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Expanding Blue Intensity applications: Exploring compression wood proportions in cross-sections of treeline *Picea abies* seedlings

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ABSTRACT

Blue Intensity (BI) has been widely used as a proxy for wood density in dendrochronology, yet its applications remain underexplored in treeline ecology. Moving beyond traditional growth ring analyses, we expanded the Blue Intensity scope by quantifying the high wood density proportions (HighWD), including two HighWD subcategories: latewood and compression wood, in the stems of *Picea abies* seedlings from Central European treelines. We used BIcounter, a novel tool based on the CooRecorder BI algorithm, to quantify pixel distributions across blue light intensities and estimate HighWD areas as proportions of the total cross-section. Our approach combined BIcounter with densitometry analysis in CooRecorder (both based on BI measurements), allowing us to study compression wood occurrence as a continuous variable, which enhances statistical models' robustness. To estimate and compare means and variance of HighWD, latewood and compression wood proportions, and to quantify those estimates' uncertainty, we constructed Bayesian generalized linear models. HighWD occupied nearly half of the cross-section of treeline seedlings (Mean = 0.39, S.D. = 0.04) and did not differ between treeline sites. Stem eccentricity was generally high and did not correlate with compression wood proportions. As compression wood accounted for up to 43 % of the treeline seedlings' stem cross-section, its occurrence could affect growth and survival, highlighting the importance of considering compression wood proportions in future ecological research. With this study, we outline the potential of BI for applications in and dendroecology, and suggest that future research could explore its use in other dendrochronological sub-disciplines, such as dendrogeomorphology.

1. Introduction

Blue Intensity (BI) has been widely used as a proxy for wood density in dendrochronology, yet its applications remain underexplored. The new approach introduced in this study expands the traditional applications of BI by shifting the method utilization from cores and growth ring analysis (Fig. 1a) to evaluating entire cross-sections (Fig. 1b-d). We used a novel program (BIcounter; Fig. 1e) based on the CooRecorder™ BI algorithm to analyze stem wood density variations of seedlings in European treelines. Combining BI tools (densitometer in CooRecorder, Fig. 2a, and BIcounter, Fig. 2b), enabled us to analyze stem compression wood proportions by treating it as a continuous variable, rather than a categorical variable (i.e. indicating presence or absence, or describing abundance using ordinal scales).

There are currently several methods to measure wood density (WD) variations. Some examples include the gravimetric method (Björklund et al., 2021b, Williamson and Wiemann, 2010); X-ray densitometry (Schweingruber et al., 1978; Björklund et al., 2019); computed tomography (De Mil and Van den Bulcke, 2023); reflected-light imaging based on microscopy and photography (e.g., García-Hidalgo et al., 2022; Rydval et al., 2024; Sheppard et al., 1996); anatomical WD (Pritzkow et al., 2014); and more recently, BI (Kaczka and Wilson, 2021). BI is a relatively new and simple technique that quantifies the amount of blue light absorbed by the surface of wood in scanned images, which correlates strongly with wood density (Björklund et al., 2024).

BI represents a promising approach among the available methods, as it allows for the quantitative assessment of WD variations. However, its potential to expand its reach is currently restricted by certain limits in terms of its application. One of these limits is that it has almost exclusively been applied in studies focusing on adult trees, which creates a

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bias toward the oldest and largest individuals in a forest population. Furthermore, existing software is primarily designed to measure BI parameters at the tree-ring level (e.g., Campbell et al., 2011; Rydval et al., 2014). While various programs and approaches exist for measuring BI, including WinDendro™ (Regent Instruments, Canada) and LignoVision™ (Rinntech, Germany), CooRecorder™ (Cybis Elektronik & Data AB, Sweden) represents the most widely used software package (Björklund et al., 2024). CooRecorder measures BI at the annual growth ring level by analyzing segments or small rectangular regions ("frames"; e.g., Fig. 1a) along cross-sections of tree stems obtained from increment cores (Cybis, 2024; Heeter et al., 2022). While the study of annual growth rings is valuable for specific analyses, they only represent a small subdivision of the wood within the stem, which functions as a unit with structural variations and connections (Wason et al., 2019; Pretzsch, 2021; Bouda et al., 2022).

Seedlings from treelines, which represent a key component of those ecosystems (Lett and Dorrepaal, 2018), are ideal for exploring BI beyond its traditional applications and testing this new BI method. Because seedlings are small, whole cross sections can easily be obtained (Fig. 1b-c) and variations in their wood density have not been studied before. Additionally, the environmental conditions of growth at treeline sites (such as strong winds, steep slopes, and thick winter snowpack) cause mechanical stress on seedling stems. This continuous mechanical stress on treeline seedlings induces modifications in their stem shape, as well as their wood anatomy and chemical composition (Gardiner et al., 2016; Thibaut and Gril, 2021). As a result, it is common for treeline trees to produce reaction wood (*i.e.* compression wood in gymnosperms) in the parts affected by mechanical stress, and to have eccentrically shaped

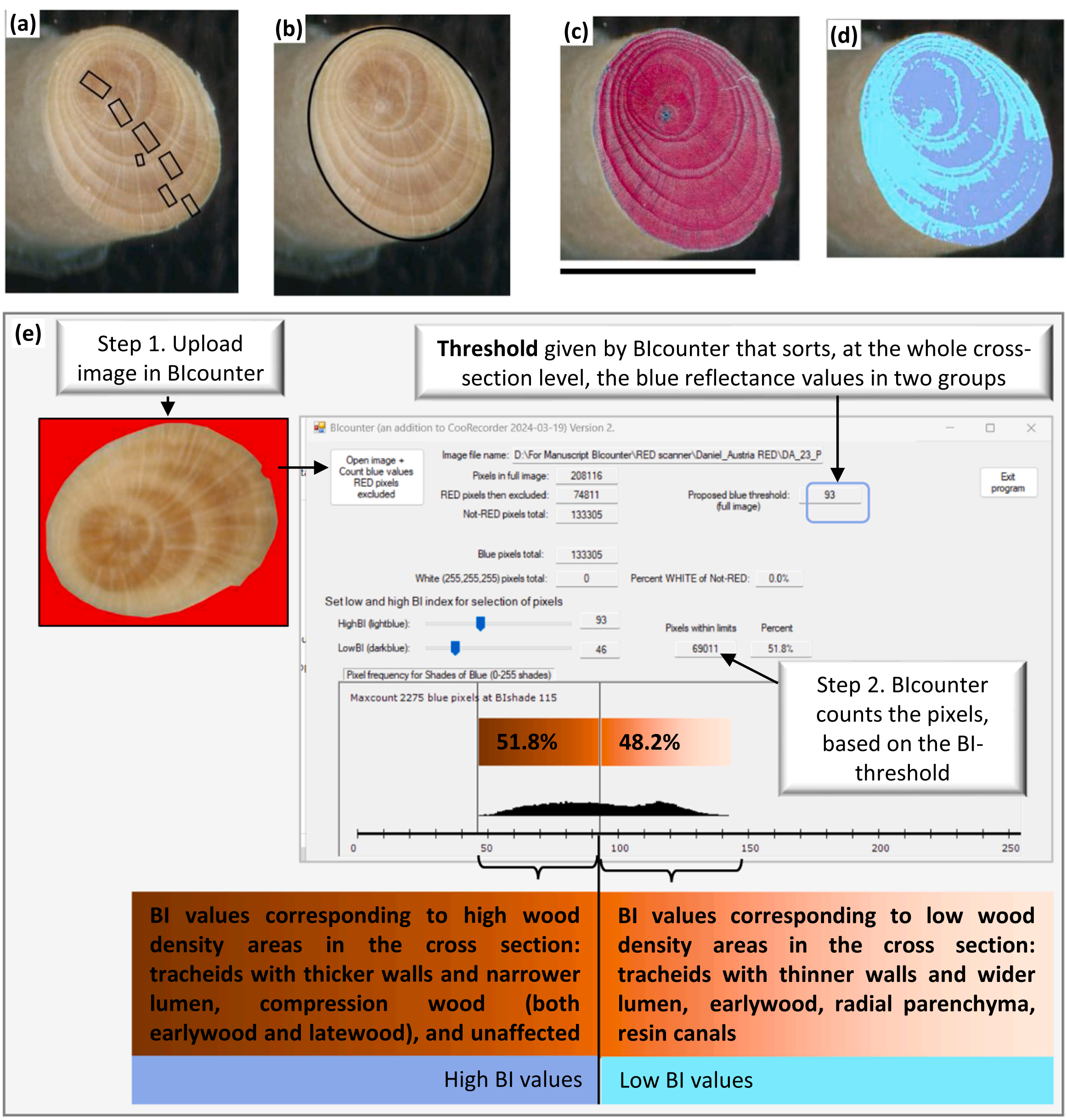

Fig. 1. Sample of the stem of a *Picea abies* seedling, with high proportion of compression wood, collected from the treeline at the Daniel site (DA23) in Austria; the sample was analyzed using the BIcounter program. (a) cross-section scanned with an EPSON scanner, rectangles represent CooRecorder frames for measuring Blue Intensity (BI) at the tree ring level. (b) ellipse represents the modified area for BI measurements at the whole-cross level. (c) cross anatomical section (scanned with a NIKON microscope with 20X zoom) used to verify the presence of severe compression wood; scale = 0.2 cm. (d) an image (generated with densitometer in CooRecorder) that solely provides an approximate visual reference of areas with high wood density (HighWD; dark blue) and low wood density (LowWD; light blue) sorted with the BI color segmentation algorithm. (e) Steps of BI measurements using the BIcounter program, to assess the proportion of high wood density areas (HighWD = unaffected latewood + compression wood in both early- and late-wood) across the entire cross-section of the treeline seedling stem.

stems (Palombo et al., 2018). Compression wood can develop in earlywood and latewood, and exhibits structural, functional, and chemical modifications related to wood density (WD), strength and elasticity (Timell, 1982; 1986; Mayr and Cochard, 2003; Mayr et al., 2005; Fernandes et al., 2017; Lu et al., 2021; Yang et al., 2024). This results in significant variations in WD, although no cheap and fast objective methods were available to quantify this variation in the past.

The main objectives of this study were: (1) to assess variations in stem wood density (WD) in seedlings collected from seven Central European treelines using a novel BI-based approach; and (2) to introduce and demonstrate our new BI-based technique, which includes the first implementation of a new BI tool (BIcounter), used in conjunction with the densitometer tool available in CooRecorder (both based on BI measurements). CooRecorder software has been widely used for

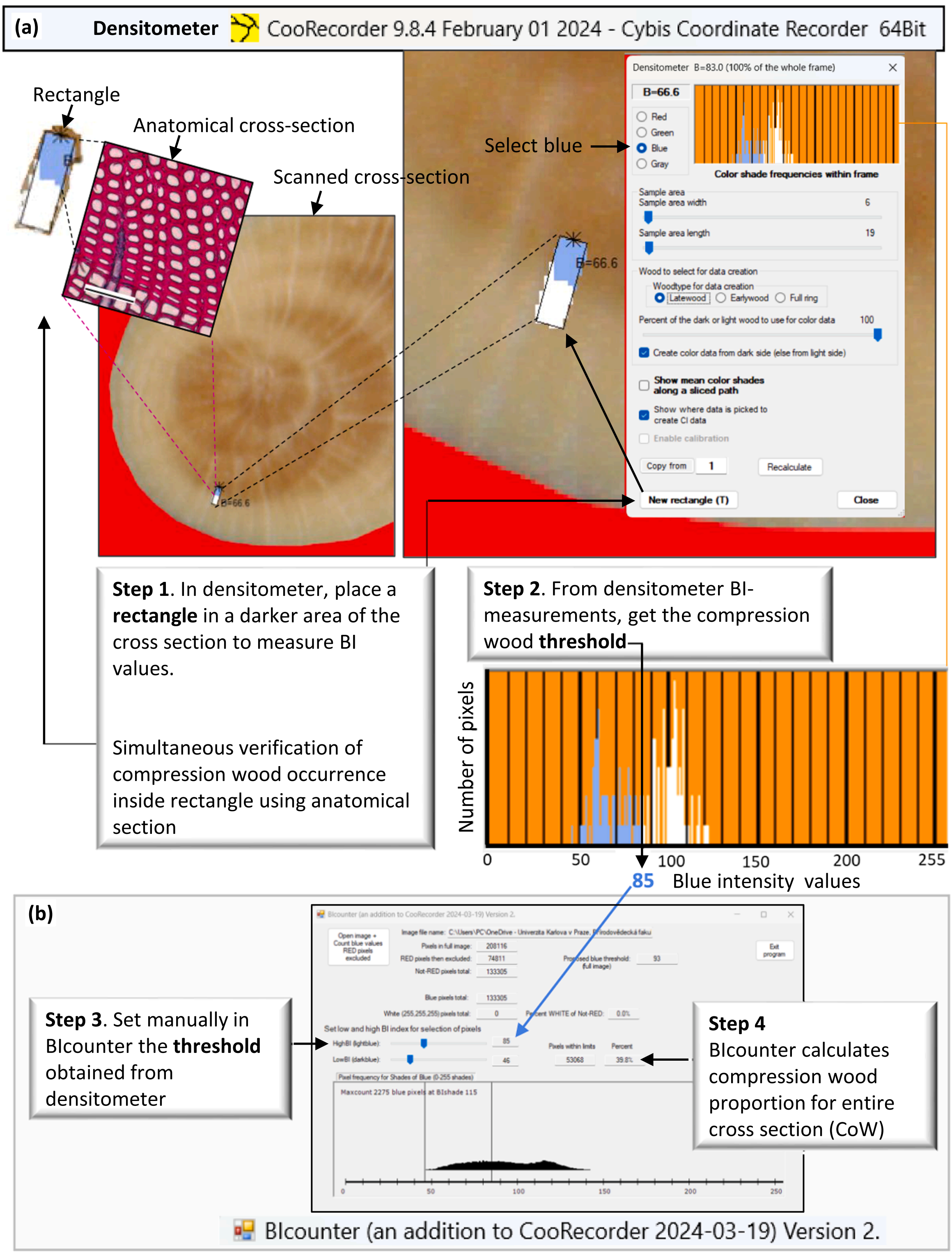

Fig. 2. Estimation of proportions of severe theoretical compression wood (CoW) using densitometer in CooRecorder and BIcounter new tool. A seedling with a high proportion of CoW is depicted as an example (39.8 %). (a) Steps 1 and 2 in densitometer. (b) steps 3 and 4 in BIcounter. CW: compression wood.

measuring BI by utilizing a color segmentation algorithm. While the software was originally developed to measure BI at the tree-ring level (e.g., Fig. 1a), BIcounter (Figs. 1e, 2b), is a stand-alone program (available at https://www.cybis.se/bicount) that utilizes the same color segmentation algorithm as CooRecorder, and calculates the absolute number of pixels with specific blue light intensity values. BIcounter enables the analysis of full cross-sections (Fig. 1e), and the quantification of the blue light pixels based on their intensity, which is not possible in current versions of CooRecorder. Particularly, we aimed to estimate and compare variations in stem eccentricity and in the proportions of high WD and low WD, including compression wood (a subset of high WD; Fig. 2), in complete stem cross-sections of seedlings, across different treeline sites. We expected high CoW proportions in all seedlings due to the mechanical forces typical of treeline ecosystems (slope, snow packs, wind). By utilizing 2D images obtained from a standard scanner, our approach enabled the quantification of areas with varying WD in just minutes, eliminating the need for subjective approaches (i.e. manual sketching). Additionally, we explored the potential correlations between compression wood proportions (CoW) and the following variables: stem eccentricity, high WD proportions (HighWD), and unaffected latewood proportions (LW).

2. Materials and methods

2.1. Study site and sample collection

We analyzed 59 *Picea abies* (L.) H. Karst seedlings from seven Central-European treelines, characterized by a range of temperature conditions (Fig. S1 and Table S1 in Appendix). *Picea abies* was the dominant tree species in all of the studied treeline sites. Here, we define the treeline as the elevation zone where trees cease to grow due to conditions associated with low growing season temperature (Körner, 2012), and winter conditions causing high seedling mortality (Brodersen et al., 2019). All sites were characterized by a relatively short growing season (123 ± 26 days) and a low growing season temperature (7.4 ± 1.1 °C) based on the 1980–2010 climatology of the CHELSA bioclimatic dataset (Karger et al., 2017). All sampling sites were located in open areas of the treeline ecotone, free from the influence of mature trees, and depressions and convex regions were avoided. We defined seedlings as individuals without reproductive structures, with basal diameter between 0.2 and 2.2 cm (mean = 0.87 cm, standard deviation = 0.45 cm) and height between 11 cm to 43 cm (mean = 25 cm, S.D. = 7 cm). Details per site are provided in Table S2. Five to ten sun-exposed seedlings were collected at each treeline site.

2.2. Sample scanning and image acquisition for Blue Intensity measurement

We scanned each of the 59 whole cross sections (e.g. Fig. 1b) using an Epson Perfection V850 Pro scanner with medium sharpness and a resolution of 4800 dpi. Samples were covered with black fabric to create dark conditions during scanning. Before scanning the samples, we calibrated the color of all RGB high-resolution images using the software i1Profiler v. 3.8.1.17800, together with a calibration plate (R221105 Wolf Faust reflexive, scanned at 200 dpi).

We edited each picture with a GNU Image Manipulation Program (GIMP, 2025 Version 2.10.38, Free Software, 2025): the background and bark of each cross section were removed and filled with red color (HTML ff0000) to exclude this area from BI measurement (e.g. Fig. 1e, step 1). The influence of any cracks in the wood was eliminated by also changing the color of these features to red. The images were scaled using GIMP to fit the maximum size of the densitometer frame (width ≤ 800 and length ≤ 1600 pixels) and exported in TIFF format. To ensure compatibility with the BIcounter program (an addition to CooRecorder 2024–03–19, Version 2), which we used for measuring the light intensity of blue pixels, the image was saved as a single layer during the export to TIFF (i.e., by deselecting the multiple layers option). Further details on blue pixel analysis are provided in Section 2.4 below.

2.3. Eccentricity index

We measured the diameter of each cross-section in cm using GIMP software. We then calculated the eccentricity index following Schweingruber (1996), where the value of a circle corresponds to 1:

$$\text{Eccentricity} = r1/r2 \tag{1}$$

In Eqs. 1, r1 denotes the longest radius in the cross-section, usually corresponding to the darker areas affected by compression wood, and r2 refers to the opposite (typically shorter) radius to r1.

2.4. Blue Intensity measurements

To assess the proportion of HighWD (unaffected latewood plus compression wood located both in earlywood and in latewood), we used the BIcounter program mentioned above (Fig. 1e; Fig. 2b; Fig. S2). All references to BI measurements performed with BIcounter represent raw (non-inverted) blue light intensity measurements (Rydval et al., 2014) equivalent to Blue Reflectance (BR) as defined in Björklund et al. (2024). Each edited image was uploaded to BIcounter (Fig. 1e, step 1). For each full cross-section, BIcounter produced a threshold segregating high WD areas (HighWD) from low WD areas (LowWD; Fig. 1e); red pixels were excluded. BIcounter then counted the total number of blue pixels with BI values corresponding to HighWD and calculated its proportion within the cross-section (Fig. 1e, step 2). LowWD, which we assumed may contain: unaffected earlywood, radial parenchyma, and/or resin canals, was calculated as follows:

$$\text{LowWD} = 1 - \text{HighWD} \tag{2}$$

2.5. Estimating proportions of severe compression wood (CoW) and unaffected latewood (LW)

We subdivided HighWD in each cross-section into the proportion of CoW and the proportion of unaffected latewood (LW). CoW was estimated using the densitometer within CooRecorder™ (Larsson, 2005; Fig. 2a) in combination with BIcounter new tool (Fig. 2b; details in Fig. S3). LW was calculated as follows:

$$\text{LW} = \text{HighWD} - \text{CoW} \tag{3}$$

2.6. Verification of compression wood-threshold inside densitometer's rectangle

In wood anatomical sections, the same darker areas selected in the densitometer were observed to verify CoW occurrence (Fig. 2a, Fig. 3). Thin cross-sections (one per each of the 59 samples), between 5 and 15 µm were cut with a rotary microtome, stained with Safranin-Astra blue, and the semi-permanent slides were scanned with a NIKON microscope at 20X resolution. We defined severe compression wood by observing and comparing the anatomical characteristics of affected and unaffected tracheids within the same cross-section, considering definitions by Tarmian and Azadfallah (2009), and Janecka et al. (2020). For this study, the key characteristics of tracheids affected by compression forces (e.g., Fig. 3a, d, h) were discerned through comprehensive examination of entire cross-sections and detailed anatomical comparisons: affected tracheids typically exhibited enlarged intercellular spaces among their rounded profiles, and their cell walls appeared swollen. These tracheids commonly occurred along the larger radius when strong eccentricity was observed. In contrast, unaffected tracheids had a

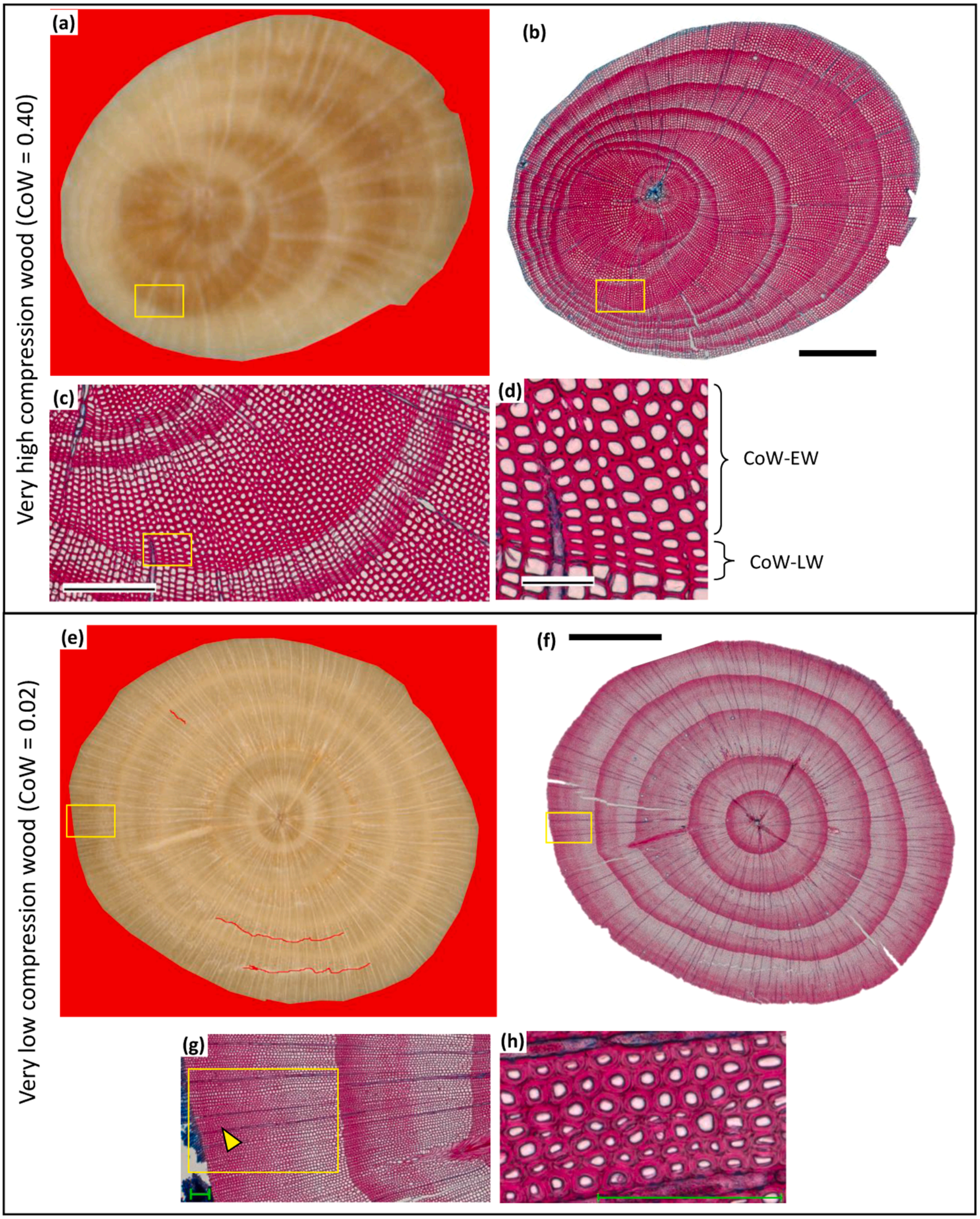

Fig. 3. Cross-sections of the stem of *Picea abies* treeline seedlings, displaying extreme proportions of compression wood (CoW). Panels (a)–(d) show sections from a seedling collected in Austria (DA23), with very high CoW (0.40). (e)–(h) from a seedling collected in the Czech Republic (JE22) and presents a very low CoW (0.02). (a) and (e) sections scanned with an EPSON scanner for Blue Intensity (BI) measurements using CooRecorder. (b) and (f) anatomical sections (scanned using a NIKON microscope, 20X) of the same sections in (a) and in (e), respectively. Anatomical cross-sections were used to verify the presence of compression wood in the selected areas with the densitometer to measure BI values corresponding to CoW as shown in (c), (d), (g), (h). (d) Depicts compression wood tracheids in earlywood (CoW-EW) and CoW-LW. (h) Indicates compression wood in latewood (CoW-LW). Scales: (b) = 300 μm, (c) = 200 μm, (d) = 50 μm, (f) = 0.2 cm, (h) = 0.01 cm.

relatively more rectangular shape, with the tangential diameter clearly exceeding the radial, and were found usually along the shorter radius under strong eccentricity.

2.7. Statistical analyses

We used Bayesian statistical models (for details see Appendix, Supplementary notes) to estimate the mean values of the examined variables (Eccentricity Index, HighWD, LW, and CoW). Rather than relying on a single mean per variable (e.g. Fig. S4), we generated full distributions of possible mean values, which allowed us to represent uncertainty, and also estimated the degree of data variability. To avoid bias from prior assumptions, we used weakly informative priors in our models (Bürkner, 2017). Next, we tested whether means and variance differed between treeline sites by comparing models that either included or excluded "site" as a factor. To choose the best model, we used the PSIS-LOOIC method (Vehtari et al., 2017). If the best model showed that site had no effect on each variable (Eccentricity Index, HighWD, LW, and CoW), this meant the mean value was consistent across all sites. Finally, we looked at how CoW was related to Eccentricity, HighWD, and LW, using another Bayesian model that included correlations. All analyses were performed in R (R Core Team, 2024) using the *brms* package (Bürkner, 2017).

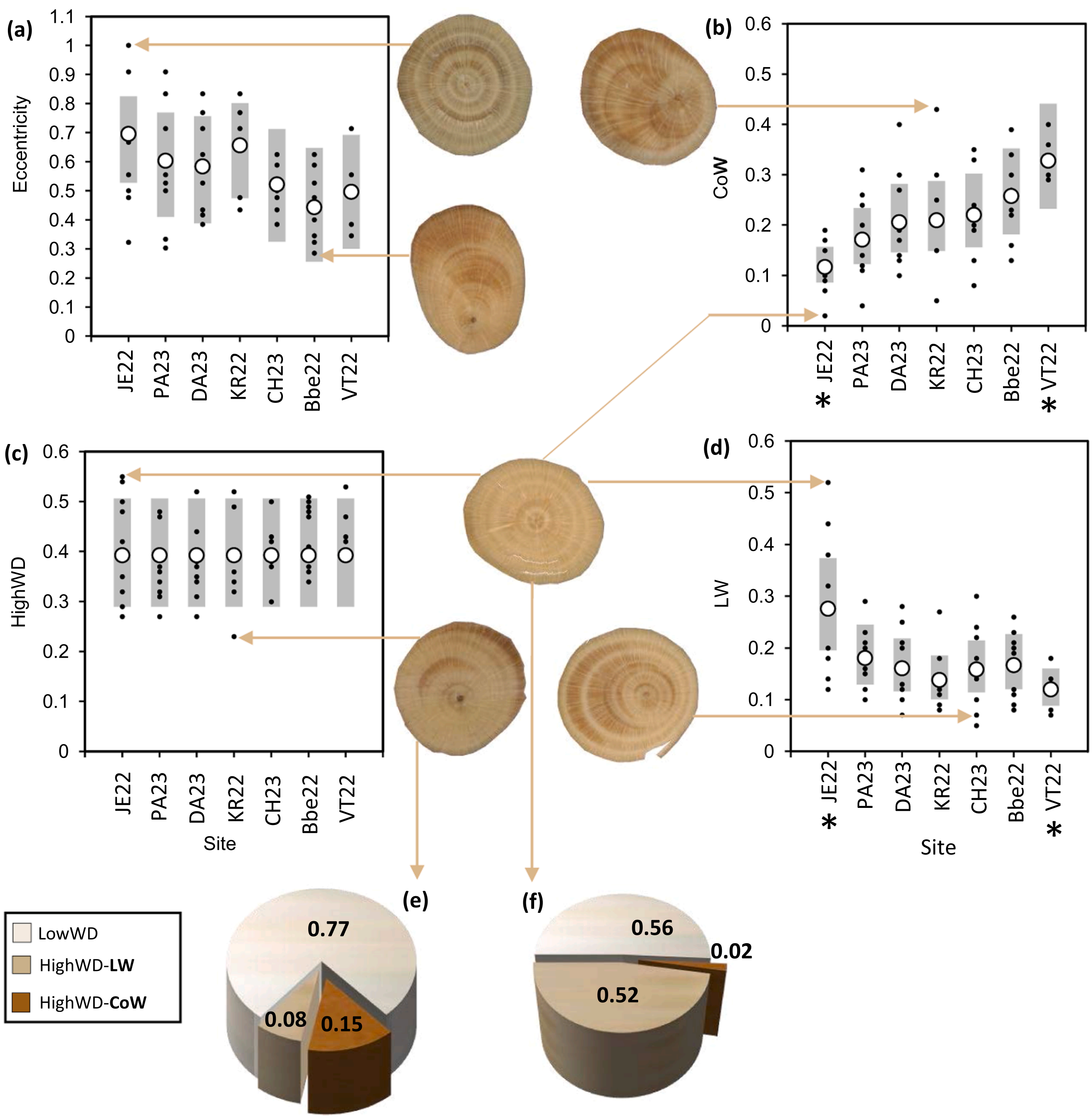

Fig. 4. Estimated mean proportions of wood density variations, and eccentricity, in stem whole-cross-sections of *Picea abies* treeline seedlings: Czech Republic, Jeseniky (JE22) and Krkonoše (KR22); Austria, Innsbruck (PA23); Austria, Daniel (DA23); Italy, Chiavenna (CH23); and Bulgaria, Boatin (Bbe22). (a) An eccentricity value of 1 corresponds to a circular stem shape. (b) Estimated proportion of compression wood (CoW), determined with Blue Intensity (BI), using densitometer in CooRecorder. (c) Proportion of high wood density (HighWD) obtained using BI measurements from the BIcounter program. The null model (site has no effect on mean or variance parameters) was the best supported for HighWD (estimated mean and variance were consistent across all sites). HighWD was subdivided into (b) CoW, and into (d) unaffected latewood proportion (LW; calculated as HighWD minus CoW); both CoW and LW are expressed in relation to the entire cross-section. Dots represent the estimated means from the best Bayesian model; horizontal gray bars indicate the estimated standard deviations. Cross-sections (not to scale) in (a) - (d) illustrate seedlings with extreme observed values. Asterisks in (b) and (d) represent means with clear statistical differences. (e) and (f) illustrate examples of seedlings with the highest and lowest proportions of low wood density (LowWD) across the entire cross-sectional area.

3. Results

3.1. Eccentricity index within and across treelines

Among the sites analyzed, JE22 had the least eccentric seedling stems, with a mean value of 0.69 (where a value of 1 represents a circular stem; Fig. 4a). In contrast, Bbe22 had the highest eccentricity with a mean of 0.44. These were the only clear statistical differences in mean stem eccentricity across the sites (Fig. S5a). Notably, the estimated variances for site JE22 were significantly different from those of the other sites (Fig. S6a). Sites CH23 and Bbe22 had the highest coefficients of variation (CV), while JE22 had the lowest CV (Fig. S7a).

3.2. Proportions of severe compression wood (CoW) within and across treelines

Among the sites, JE22 exhibited the lowest CoW proportion with a mean of 0.12 (with population estimates as low as 0.08; Fig. 4b). In contrast, VT22 had the highest proportion, showing a mean of 0.33 and a potential estimated mean up to 0.42. Statistical analyses confirmed significant differences in mean CoW across sites, notably between JE22 and VT22 (Fig. 4b). Although the overall variance of CoW was low (0.03; Fig. S5b), reflecting consistent variability regardless of site, the coefficient of variation differed (being highest in JE22; Fig. S7b).

3.3. High WD proportions, within and across treelines

The null model (i.e., where the site has no effect on mean or variance parameters) was best supported for HighWD, indicating consistent mean and variance estimates across all sites (Figs. S5c and S6c). The regional mean estimate proportion for this variable was 0.39 (Fig. 4c). The estimated population range was between 0.29 and 0.51. The variance was very low and consistent across all sites (0.04; Fig. S6c; S7c). One seedling in JE22 exhibited the highest proportion of unaffected latewood, 0.52 (Fig. 4f).

3.4. Proportions of unaffected latewood (LW), within and across treelines

LW had generally low estimated mean values across treelines (Fig. 4d). Among the sites, VT22 displayed the lowest LW proportion, with a mean of 0.12, though the population mean could potentially be as low as 0.07. Conversely, JE22 exhibited the highest LW proportion, with a mean of 0.28, which may reach a population mean of up to 0.34. Statistical analysis indicated significant differences only between the mean LW of JE22 (the highest proportion) and VT22 (the lowest proportion; Fig. S5d). Site differences did not significantly influence the LW variance (0.03 for all sites; Fig. S6d). Notably, the coefficient of variation for LW was lowest in JE22 (Fig. S7d).

3.5. Correlations between CoW and: stem eccentricity, LW and HighWD

The analysis found no statistically significant correlations between the estimated mean CoW and eccentricity (Fig. S8a), HighWD (Fig. S8b), or LW (Fig. S8c) across all study sites. Credibility intervals for all variable pairs included zero, confirming the lack of significant correlations (Fig. S8). Correlations between CoW and eccentricity were weak and close to zero, indicating a non-significant relationship (Fig. S8a). Similarly, correlations between CoW and both HighWD and LW were weak and non-significant, ranging from near zero to about 0.20 (Fig. S8b, S8c).

4. Discussion

In this study, we analyzed the wood density (WD) variations in stems of Norway spruce seedlings from Central European treelines. Our main challenge was to overcome the limitations of conventional methods in objectively assessing WD values. By utilizing BI measurements that encompass entire cross-sections rather than focusing solely on individual tree rings, we developed an innovative approach to quantify the proportions of WD variations in the seedlings' stems. By employing the BI tool in CooRecorder (densitometer) together with the newly introduced BIcounter program, we effectively quantified pixels corresponding to various WD categories (HighWD, LowWD, LW, CoW). This study is the first to test these tools on treeline seedlings, providing a quick and effective way to measure WD variations in cross-sections. However, we acknowledge certain limitations and the need for further research.

4.1. Stem eccentricity and WD variations across and within the treeline seedlings

Our results indicate that mechanical forces in the extreme treeline environment strongly influence seedlings' wood formation. High percentages of compression wood were observed across all study sites, accompanied by notable eccentricity, regardless of the location.

We found that the proportion of HighWD (mainly composed of unaffected latewood and compression wood) was similar across treeline sites, consistently accounting for 39 % of the stem cross-section. Because HighWD and LowWD together make up 100 % of the analyzed area, the proportion of LowWD (primarily consisting of earlywood, resin canals, and radial parenchyma) also remained constant. The amount of latewood (LW) present may reflect environmental conditions, growth patterns, or stress responses (Zhirnova et al., 2020). WD results from the interaction between carbon-based compounds and the relative proportions of wood cell types and voids, and is expressed as mass per volume (Lachenbruch and Mcculloh, 2014). It tends to decrease with higher proportions of earlywood, tracheid lumina, intercellular spaces, and resin canals (Gindl et al., 2001), and increase with more LW and compression wood (CoW), which are richer in carbon-dense compounds such as lignin in the tracheid walls (Soro et al., 2023). These relationships explain the observed variations in WD.

JE22 stands out as a warmer treeline with a longer growing season and less snowpack, supporting higher unaffected latewood production. The seedlings came from flat or gently sloped summit areas, where reduced snowpack likely lowers mechanical stress.

As the coefficient of variation (CV) of CoW could reach up to 30 %, we recommend that future studies incorporate variance parameters. This would allow for a more accurate assessment of the structural heterogeneity inherent in compression wood formation.

Our study indicates that treeline seedlings consistently exhibit high proportions of compression wood (CoW), with mean values reaching up to 33 % and individual measurements up to 43 % of the stem cross-section, whereas Duncker and Spiecker (2008) reported extreme CoW values (up to 37 %) in adult Norway spruce in less than 1 % of cases with averages typically below 4 %. Since compression wood differs from normal wood in its physical, mechanical, and chemical properties, and can affect tree growth by, (e.g., increasing hydraulic vulnerability; Mayr and Cochard, 2003), its consistent presence at the treeline may have important implications for understanding seedlings' growth and survival, and may also be of interest for geomorphological studies where CoW helps identify and date disturbances (e.g., Bräuning et al., 2016; Šilhán and Plavcová, 2021).

We expected a correlation between compression wood (CoW) and stem eccentricity, as CoW typically forms in response to mechanical forces in treeline environments. However, no clear relationship was found, which deviates from existing knowledge (though such a lack of correlation can occasionally occur; Duncker and Spiecker, 2008), and might be specific for seedlings' life stage.

Despite finding no significant relationships between the parameters we investigated (CoW, eccentricity, HighWD, and LW), our study provides valuable insights, as it reveals natural variability that larger datasets might overlook. For example, Fig. 4f shows an outlier from the JE22 treeline seedling, where lignin-carbon was mainly concentrated in latewood, especially in the absence of compression wood.

4.2. Using Blue Intensity for assessing WD variations

Visual assessment enables us to distinguish between lighter and darker portions (representing lower and higher density wood) of the cross sections of all examined seedlings. Our analysis effectively reflected this dichotomy, which represented an integral part of our research objectives. We segmented the images based on BI values and utilized specific settings in CooRecorder to identify a suitable threshold in order to categorize the pixels into two light intensity ranges. BIcounter played a crucial role in this process by specifying the threshold, which delineates these perceived light intensity ranges, facilitating a more objective assessment than visual inspection alone. While the precision of this method may not be optimal at present, our focus was not on achieving superlative accuracy but rather on establishing a more consistent metric. Thus, BIcounter enabled us to achieve a necessary level of objectivity in our analysis, laying the groundwork for potential advancements in future research.

The context of our investigation involved analyzing compression wood, which is characterized by higher density, higher lignification and lower cellulose content compared to normal wood (Côté et al., 1967). The differences in WD are reflected in blue light intensity, with higher (lower) density wood appearing darker (lighter). It is important to clarify, however, that while latewood and compression wood cell walls are associated with higher lignification (Gindl et. al. 2000), this property probably does not translate to higher WD. Björklund et al. (2021a) noted that the relationship between lignin content and density may be overstated in past literature, suggesting that the primary determinant of density seems to be the ratio of the area containing cell wall material to the area of empty space within the wood structure. Therefore, although higher lignin content is typically found in latewood compared to earlywood, the lignin itself likely does not directly control density. However, further investigation of these relationships is required to definitively settle this matter.

4.3. Advantages, perspectives, and limitations of BIcounter

In our study, one of the aims was to calculate the proportion of compression wood in each cross-section. Previously, the identification of compression wood was not well reproducible when relying solely on observing tracheid anatomical features in cross-sections. To address this limitation, we assessed the higher density values (typical of latewood and compression wood; Soro et al., 2023) in relation to lower density values. For this purpose, BI measurements were particularly suitable.

Compression wood has traditionally been analyzed as a discrete variable despite its inherently continuous nature (Wimmer, 2002). Our method enables us to analyze this property by appropriately treating it as a continuous variable, as it was calculated based on the number of pixels occupied by compression wood in a cross section image, allowing for fine-scale variation without categories. This advancement was made possible by using the relationship between WD and the algorithm employed in CooRecorder, which identifies the division point between latewood (LW) and earlywood (EW). BIcounter is based on the same principles as that used in BI quantification and LW, EW subdivisions.

By utilizing BI measurements from the BIcounter, together with the densitometer integrated within CooRecorder, we present a useful resource for quantifying WD properties and variations. The densitometer in CooRecorder already represents a useful tool, for example, to distinguish tree ring borders by the identification of earlywood and latewood (Frank and Nicolussi, 2020). By integrating the functionality of the densitometer tool within BIcounter, we expanded its usefulness to assess the proportions of compression wood.

The BIcounter tool, introduced herein, could have a broad range of potential applications in various sub-disciplines of dendrochronology (incl., dendroecology, dendrogeomorphology, dendroclimatology; Šilhán and Plavcová, 2021), and perhaps even wood industry applications involving assessments of wood quality for commercial purposes. Our approach may also provide insights of how WD influences biomass allocation and function in gymnosperms. Prior studies, such as Zanne et al. (2010), have examined similar relationships in angiosperms, underscoring the importance of these investigations.

Currently, it is possible to utilize BIcounter as a standalone tool, which utilizes the light intensity (i.e., pixel brightness) detection algorithm of CooRecorder for calculating proportions of areas with gradients of WD (for example to evaluate low and high-density areas as demonstrated here). A distinct advantage is that the technique can be applied to full cross-sections as well as annual rings. Furthermore, images can easily be adjusted using image editing software (e.g., GIMP or ImageJ) to select only the area of interest (excluding bark, for example) and to exclude certain undesirable regions/features within the area of interest, such as cracks. In future studies, if BI values are required instead of the raw Blue Reflectance (BR) values provided by BIcounter, the Eq. (1) in Rydval et al. (2014) can be applied to invert the raw BR measurements.

Our approach has the potential for broader applications beyond young individuals; it can also be employed for adults, utilizing radial cores (or possibly even whole cross-sections) for diverse WD evaluations. Additionally, inter- and intra-annual assessments of WD could provide a more comprehensive understanding of density variations within stems and trees. This enhanced knowledge may facilitate the development of more accurate estimates regarding the carbon storage functions of trees and forests.

While we present several promising perspectives and successfully quantify the variations in WD across the cross sections of treeline seedlings, we acknowledge the limitations inherent in our approach. Additional in-depth testing and experimentation might be required to uncover its full potential (e.g. with methods similar to Duncker, and Spiecker, 2009), and identify any limitations and shortcomings of this approach, highlighting specific aspects for future improvement.

5. Conclusions

In this study, we presented a quantitative method for assessing WD variations across the entire stem cross-section of European treeline seedlings. Our method effectively detects and estimates the proportions of LowWD which contains wide tracheid lumina, radial parenchyma, and resin channels, and HighWD including severe compression wood and unaffected latewood. Analyzing the entire cross-section marks a fundamental departure from conventional approaches, such as those used in CooRecorder for latewood and earlywood identification. Standard dendrochronological image analysis tools typically focus on the assessment of separate growth rings in adult tree cores rather than complete cross-sections of seedlings. Additionally, our approach represents compression wood as a continuous variable, offering significant advantages in statistical power and analysis.

We found that high WD proportions occur consistently in seedlings across treeline ecosystems, likely due to climatic and mechanical constraints. High stem eccentricity seems to be a natural feature at the regional treeline scale, which is unsurprising given the influence of strong mechanical forces. As CoW in treeline seedlings was generally high, we suggest that further studies incorporate severe compression wood proportions and its potential impact on plant growth and survival.

CRediT authorship contribution statement

Václav Treml: Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition. **Miloš Rydval:** Writing – review & editing, Validation, Methodology, Conceptualization. **Edgar J. González:** Writing – review & editing, Validation, Formal analysis. **Eunice Romero:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.dendro.2025.126394.

Data availability

Data will be made available on request.

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