The authors declare no conflict of interest.

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