










Temporal and spatial variability of phloem structure in *Picea abies* and *Fagus sylvatica* and its link to climate

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Abstract

Using a unique 8-year data set (2010–2017) of phloem data, we studied the effect of temperature and precipitation on the phloem anatomy (conduit area, widths of ring, early and late phloem) and xylem-ring width in two coexisting temperate tree species, *Picea abies* and *Fagus sylvatica*, from three contrasting European temperate forest sites. Histometric analyses were performed on microcores taken from tree stems in autumn. We found high interannual variability and sensitivity of phloem anatomy and xylem-ring widths to precipitation and temperature; however, the responses were species- and site-specific. The contrasting response of xylem and phloem-ring widths of the same tree species to weather conditions was found at the two Slovenian sites generally well supplied with precipitation, while at the driest Czech site, the influence of weather factors on xylem and phloem ring widths was synchronised. Since widths of mean annual xylem and phloem increments were narrowest at the Czech site, this site is suggested to be most restrictive for the radial growth of both species. By influencing the seasonal patterns of xylem and phloem development, water availability appears to be the most important determinant of tissue- and species-specific responses to local weather conditions.

KEYWORDS

anatomy, early phloem, European beech, late phloem, Norway spruce, sieve element area, xylem-ring width

1 | INTRODUCTION

Xylem and phloem form vascular systems in woody plants. Although the two tissues perform different functions in a tree, they are closely linked through rays (Pfautsch et al., 2015). Phloem plays a critical role in the long-distance transport and allocation of nutrients, resources

and signals that are important for regulating organ growth, development and adaptation to stresses (Dinant & Lemoine, 2010). A fair amount of research has recently been published on phloem phenology and/or structure (e.g., Balzano et al., 2020; Dannoura et al., 2019; Jyske & Hölttä, 2015; Kiorapostolou et al., 2020; Savage & Chuine, 2021; Shtein et al., 2023) but few of these studies include

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multi-year data that provide insight into the impact of weather conditions on the seasonal development of the phloem growth ring (e.g., Gričar et al., 2022; Prislán et al., 2013). Moreover, with few exceptions (Gričar et al., 2014; Miller et al., 2020; Swidrak et al., 2014), comparison of seasonal trends in phloem between different locations and coexisting species is particularly understudied, although studying the weather-phloem interaction is necessary for better understanding tree physiological limits in terms of species-specific plasticity of phloem development and its connection to leaf and xylem growth (Savage, 2020). Furthermore, phloem structure, such as sieve element characteristics (e.g., their number, lumen size) and axial parenchyma frequency, largely affect phloem transport capacity and carbohydrate and water reserve storage, respectively (Savage et al., 2016; Spicer, 2014). Deciphering how unfavourable weather conditions, such as drought stress, influence phloem anatomy and consequently phloem functioning is crucial for elucidating constraints on tree growth and mortality (Woodruff, 2014). This information is especially relevant for economically important and widespread tree species, such as Norway spruce (*Picea abies*) and European beech (*Fagus sylvatica*), which have recently suffered in terms of growth decline, dieback and calamities on numerous forest sites across Europe, due to various natural disturbances, resulting in large uncertainty in future growth predictions for them (Jevšenak et al., 2021; Martínez del Castillo et al., 2022; Tremblé et al., 2022). These climate change-induced changes in the growth stock of Europe's two main tree species could affect timber production and supply, which in turn is likely to have implications for the global wood-based bioeconomy (Daigneault et al., 2022).

Nevertheless, compared to other tree species, seasonal phloem development and structure in *P. abies* (Jyske & Hölttä, 2015; Petit & Crivellaro, 2014; Rosner et al., 2001) and *F. sylvatica* (Gričar & Prislán, 2022; Prislán et al., 2013) have been frequently studied by various research groups in the last decade. It has been shown that, in addition to internal growth regulators (e.g., hormones, signalling molecules), environmental factors have a significant impact on cambial rhythm and cell development (Balzano et al., 2020; Gričar & Jevšenak, Hafner, et al., 2022; Prislán et al., 2016). In *P. abies* and *F. sylvatica*, differences in phloem structure are mainly reflected in the anatomy and width of late phloem, while early phloem is less variable (Gričar et al., 2016). Although very important for the insight into the seasonal dynamics of phloem formation, these initial studies were limited primarily by the short time series of the data (a maximum of three growing seasons), which did not allow a more detailed insight into the temporal dynamics over several years and the relationships between weather and phloem traits at a single site (Gričar et al., 2015; Prislán et al., 2013). Moreover, capturing extreme weather events in the study is essential to revealing how a particular tree species adapts phloem structure to ensure optimal functioning under given conditions (Gričar & Jevšenak, Hafner, et al., 2022). Answering these questions requires intense multi-year sampling of developing phloem tissue, laborious section preparation and quantitative phloem anatomy. With few exceptions, these studies must be performed on the youngest phloem increment, because age-related changes in

bark tissue can greatly affect the morphology of phloem cells (Jyske et al., 2016). Significant improvements in sample preparation, the development of imaging techniques and the rate of knowledge and data exchange among different research laboratories (Shtein et al., 2023) are making a major contribution to improving our understanding of phloem phenology and anatomy.

In this study, we investigated the effect of weather conditions on the xylem-ring width and structure of phloem increments in two coexisting temperate tree species, *P. abies* and *F. sylvatica*, from three contrasting European temperate sites differing in altitude and latitude. Two sites were located in Slovenia and one in the Czech Republic. For this purpose, a unique 8-year data set of phloem anatomy data, covering from 2010 to 2017 was used. Based on the findings of obtained for both species from a shorter data set (max. three years) (Gričar et al., 2015; Prislán et al., 2013) we hypothesised that: (H1) Phloem structure will differ between the two species and sites due to a species-specific plastic response of phloem traits to local conditions (moist and wet sites). This plasticity will be reflected in particular in late phloem traits, irrespective of species. (H2) At the three sites, temperature and precipitation in the previous and current year will have different effects on the phloem anatomy of the two species. However, at an individual site, year-to-year variability in the phloem structure will be small and will be mainly expressed in extreme years (i.e., summer drought). (H3) Because of the different internal and external controls on the formation of xylem and phloem and the different priorities of their formation for the tree functioning, weather factors will affect the annual width of the two tissues differently. This would suggest different adaptation strategies of phloem and xylem formation to local conditions (moist and wet sites).

2 | MATERIAL AND METHODS

2.1 | Study site characteristics

The study was conducted at three forest sites, which greatly differ in terms of elevation and local climatic conditions: two in Slovenia (Panška reka–PAN and Menina planina–MEN) and one in the Czech Republic (Rájec–Němčice–RAJ) (Table 1). In Slovenia, sampling of *Fagus sylvatica* L. and *Picea abies* (L.) H. Karst. was carried out at two uneven-aged mixed forest stands at different elevations. PAN is a low elevation site, located near Ljubljana and dominated by *Fagus sylvatica* L., *Acer pseudoplatanus* L. and *Picea abies* (L.) H. Karst. MEN is a high-elevation site located on a pre-Alpine Karst plateau in the Kamnik–Savinja Alps and dominated by *F. sylvatica*, *P. abies* and *Abies alba* Mill. The site in the Czech Republic, RAJ ecological station, is located north of Němčice in the South Moravian region, located in the hills of the Dražanská highlands, ca. 400 km from the Slovenian sites. The site is a spruce monoculture (the first generation after mixed forest) (Fabiánek et al., 2009). Study site characteristics are presented in Table 1. Climate diagrams for PAN, MEN and RAJ for 1950–2021 are presented in Figure S1. In the 1950–2021 period, the average annual air temperature at PAN was 10.0°C, at MEN 7.8°C

TABLE 1 Study site characteristics. Weather data are for the study period 2010–2017.

Site ID	Site	Latitude	Longitude	Altitude (m a.s.l.)	Annual mean temperature [°C]	Annual precipitation sum [mm]	Soil characteristics	Forest association
PAN	Panška reka	46°00' N	14°40' E	400	15.9	1306	Dolomite, rendzic leptosols	<i>Hacquetio-Fagetum typicum</i>
MEN	Menina planina	46°16' N	14°48' E	1200	13.3	1534	Limestone, dystric cambisols	<i>Abieti-Fagetum prealpinum typicum</i>
RAJ	Rájec – Nēmčice	49°27' N	16°42' E	650	12.1	578	Acid granodiorite, loam-clay cambisols	<i>Abieto-Fagetum mesotrophicum</i> with <i>Oxalis acetosella</i> L

and at RAJ 7.3°C. Site RAJ is the driest, with close to 600 mm of annual precipitation, while MEN and PAN on average receive between 1300 and 1500 mm of precipitation. The 8-year (2010–2017) study covered a wide range of weather conditions, from the very dry year in 2012 in Slovenia to the rather wet year in 2014. The driest year at RAJ was 2015 and the wettest was 2010, with 450 mm of precipitation from February to July.

2.2 | Sample collection and preparation of cross-sections for light microscopy

Analysis of the youngest phloem structure in *P. abies* and *F. sylvatica* was performed for the years 2010–2017. Tree-level properties (mean diameter at breast height, tree height and age) of both species at all three study sites are presented in Table S1. In all studied years, six trees per species and site were sampled for analyses of the seasonal dynamics of xylem and phloem formation (for details, see Gričar et al., 2021) using a Trephor tool (Rossi et al., 2006) to extract microcores (2.4 mm in diameter) from tree stems at a height of 1.0–1.7 m above the ground. The measurements of the xylem and phloem widths, as well as phloem anatomy, were performed on samples taken at the end of cambial cell production, that is, in August or September. Each microcore contained inner living phloem (uncollapsed and collapsed parts), cambium, and at least two recent xylem growth rings. Immediately after collection, the microcores were transferred to FAA (formaldehyde/50% ethanol/acetic acid solution) for 1 week. Tissue processing steps for the preparation of cross-sections embedded in hydrophobic paraffin included removing water from the samples. This was achieved by immersing the samples in a series of ethanol solutions of increasing concentration (70, 90% and 95%) until pure, water-free alcohol was reached. The samples were then embedded in paraffin. Afterwards, 8–12- μ m-thick permanent cross-sections were cut using a Leica RM 2245 rotary microtome (Leica Microsystems, Wetzlar, Germany) and stained with a safranin (Merck) (0.04%) and Astra Blue (Sigma-Aldrich) (0.15%) water mixture (for details, see Prislán et al., 2022). Cross-sections were mounted in Euparal (Waldeck) and were observed under a Leica DM 4000 light microscope or Leica DM 2000 light microscope (Leica Microsystems) using transmitted and polarised light modes. Histometric analyses

were performed at 4 and 10 \times magnifications using a Leica DFC 280 digital camera and the LAS (Leica Application Suite) image analysis system (Leica Microsystems) or a Leica DFC 295 digital camera (Leica Microsystems) and a public-domain image processing program ImageJ (Abramoff et al., 2004).

2.3 | Histometric analyses

Quantitative phloem anatomy analysis was performed on two samples (i.e., cross-sections) per tree taken on different dates after the cessation of cambium activity (i.e., August or September) when the width of the phloem increment and the conduit size were finally determined. In each cross-section, the following widths of tissues were measured for three radial files (parallel to the rays) and then averaged (Figure 1): (1) the width of the youngest xylem increment (XR); (2) the width of the youngest phloem increment (PR); (3) the width of early phloem (EP) and (d) the width of late phloem (LP). To assess the year-to-year, site-specific and species-specific differences in conduit size in the phloem, the areas of 10 randomly selected early and late phloem conduits were measured on each cross-section, making a total of 20 measurements for each variable per tree. The mean values on a tree level were then calculated separately for early and late phloem for each growing season. The mean values of the variables for individual trees were then combined into values for sites, separately for each year and tree species.

2.4 | Data analysis

To assess the effects of site and year-to-year variability on xylem increment width and phloem anatomy parameters, we applied mixed-effects models (Bates et al., 2015). Separate models were created for each tree species with year, site and their interaction as fixed effects and trees within sites as random effects. Log transform on the response variable was used when necessary to better meet the homogeneity of variance assumption. The effect of climate was further evaluated using monthly correlations calculated with the dendroTools R package (Jevšenak, 2019, 2020). We used non-parametric Kendall's rank correlation coefficient (τ), which is robust

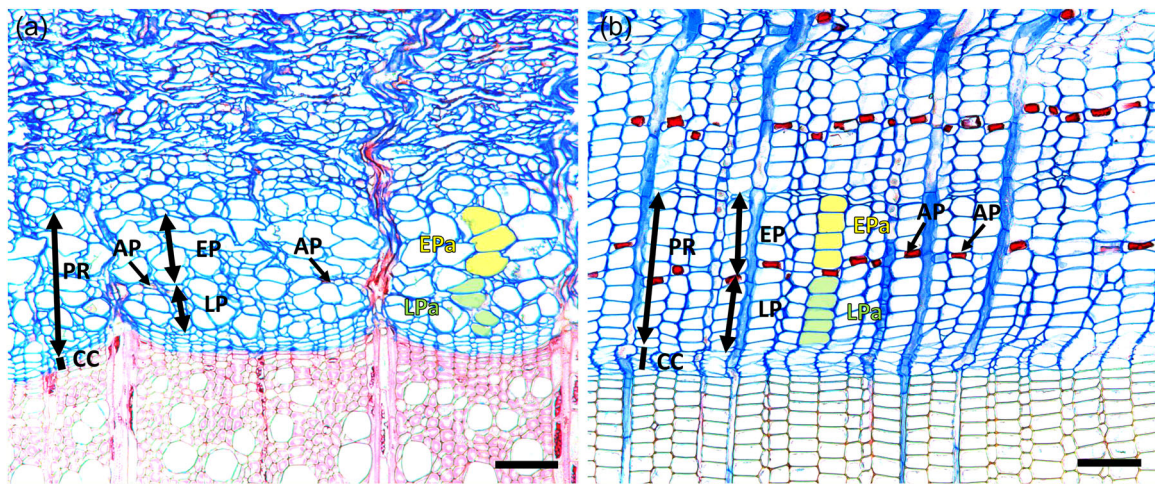


FIGURE 1 Illustration of performed measurements in phloem in *Fagus sylvatica* (a) and *Picea abies* (b): width of the youngest phloem increment (PR), width of early phloem (EP), width of late phloem (LP), area of conduit in early phloem (EPA; coloured yellow), area of conduit in late phloem (LPA; coloured green) and axial parenchyma separating early and late phloem (AP). CC, cambial cells. Scale bars = 100 μm . [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/pce.14811)]

to outliers, does not assume homoscedasticity and accounts for a smaller sample size (Newson, 2002). We considered up to 16 consecutive months, starting from the previous July and ending in the current May (early phloem variables) or August (late phloem variables). The resulting correlation matrices were plotted as heatmaps and are shown in a supplementary figure, while in the main text we highlight the highest absolute τ for each parameter and climate variable, separately for the previous and current growing seasons. The correlation matrices were also analysed with Principal Component Analysis. The resulting biplots were used to derive the relative position of each parameter, site and species in terms of their climate-growth response. To evaluate the effect of climate on the studied parameters (xylem increment width and phloem anatomy) further, we compared the studied parameters in the driest and the wettest years, following a 6-month Standardised Precipitation-Evapotranspiration Index (SPEI₆), ending in May (Vicente-Serrano et al., 2010). Response variables were compared between a preselected wet and dry year for each site using planned contrasts as follow-ups after the above-mentioned mixed models. For the Slovenian sites, 2013 and 2017 were selected as wet and dry years, respectively, and 2010 and 2014 as wet and dry years, respectively, for the Czech sites (Figure 2).

3 | RESULTS

3.1 | Site-specific, year-to-year and species-specific differences in phloem anatomy

The linear mixed models and *post-hoc* tests confirmed significant site differences, year-to-year variability and their interaction for all of the studied parameters (phloem anatomy and xylem increment width) (Table S2). Boxplots of the studied phloem and xylem parameters,

presented separately for each tree species, site and year, are shown in Figure 3. Species-specific radial growth patterns of the two coexisting tree species were observed at all three locations (Figure S2). Generally, *F. sylvatica* and *P. abies* from the warmest site, PAN, had higher values of all phloem and xylem parameters than at MEN and RAJ. In addition, *P. abies* from the driest site, RAJ, had consistently lower values for all measured xylem and phloem parameters compared to PAN and MEN. In the case of *F. sylvatica* the lowest values were recorded either at MEN (area of early phloem conduits, early phloem width and xylem width) or RAJ (area of late phloem conduits, late phloem width and phloem width).

Except for *P. abies* at PAN, the early phloem was wider and less variable than the late phloem. In *F. sylvatica*, the early phloem occupied on average 63–75% of the phloem increment width, while in *P. abies* it was 47%–60%. The area of early phloem conduits was larger in *F. sylvatica* than in *P. abies* at all three sites, whereas the opposite trend was observed in the case of late phloem conduits. Except for *P. abies* at RAJ, the average values of early phloem conduits exceeded 1000 μm^2 , whereas the average values of late phloem conduits were 530–655 μm^2 in *F. sylvatica* than and 340–716 μm^2 in *P. abies*, respectively. The size of late phloem conduits was 60%–75% and 25%–45% smaller than of early phloem conduits in *F. sylvatica* than in *P. abies*, respectively.

The phloem increments were in all cases wider in *P. abies* than in *F. sylvatica* regardless of the site. Xylem increments were wider than phloem increments. In the case of xylem, only at MEN were the increments wider in *P. abies* than in *F. sylvatica*.

3.2 | Weather-phloem relationship

In terms of correlations between weather conditions in the previous and current growing seasons, responses were generally heterogeneous across

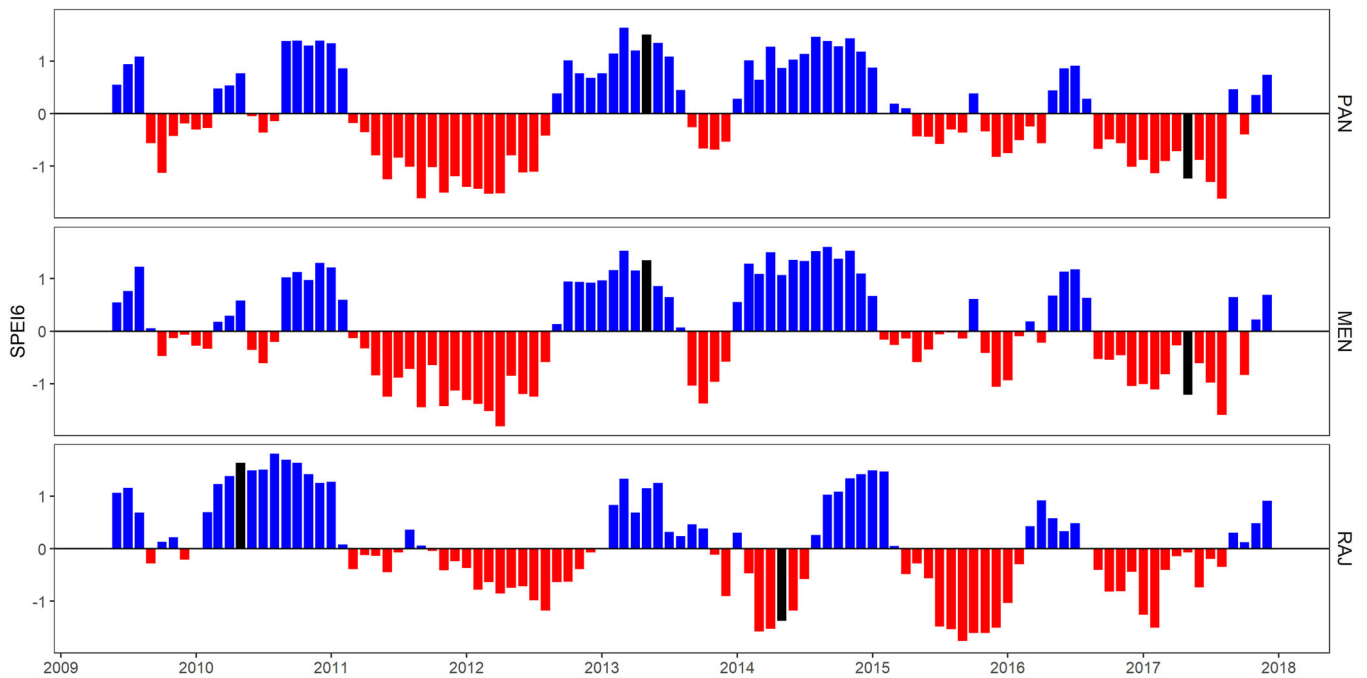


FIGURE 2 Six-month Standardised Precipitation-Evapotranspiration Index (SPEI6). Black colour indicates the wettest and driest early seasons (previous December–current May) in the period between 2009 and 2017. [Color figure can be viewed at wileyonlinelibrary.com]

sites and species (Figure 4, Figure S3). All phloem parameters of *F. sylvatica* at wetter sites (PAN and MEN) were negatively correlated with high temperatures in the previous growing season (July–December), while xylem ring width showed a positive response. The opposite pattern was seen at the dry site (RAJ), where warm temperatures in the previous year resulted in larger phloem conduits and phloem widths but reduced xylem ring width. Furthermore, higher temperatures in the current year resulted in smaller phloem conduits and phloem/xylem increment widths at the dry site, RAJ, while higher precipitation rates were positively correlated with all phloem parameters and xylem ring widths. Higher precipitation rates at wetter sites, especially PAN, negatively affected phloem increment and conduit growth.

For *P. abies*, higher precipitation in the current and previous growing seasons generally correlated negatively with phloem conduits and increment widths, except at PAN, where early phloem width and conduit areas correlated positively with higher precipitation in the previous year. In contrast, the effect of temperature was generally positive for dry site and negative for the two sites in Slovenia that received higher rainfall.

These differences in weather-growth relationships suggest heterogeneous growth strategies of coexisting *F. sylvatica* and *P. abies* (Figure 4). In many cases, we found a contrasting response of phloem parameters and xylem ring width, i.e., the temperature of the previous year for *P. abies* and precipitation of the current year for *P. abies* at moist sites (PAN and MEN). Of all the parameters analysed, regardless of tree species or location, an absence of a climate signal was observed in the early phloem parameters. To further assess the effects of weather conditions (especially precipitation) on xylem ring

width and phloem anatomy, we checked the impact of precipitation on the measured parameters in two extreme years, the driest and the wettest in the analysed period 2010–2017 (Table S3, Figure 5). Based on calculated SPEI6, the dry year for PAN and MEN was 2017 and for RAJ 2014. The wet year for PAN and MEN was 2013 and for RAJ 2010 (Figure 2). Only *F. sylvatica* from the driest site, RAJ, responded positively to increased precipitation (Figure 5).

Principle component analysis (PCA), that is, PC1 and PC2 components, revealed differences in the site-specific response of the xylem increment width and phloem variables to weather conditions (temperature and precipitation) (Figure 6). The position of PC1 and PC2 loadings generally indicated similar climatic effects for PAN and MEN (Figure 6), while *F. sylvatica* from the RAJ site showed the most unique response to climate. These results were expected, as the PAN and MEN sites have more similar climatic conditions compared to the RAJ site (Figure S1). For many parameters, for example, phloem ring width, early phloem width and area, late phloem width; the loadings of *F. sylvatica* and *P. abies* from RAJ form an angle close to 180°, indicating the contrasting climate effects. PAN and RAJ were generally the most different in the response of an individual anatomical parameter to weather conditions. For some analysed phloem variables (e.g., late phloem width), site-variability was small, while for others (early phloem sieve element areas), the variability was much greater, both for precipitation and temperature. Graphical depiction of phloem structural differences in *Fagus sylvatica* and *Picea abies* as a result of extreme dry and wet years. *F. sylvatica* at RAJ consistently showed different patterns compared to PAN and MEN (Figure 7).

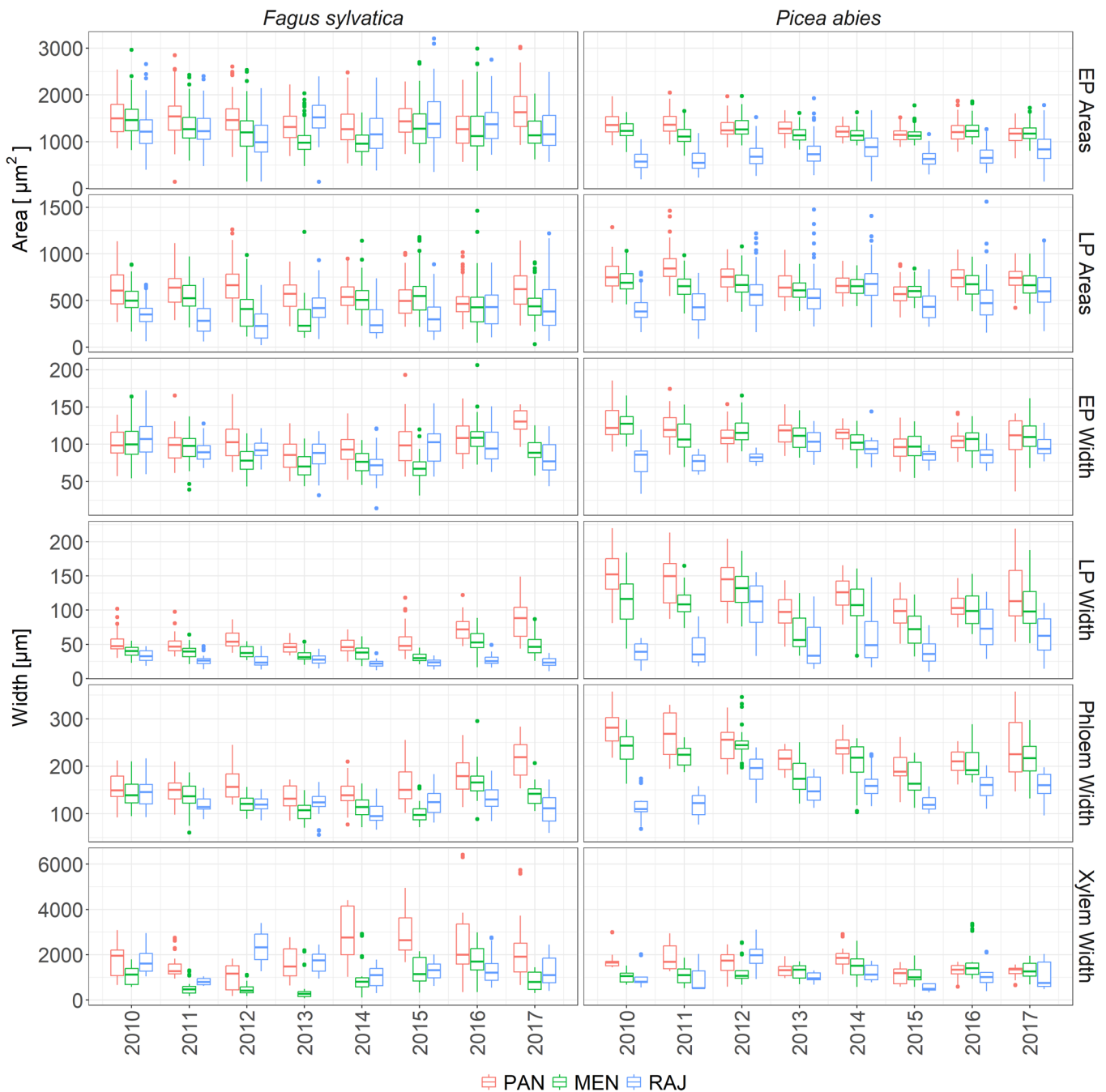


FIGURE 3 Boxplots of studied phloem and xylem parameters, shown separately for each tree species, site and year. EP, early phloem; LP, late phloem. [Color figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

Overall, we found site-, species- and tissue-specific responses to local weather conditions of the two co-existing temperate tree species from three contrasting European temperate sites. Our analyses showed that precipitation and temperature in the previous growing season seem to be most important for the size of early phloem conduits in *P. abies*. In the case of *F. sylvatica*, in addition to temperature in the previous growing season, precipitation in the current growing season also greatly affected

the size of the early phloem conduits. At PAN and MEN, well supplied with precipitation, both tree species responded in the same direction to changes in temperature and precipitation. However, the response of the widths of xylem and phloem growth rings to identical weather conditions was contrasting at these two sites. In contrast, at the driest site, RAJ, growing conditions seemed to be most restrictive for the radial growth of the selected tree species, which was evident from the narrowest xylem and phloem increments and their synchronised response to weather conditions.

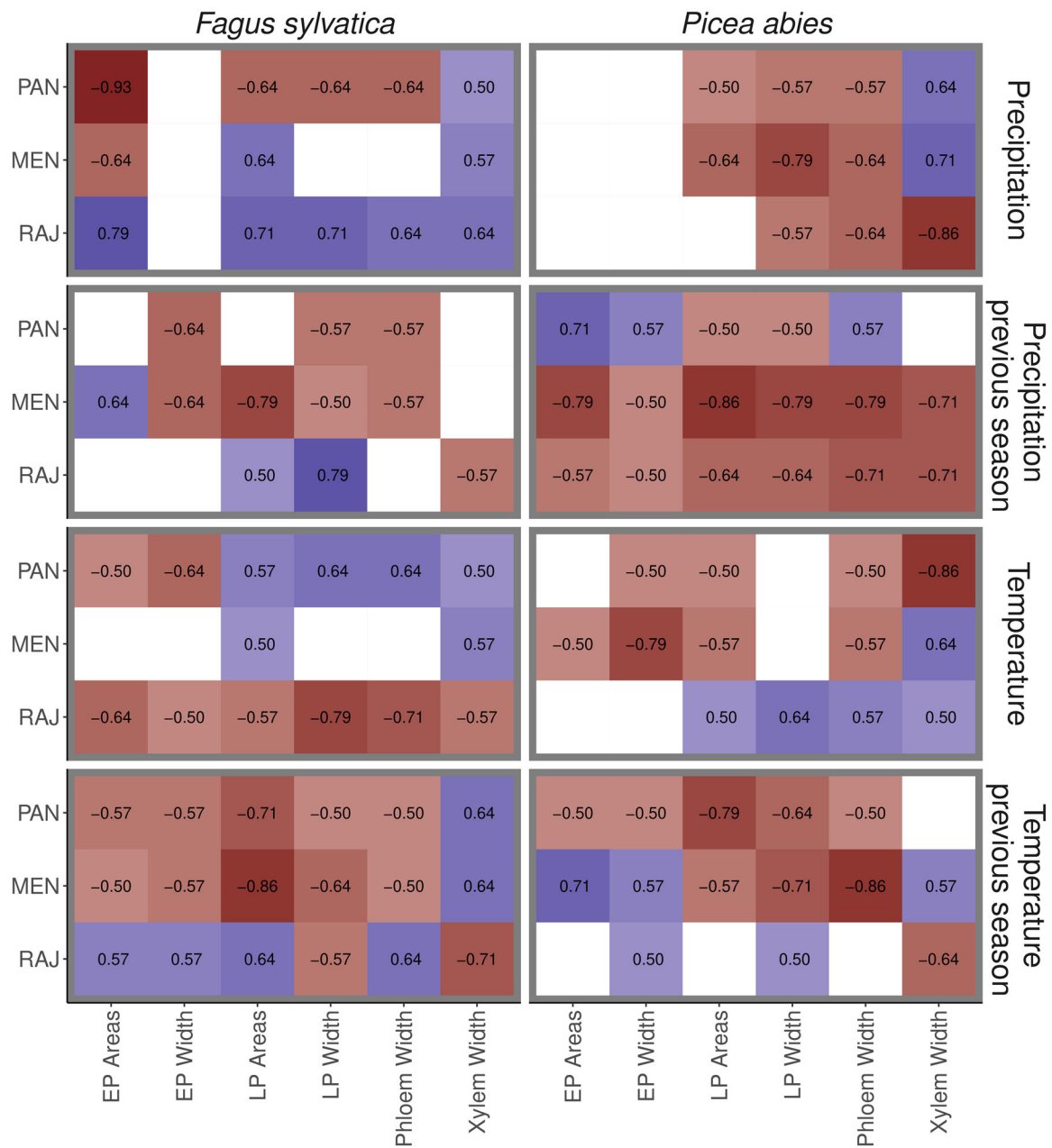


FIGURE 4 The highest absolute Kendall's tau correlation between mean temperature and precipitation sum, extracted separately for the previous and current growing seasons. Kendall's tau correlation is a nonparametric measure of the strength and direction of association that exists between two variables. Only significant correlations with $p < 0.05$ are shown. EP, early phloem; LP, late phloem. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

Although the number of conduits in early phloem is relatively stable, the area of early phloem and late phloem conduits as well as phloem ring widths were confirmed to be highly controlled by weather factors, which highlights the link between climate and key functions of the phloem, such as photosynthate translocation and storage (Savage et al., 2016). The species- and site-specific sensitivity of phloem traits to precipitation and temperature as well as high interannual variability of phloem anatomy confirmed the high plasticity of radial growth patterns in both species to ensure optimal

functioning under the given conditions. As explained in more detail below, all three set hypotheses (H) were only partially confirmed (H1 and H3) or rejected (H2).

H1. Phloem structure differed between the two species and sites due to a species-specific plastic response of phloem traits to local conditions (moist and wet sites). This plasticity is reflected in particular in late phloem traits, irrespective of species. (Partly confirmed)

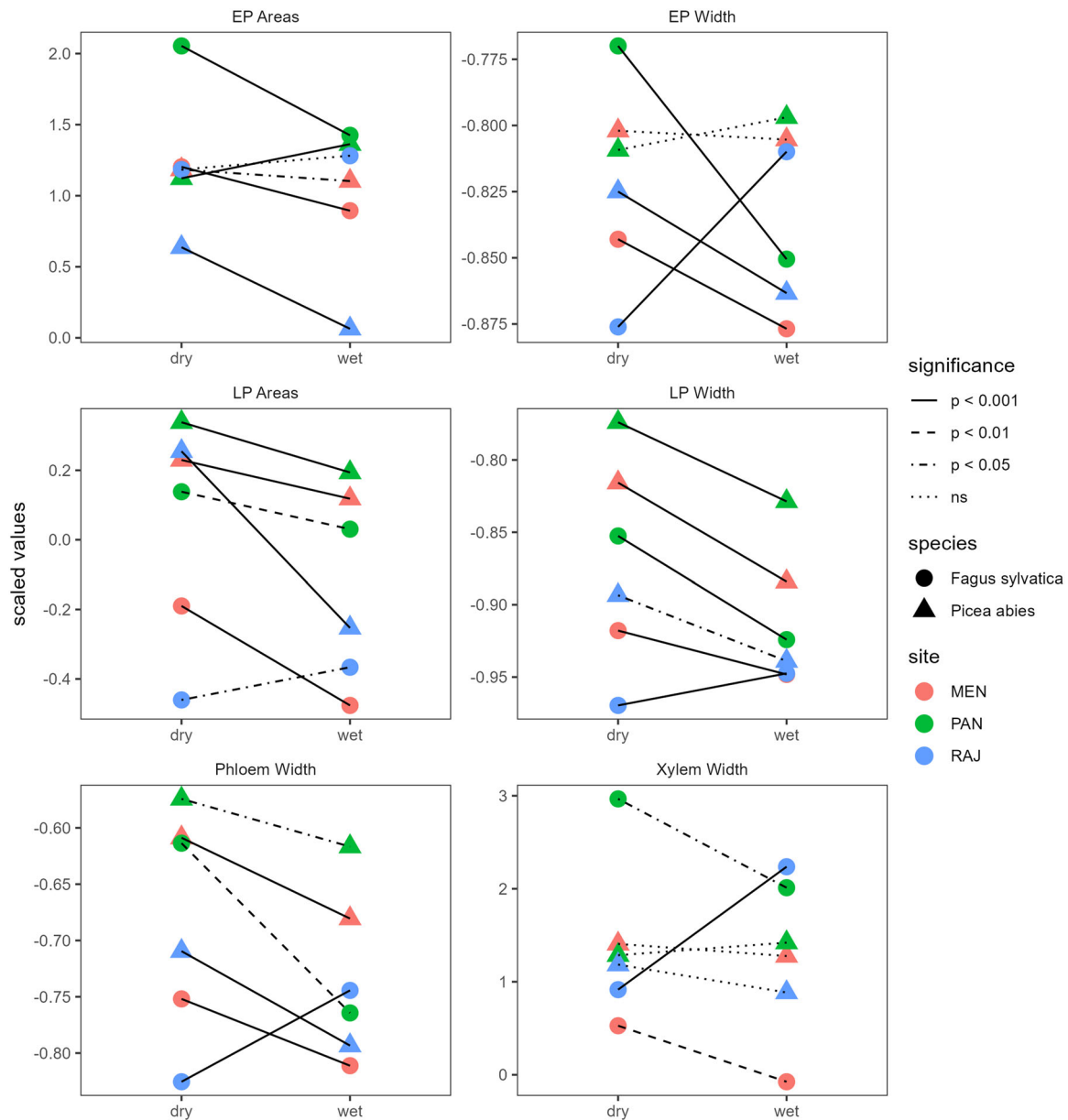


FIGURE 5 Comparison of phloem parameters and xylem ring width between the driest and the wettest seasons in the period 2010–2017. The y-axis is scaled for visual purposes. Drought was characterised based on the SPEI6 ending in May. EP, early phloem; LP, late phloem; FASY, *Fagus sylvatica*; PCAB, *Picea abies*. [Color figure can be viewed at wileyonlinelibrary.com]

The results support our assumption that the phloem structures of *F. sylvatica* and *P. abies* differ due to their different sensitivity to weather (site) conditions (Figure 4). However, the 8 years of data and more detailed analyses refuted our surmise that early phloem is relatively stable, as we had previously concluded for both species from a shorter data set (max. three years) in which only the number of early phloem cells was assessed (Gričar et al., 2015; Prisljan et al., 2013). Indeed, the constant number of early phloem (i.e., 3–5 cell layers) was reconfirmed, but additional measurements of conduit area showed that conduit size varies greatly between sites and years, affecting the final width of early phloem and especially expected hydraulic conductance of the phloem (H1 partly confirmed).

The radial conduit size is generally related to turgor pressure in the expanding cells, which is controlled by water potential, osmoregulation and hormonal signals (Jyske & Hölttä, 2015). In *P. abies*, the early phloem conduits were 40% narrower at the driest site RAJ than at PAN and MEN, while the environmental differences at the study sites were not reflected in the size of early phloem conduits in *F. sylvatica*. The impact of weather conditions on conduit size is addressed in the next subchapter. In both tree species, the area of early phloem conduits was larger, and their number was less variable than in late phloem, which is consistent with previous studies (Jyske & Hölttä, 2015; Petit & Crivellaro, 2014). The transition from early phloem to late phloem was identified by the presence of a tangential

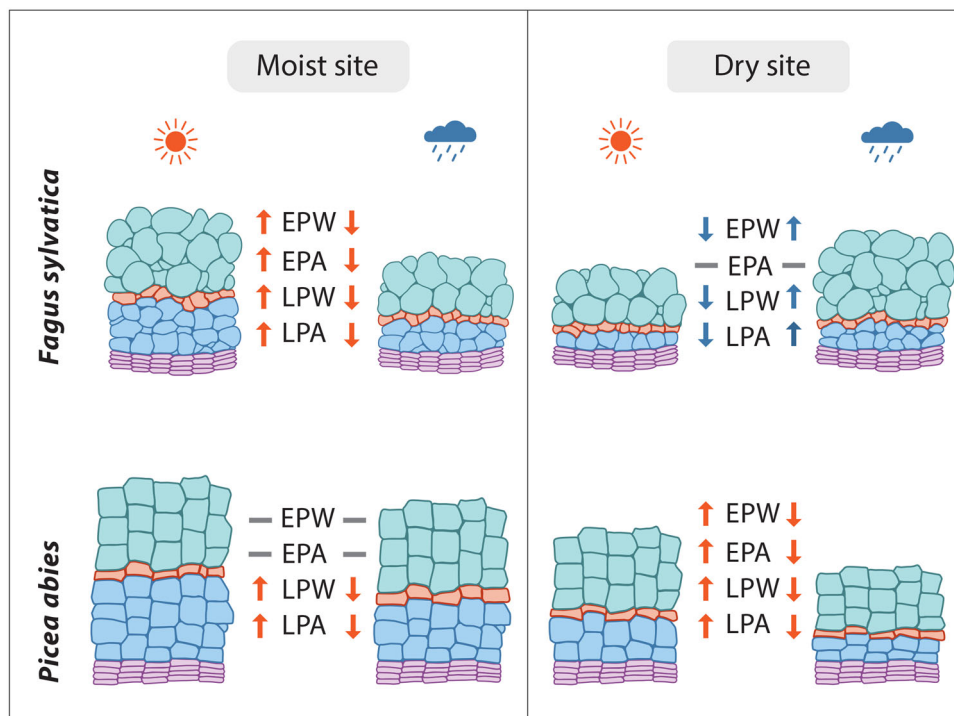


FIGURE 7 Graphical depiction of phloem structural differences in *Fagus sylvatica* and *Picea abies* as a result of extreme dry (sun) and wet (rain cloud) years. An arrow pointing up indicates an increase in conduit size/tissue width, whereas an arrow pointing down denotes a decrease in conduit size/tissue width. The grey line indicates an unchanged dimension/width. Blue-green colour denotes early phloem cells, blue colour denotes late phloem cells, orange colour denotes axial parenchyma separating early and late phloem parts, and pink colour indicates cambium cells. *Fagus sylvatica* from the Czech Republic showed consistently different response to precipitation. EPW, early phloem width; EPA, area of early phloem conduits; LPW, late phloem width; LPA, area of late phloem conduits. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

band in the axial parenchyma in both species (Figure 1). Shortly after the end of cambial cell production, the collapse of early phloem sieve elements formed in the current year began (Gričar & Prislan, 2022). In contrast, late phloem sieve elements maintained their shape until the following spring. Smaller conducting elements and the presence of axial parenchyma support the dominant storage (sugars) role of late phloem (De Schepper et al., 2013; Prislan et al., 2018b), in which the accumulation of sugars continues in autumn, after the completion of radial growth (Gričar et al., 2018; Rezaie et al., 2023). Despite the division of the predominant roles of early and late phloem in a tree (conducting and storing), the importance of the conductive function of the latter becomes obvious in early spring, at the beginning of cambial cell production, when most of the conduction is done by the late phloem formed in the previous growing season (Gričar & Prislan, 2022). From the seasonal variations in the structure of the noncollapsed phloem, it can be concluded that the predominant role

of conduction or storage most likely changes in the case of the late phloem in contrast to the early phloem, where the conductive function always seems to predominate.

Even though analyses of the structure and width of the noncollapsed phloem were not part of the present study, these characteristics have a considerable influence on the conducting capacity of the phloem. Indeed, it was recently shown that the seasonal dynamics of phloem formation and the collapse of sieve elements significantly affect the structure and width of the noncollapsed phloem in temperate species regardless of wood porosity (Gričar & Prislan, 2022). Noncollapsed phloem is considered to be a conducting tissue in which the sieve elements are still functional (Angyalossy et al., 2016). In addition to the sieve elements, the parenchyma cells are involved in the storage and mobilisation of starch and other metabolites. In collapsed phloem, conducting capacity is lost; the sieve elements are collapsed. However, the

FIGURE 6 Loading plot of first two principal components (Dim1 and Dim2) of PCA showing a site-specific response of the xylem ring width and phloem anatomy variables to temperature (a–f) and precipitation (g–l). Positively correlated variables point in the same direction, negatively correlated variables diverge and form an angle close to 180°, while unrelated variables meet each other at approximately 90°. The colour of the arrows indicates the % of the variable contribution. The length of the arrow shows the association of each anatomical variable with each component (PC1 and PC2). EP, early phloem; FASY, *Fagus sylvatica*; LP, late phloem; PCAB, *Picea abies*. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

parenchyma cells continue to function and retain their meristematic capacity in the collapsed phloem. Part of the parenchyma cells are involved in dilation growth, sclerification and the accumulation of secondary metabolites (Angyalossy et al., 2016). The noncollapsed phloem is generally composed of the phloem cells formed in the previous and the current growing seasons with the width and proportion of both increments changing seasonally (Gričar & Prislan, 2022). At the onset of the growing season, the phloem increment of the previous year is crucial for the function and account for over 80% of the noncollapsed phloem, whereas at the end of the growing season, the phloem increment of the current year constitute the majority of the noncollapsed phloem. In addition, the noncollapsed phloem width is narrowest in spring, before cambial reactivation, and widest at the time of cessation of cambial activity. The width of the noncollapsed phloem is the result of two opposing processes, cambial cell production and collapse of older phloem cells. The unsynchronised course of these two processes leads to seasonal variation in the noncollapsed phloem width (Gričar & Prislan, 2022). The formation of new phloem cells by the cambium is seasonal in temperate tree species and contribute positively to the width of the noncollapsed phloem. In contrast, the collapse of older phloem tissue may be a continuous process, which can occur throughout the year with different intensity. Its effect on the noncollapsed phloem width is even more pronounced during the winter when no new phloem cells are produced to 'compensate' for its progress.

At all three sites, late phloem at the driest site RAJ was narrowest in both species in all study years (Table S2, Figure 3). Consistently narrowest late phloem width and smallest sieve element area probably affected the trade-off between conducting (conduits) and storing functions (parenchyma) of the annual phloem increment, since the axial parenchyma fraction, being mainly located in late phloem, is consequently also reduced, as was evidenced from the discontinuous tangential band of axial parenchyma. This may also be related to the different duration of the two functions; phloem conduits are functional only for one to two growing seasons, while axial parenchyma usually continues to serve as storage tissue in the nonconducting living phloem for several years (Franceschi et al., 2000). Thus, the priority of the formation of conducting cells over storage cells in thereby evident. However, such a long-term decrease in the amount of phloem axial parenchyma could amplify tree susceptibility to drought stress or pathogen attack (Rosell et al., 2014; Secchi et al., 2017). In Norway spruce, for example, axial phloem parenchyma accumulates stilbenes, secondary metabolites with antifungal and antibacterial properties, which are vital to resistance to pathogens (Jyske et al., 2020). Nevertheless, the role of parenchyma in different tree processes may also depend on its location (root vs. stem, xylem vs. bark) (e.g., Richardson et al., 2015).

Although the parenchyma fraction in phloem and xylem was not the subject of our research, this aspect would be necessary to include in future analyses when assessing the trade-off between different needs in a tree, such as growth, storage and defence. In particular because nonstructural carbohydrates are stored in the parenchyma, these cells are recognised as a valid proxy for the assessment of

potential storage reserve capacity in tree tissues and organs (Rezaei et al., 2023). The xylem and bark axial and ray parenchyma fraction greatly depends on tree species. Angiosperms generally have higher quantities of parenchyma than gymnosperms, with their different distribution in tree tissues/organs. In angiosperms, xylem contains a significant amount of axial and ray parenchyma (Morris et al., 2016), while in gymnosperms, having generally a negligible proportion of parenchyma in xylem, bark parenchyma is crucial for storage and transport of nonstructural carbohydrates, nutrients and water (Spicer, 2014). However, in *F. sylvatica*, with only one periderm and a space-saving strategy of the formation of new phloem cells (Holdheide, 1951; Prislan et al., 2012), the phloem parenchyma fraction is assumed to be much smaller than in *P. abies*, with thick bark and wide nonconducting phloem.

H2. At the three sites, temperature and precipitation in the previous and current year have different effects on the phloem anatomy of the two species. However, at an individual site, year-to-year variability in the phloem structure is small and is mainly expressed in extreme years (i.e., summer drought). (Rejected)

Both the general correlation analysis between phloem anatomical traits and precipitation and temperature (Figure 4), and the comparison of extreme wet and dry years (Figures 5 and 7) rejected our hypothesis H2. The environmental conditions at the three sampling sites are different; PAN and MEN are generally well supplied with precipitation (Prislan et al., 2018a), especially at the higher elevation site, MEN, where summer drought events are currently rarer compared to the other two plots. However, PAN and MEN differ in temperature. The site RAJ lies between PAN and MEN in terms of altitude. However, it is at a higher latitude and the average rainfall is half that of the other two temperate sites. These differences affected the sensitivity of phloem traits to temperature and precipitation. Generally, at the warmest site, PAN, *F. sylvatica* and *P. abies* had higher values for all phloem parameters than at the other two sites. At the driest site, RAJ, *P. abies* had consistently lower values for all measured phloem parameters. For *F. sylvatica*, the late phloem part seemed to be most negatively affected by the conditions at this site, while the values for the early phloem (conduit area and tissue width) were the lowest at the high elevation site MEN.

Precipitation and temperature in the previous growing season seemed to be most important for the size of early phloem sieve cells in *P. abies*. In the case of *F. sylvatica*, in addition to temperature in the previous growing season, precipitation in the current growing season also greatly affected the size of the early phloem conduits. In both species, the response of the conduits differed depending on the site. In *P. abies*, PAN (warmest site) stood out and in *F. sylvatica* RAJ (driest site) (Figure 4). Furthermore, phloem structural differences in *F. sylvatica* at RAJ consistently showed different patterns compared to the other two sites (Figure 7).

Differences between species in response to weather conditions can be partly ascribed to foliar characteristics, with *P. abies* being an evergreen conifer and *F. sylvatica* a deciduous broadleaved tree

species, thereby affecting seasonal variations in photosynthesis and transpiration in needles/leaves. Both processes influence sugar transport in the phloem and water transport in the xylem, since both vascular tissues are strongly hydraulically coupled and interdependent (Holttä et al., 2010). Furthermore, the different timing of leaf and cambial phenology may also lead to different climatic influences on the early phloem conduits. Site conditions can strongly influence cambial rhythm and thus phloem development patterns (Gričar et al., 2015; Prisljan et al., 2013). Different responses to weather within both species at the selected sites and high interannual variability of phloem anatomy demonstrate the high plasticity of radial growth to adapt phloem structure to local environmental conditions for assuring ensure optimal tree functioning (Gričar et al., 2016). This has already been noted previously in xylem anatomy in both species (e.g., Arnič et al., 2021; Castagneri et al., 2017; Stojnic et al., 2013). In addition to a weather influence, the size of phloem conduits may also depend on tree age and size, environment (humid vs. dry), tree species (desiccation-avoidant vs. desiccation-tolerant) or tree condition (healthy vs. dying) (Dannoura et al., 2019; Kiorapostolou et al., 2020; Sevanto, 2018).

Not only climatic conditions (especially precipitation), soil properties, especially water holding capacity, may also have a major influence on radial growth patterns and tree anatomy (Gričar et al., 2018), so these data should be included in future studies whenever possible. Furthermore, potential genetic differences of *P. abies* and *F. sylvatica* between the three sites may also be a reason for a different phloem anatomical response to weather conditions, since numerous wood anatomical studies in international provenance *F. sylvatica* trials have clearly shown that different provenances can respond differently to identical climatic conditions (e.g., Eilmann et al., 2014; Krajnc et al., 2022). These studies confirmed that the plastic response of radial growth in *F. sylvatica* adjusts the vascular structure to given environmental conditions (Stojnic et al., 2013) and we can only assume that this also applies to phloem.

H3. Because of the different internal and external controls on the formation of xylem and phloem and the different priorities of their formation for the tree functioning, weather factors affect the annual width of the two tissues differently. This suggests different adaptation strategies of phloem and xylem formation to local conditions (moist and wet sites). (Partly confirmed)

Similarly to phloem, high interannual variability of xylem-ring width was detected at all sites (Table S2, Figure 3). In *F. sylvatica*, the differences in xylem width were significant between all sites and years, while in *P. abies* the differences were expressed only in certain (extreme) years at the individual site (Table S3, Figure 5). The comparison of phloem structure and xylem-ring width in the two most extreme years in relation to annual precipitation showed species- and site-specific differences in the response to rainfall shortage/excess. At PAN and MEN, well supplied with precipitation, a contrasting response of phloem and xylem increment widths to the

weather conditions was found for both species, while at the driest site RAJ it was generally the same (H3 partially confirmed) (Figures 4 and 6). The contrasting response of xylem and phloem annual increments to identical weather conditions at PAN and MEN demonstrate that phloem and xylem formation patterns are under different climatic control. Although with different significance levels, the response of the same tree species to temperature and precipitation was mainly coherent at PAN and MEN, while it was generally different for RAJ having much lower annual rainfall.

It has been previously shown that water availability is not a limiting factor for the radial growth of *P. abies* and *F. sylvatica* at the two Slovenian sites, with an annual amount between 1300 and 1500 (Gričar et al., 2015; Prisljan et al., 2019), while this is not the case for RAJ, at least not for all growing seasons (Giagli et al., 2016). A previous study by Prisljan et al. (2019) showed that xylem growth of *F. sylvatica* at PAN and MEN is generally accelerated in the case of a higher minimum temperature in the previous autumn and current spring. In addition, precipitation at the beginning of the current year has a positive effect on xylem growth. In contrast, a higher maximum temperature in August and September has a negative effect on xylem increments. Recent dendrochronological studies by Arnič et al. (2021) on *F. sylvatica* at three optimal sites in Slovenia showed positive and negative effects of winter maximum temperature and precipitation, respectively, on xylem-ring width. In the case of *P. abies*, sampled at twelve sites in Slovenia differing in climate regimes and ranging in elevation between 170 and 1300 m a.s.l., different climate signals in xylem-ring widths were observed (Jevšenak et al., 2021). Xylem-ring widths of *P. abies* from drier, low-altitude sites correlated negatively with increasing summer temperature and positively with higher spring precipitation, while the response was reversed at wetter, higher-altitude sites. Based on previous and current findings, it can be concluded that the response of xylem and phloem formation in both species to local conditions is plastic and site-dependent. By influencing the seasonal patterns of xylem and phloem development, water availability (i.e., moist or dry sites) appears to be the most important determinant of tissue- and species-specific responses to local weather conditions (Figure 7).

Due to a substantially lower annual amount of precipitation, RAJ is more prone to potential water shortage compared to PAN and MEN. Consequently, the site is more subjected to a potential lack of water supply (Table 1, Figure 2). As already mentioned above, it is also necessary to take into account the soil properties, which greatly affect water availability at the site. This aspect was demonstrated in a previous study in which, under identical weather conditions, soils with higher water holding capacity enabled much more intense radial growth of *Quercus pubescens*, resulting in 40% and 60% wider xylem and phloem annual increments, respectively (Gričar et al., 2018). Despite lacking soil data, it can be concluded that the dry site RAJ is more restrictive for the radial growth of the selected tree species, which is evident from the narrowest xylem and phloem increments and a more synchronised response of development of both tissues to weather conditions (Figure 4). Common climatic signals stored in the xylem and phloem may also be an indicator of more constraining growing conditions at RAJ. Namely, trees from treeline or xeric

habitats show higher sensitivity to temperature or water availability as primary limiting factors for growth. In contrast, climatic signals in trees from favourable sites are less clear, since their growth is influenced by a multitude of biotic and abiotic factors (Carrer et al., 2012; Fischer & Neuwirth, 2013). Furthermore, a previous study on xylem formation patterns and vessel features in *F. sylvatica* from RAJ showed different responses to climatic factors in two contrasting years (2010 and 2011), characterised by different amounts of precipitation (Giagli et al., 2016). In a normal year, 2010, precipitation was not critical for xylem ring widths and vessel size, while in the dry year of 2011, the sensitivity of both anatomical features to precipitation was much higher. Our findings on radial growth of the same tree species reaffirm how the influence of genetics and environment on tree growth and xylem and phloem development can change depending on more or less favourable growing conditions at different sites or in different years.

Although the temporal dynamics of phloem and xylem formation in both species were not the subject of the present study, previous multi-year data on the same trees of both species have shown that the intensity of their growth is not synchronous, nor are their final annual widths (Prislan et al., 2013; Gričar et al., 2014). For example, the period of most intense phloem growth occurs 1 month and 2–5 weeks earlier than xylem growth in *F. sylvatica* and *P. abies*, respectively. In addition, differentiation (i.e., cell expansion) of the outermost 1–2 layers of phloem cells occurs about 1 month before cambial activity (Gričar & Čufar, 2008). These different temporal patterns in xylem and phloem formation must be considered when monitoring stem growth using continuous methods such as dendrometers (Zweifel et al., 2016). These methods always record both the new cells in the phloem and the new cells in the xylem, which cannot be distinguished per se, and which together also define the length of the growth period (Zweifel et al., 2021). This contrasts with wood anatomical studies, which refer exclusively to the xylem. The results presented here provide further important indications of the extent to which the two tissues differ in their temporal and spatial development. Moreover, numerous age-related processes in phloem anatomy explained in the previous subchapter, combined with cork cambium activity and changes in older bark tissues, present additional complexity in separating and influencing different processes on intra- and interannual variations in tree stem size, when tree species, tree age, tree vigour and site conditions are taken into account.

5 | CONCLUSIONS

Our study, based on a unique 8-year database of phloem and xylem anatomy data, showed that the phloem traits analysed may be controlled by different weather factors than those controlling the xylem traits; however, these relationships appear to be species- and site-specific. In particular, water availability (i.e., moist or dry sites) seems to act as a major constraint on radial growth and determining factor of tissue- and species-specific responses to local weather conditions. In both species, phloem transport conductivity is mainly controlled by (1) changing the size of the

early phloem conduits rather than their number and (2) by the number and size of the late phloem conduits. The analysed phloem traits in both species showed high sensitivity of phloem traits to precipitation and temperature; however, the responses were species- and site-specific. The high variability of the selected phloem traits and the divergent response to climate depending on site and/or species indicate that phloem structure contributes significantly to tree life strategies; consequently, it offers great potential for future research in this direction. As the present study shows, the number of sites and years studied has an impact on the reliability of the results. In particular, the use of a longer data set (i.e., as many years as possible) is crucial for assessing the response of phloem formation to local weather conditions. This approach enables capturing extreme weather events, which are pivotal for revealing how a given tree species responds and adapts phloem structure to ensure optimal functioning under contrasting (favourable and adverse) conditions (De Kroon et al., 2005). Answering such questions requires multi-year repeated sampling of phloem tissues at comparable developing phases and times of the year (Gričar & Prislan, 2022), as well as laborious section preparation and quantitative analyses of phloem anatomy, further confirming the importance and originality of this study, since very few phloem anatomical data are currently available on a global scale. Data based on direct measurements of phloem traits, available for different (co-existing) tree species, locations and tree properties (tree part, age and vitality) will allow more reliable modelling and prediction of intra- and interannual changes in phloem functioning as part of strategies of tree survival in various environments (Savage, 2020).

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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