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**Pia Caroline Adamič<sup>1</sup>, Matjaž, Čater<sup>1,2</sup>, Jakub Kašpar<sup>3</sup>, Domen Arnič, Peter Prislan<sup>1</sup>**

## **Variability in wood anatomical characteristics in silver fir and European beech at three different sites in the Carpathian Mountains**

**KEYWORDS:** climate change; dendrochronology; radial growth response; wood anatomy.

### **Introduction**

Although the general anatomical features are species-specific and thus genetically determined, their actual characteristics vary in different parts of xylem rings within internally determined limits due to various external factors (Rathgeber 2017). The structural adjustments of wood to environmental cues play a decisive role in defining wood's hydraulic and mechanical properties and, consequently, in tree performance and survival (Chave et al. 2009). In addition to dendrochronology, quantitative wood anatomy has been shown to provide valuable information on how trees adjust their anatomical structure in response to changing environmental conditions (von Arx et al. 2016).

Variability in wood anatomical traits and their relationships with environmental factors have not been analysed in silver fir. However, similar studies have been carried out on other conifer species; Ziaco et al. (2014) investigated the effect of drought on different pine species and found significant effect of precipitation on cell enlargement and thus on the final size of tracheids. Furthermore, Castagneri et al. (2017) found that the effect of temperature and precipitation on cell morphology (i.e. cell lumen size and wall thickness) changes during the growing season. Variability in beech anatomical characteristics has recently been investigated using data from beech provenance trials (Eilmann et al. 2014; Hajek et al. 2016). Eilmann et al. (2014) confirmed that southern beech proveniences respond differently to drought conditions compared to northern proveniences, suggesting genetic control of xylem performance in beech. Hajek et al. (2016) observed that beech responded to drought by adjusting vessel number, but not vessel diameter. Similarly, Prislan et al. (2018) recorded differences in vessel density between sites with different weather conditions, while differences in vessel dimensions were not significant. Arnič et al. (2021) found differences in growth and wood anatomical characteristics of beech growing in different forest sites in Slovenia. They concluded that changes in temperature and precipitation regimes, as predicted by most climate change scenarios, will affect tree ring growth and wood structure in beech.

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<sup>1</sup> Slovenian Forestry Institute, Department of Yield and Silviculture, Večna pot 2, 1000 Ljubljana, Slovenia

<sup>2</sup> Department of Silviculture, Faculty of Forestry and Wood Technology, Mendel University, Zemedelska 3, 613 00 Brno, Czech Republic

<sup>3</sup> Department of Forest Ecology, The Silva Tarouca Research Institute, Lidická 25/27, 602 00 Brno, Czech Republic

**Corresponding author:** Pia Caroline Adamič - [pia.adamic@gozdis.si](mailto:pia.adamic@gozdis.si)

The aim of the study was to evaluate the effect of site conditions at three different sites in the Carpathian Mountains on wood anatomical characteristics of European beech and silver fir. We hypothesised that wood anatomical characteristics (i.e. cell size, cell wall thickness, density and distribution of water conducting cells) would differ between the eastern and western populations of fir and beech. The assessment of variability in radial growth and wood anatomical characteristics at the selected sites may be crucial in assessing the range of plasticity in the species under different environmental conditions as a first step in predicting their responses to future climate scenarios.

## Material and methods

### Site description

In the Carpathians, three sites with mature fir-beech stands located between 830 and 985 m above sea level were selected and analyzed (Table 1). The selected sites were part of seven sites along the Carpathians that we recently studied as part of a dendrochronological analysis (Adamič et al., 2023). Table 2 shows the meteorological data of the selected sites.

*Table 1. Locations, forest label, altitude, and coordinates of research site locations.*

No.	County	Plot	Altitude (m)	E (DMS)	N (DMS)
A2	Suceava	Frumosu	850	25°40'60.00"	47°28'6.00"
A4	Vrancea	Soveja	830	26°36'14.00"	46° 0'5.00"
A7	Gorj	Tismana	985	22°55'1.00"	45°10'10.00"

*Table 2. Average annual air temperature, average air temperature from April to September, total annual precipitation and total precipitation from April to September for selected locations.*

No.	County	Average annual air temperature (°C)	Average air temp. April-Sept. (°C)	Total annual precipitation (mm)	Total Apr.-Sept. precipitation (mm)
A2	Suceava	5.4	12.0	738	501
A4	Vrancea	8.3	15.6	603	412
A7	Gorj	4.7	10.7	1073	695

### Sample collection and preparation

Samples (cores) of beech and fir were collected for quantitative wood anatomy analysis at the selected locations. All sampled trees were healthy trees with no visible signs of stem damage or any kind of declining tree vitality. The samples were prepared for observations under a light microscope according to the protocol suggested by von Arx et al. (2016); i.e. each core was split into sub-samples of similar length to fit on the object glass. From each sub-sample 15 to 20  $\mu\text{m}$  thick transverse sections were cut with a sledge-microtome and then stained with safranin. High-resolution images of the sections were prepared using a light microscope and digital camera. The measurements of the sections were performed with image analysis software Image Pro Plus and ROXAS (Prendin et al. 2017; von Arx et al. 2016), which provide cell dimensions (e.g. lumen size, cell wall thickness) and relative position within the dated growth ring for all selected cells (Castagneri et al., 2017). Chronologies of the following parameters were then established; for European beech and silver fir (1) mean lumen area of

vessels/tracheids (MLA), (2) tracheid or vessel density (CD) as the number of tracheids/vessels per squared mm, and (3) relative conductive area (RCTA) representing the percentage of cumulative tracheid/vessel lumen area within the measured area. In addition to the above-mentioned wood anatomical characteristics, chronologies of mean thickness of tangential cell walls (CWTTAN) were also established for silver fir.

### Statistical analysis

Raw chronologies were used to assess differences in wood anatomical characteristics between sites. The quality of site chronologies was described with common descriptive statistics, such as expressed population signal (EPS), mean inter-series correlation ( $\bar{r}$ ), and Gleichlaufigkeit (%GLK) (Cook and Kairiukstis, 1990). To test the hypothesis of equal means of tree-ring characteristics, i.e., TRW, MLA, CD, RCTA, MLA and CWTTAN we used parametric and non-parametric statistical tests. Repeated measures (rm) ANOVA was used when assumptions for parametric tests were met, while in other cases, the nonparametric Friedman test was applied.

### **Results and discussion**

For silver fir and European beech, the local chronologies of wood anatomical characteristics at sites A2, A4 and A7 ranged from 40 to 49 and 39 to 42 years, respectively (Table 3). The widest mean TRW in silver fir for the 1976–2016 period was measured at A2 ( $4654.9 \pm 916.3 \mu\text{m}$ ), followed by A4 ( $2262.8 \pm 913.0 \mu\text{m}$ ) and A7 ( $1159.4 \pm 322.5 \mu\text{m}$ ); the differences were statistically significant (Figure 1A, Figure 2A). Mean TRW for European beech was significantly lower at A2 ( $2638.5 \pm 518.1 \mu\text{m}$ ) compared to silver fir while at A4 ( $2301.6 \pm 780.9 \mu\text{m}$ ) and A7 ( $1340.8 \pm 600.1 \mu\text{m}$ ), they were slightly wider (Figure 1B, Figure 3A).

MLA values in silver fir were similar at A2 and A7, but significantly lower at site A4 (Figure 2B). Similar observations were also made for European beech, where similar MLA values were observed at A2 and A7, while the values at A4 were significantly higher (Figure 3B).

The measured wood anatomical features CD and RCTA were significantly different between all sites in case of European beech (Figures 3C–D); the highest values were observed at site A7 and the lowest at site A2 (Figure 1H–F, Figures 3C–D). In the case of silver fir, different values between sites were observed for CD; the highest values at A4 and the lowest at A7. RCTA values in silver fir were similar at site A4 and A7, yet significantly higher at A2. Mean tangential cell wall thickness (CWTTAN) in silver fir was highest at A7, and significantly lower at A2 and A4 (Figure 2E).

Our results show differences in wood anatomical characteristics (e.g. TRW, CD, RCTA and MLA) at the selected sites for both analysed species, thus confirming our hypothesis.

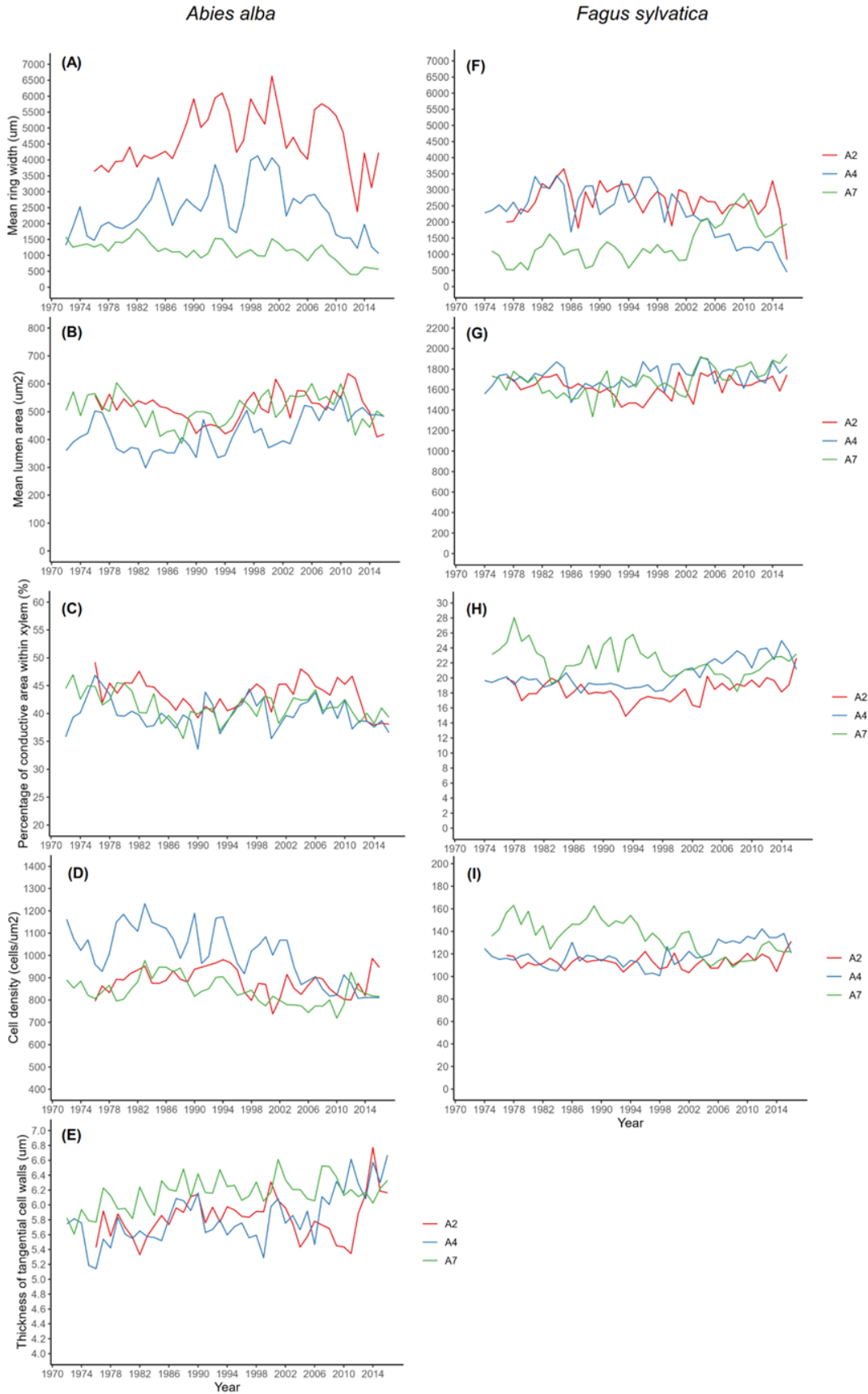


Fig. 1. Chronologies of wood anatomical characteristics in silver fir and European beech at sites Suceava (A2), Vrancea (A4) and Gorj (A7).

Table 3. Tree ring width and wood anatomy chronologies and descriptive statistics: mean values, standard deviation (SD), first-order autocorrelation (AC1), Gleichläufigkeit coefficient (%GLK), mean interseries correlation (rbar), and expressed population signal (EPS) for silver fir (ABAL) and European beech (FASY) at the selected sites; Suceava (A2), Vrancea (A4) and Gorj (A7).

Species	Site	Parameter	Start year	End year	Mean	SD	AC1	%GLK	rbar	EPS
ABAL	A2	TRW	1976	2016	4654,9	916,3	0.706	63	0.146	0.406
		RCTA	1976	2016	43,3	2,8	0.556	53	0.066	0.22
		CD	1976	2016	882,9	59,3	0.510	51	0.037	0.169
		MLA	1976	2016	515,5	56,1	0.568	51	0.019	0.072
		CWTTAN	1976	2016	5,8	0,3	0.520	50	0.09	0.285
A4	A4	TRW	1967	2016	2262,8	913,0	0.764	65	0.308	0.727
		RCTA	1967	2016	39,5	3,5	0.485	59	0.131	0.475
		CD	1967	2016	1017,1	121,3	0.680	57	0.381	0.787
		MLA	1967	2016	417,5	69,2	0.583	59	0.295	0.715
		CWTTAN	1967	2016	5,8	0,4	0.535	53	0.251	0.626
A7	A7	TRW	1968	2016	1159,4	322,5	0.751	65	0.333	0.749
		RCTA	1968	2016	41,7	2,8	0.365	55	0.229	0.598
		CD	1968	2016	836,5	59,4	0.548	47	0.115	0.395
		MLA	1968	2016	517,1	59,5	0.475	53	0.23	0.599
		CWTTAN	1968	2016	6,1	0,3	0.329	55	0.082	0.31
FASY	A2	TRW	1977	2016	2638,5	518,1	0.421	67	0.252	0.628
		RCTA	1977	2016	18,3	1,4	0.493	57	0.176	0.516
		CD	1977	2016	113,1	5,8	0.344	58	0.07	0.272
		MLA	1977	2016	1627,4	98,8	0.439	51	0.084	0.314
A4	A4	TRW	1974	2016	2301,6	780,9	0.662	58	0.553	0.881
		RCTA	1974	2016	20,5	1,8	0.532	47	0.194	0.59
		CD	1974	2016	119,4	10,3	0.545	55	0.317	0.736
		MLA	1974	2016	1726,8	102,7	0.396	57	0.106	0.415
A7	A7	TRW	1975	2016	1340,8	600,1	0.698	60	0.527	0.87
		RCTA	1975	2016	22,3	2,1	0.577	53	0.127	0.467
		CD	1975	2016	134,8	15,3	0.581	59	0.509	0.861
		MLA	1975	2016	1689,5	135,5	0.461	58	0.232	0.644

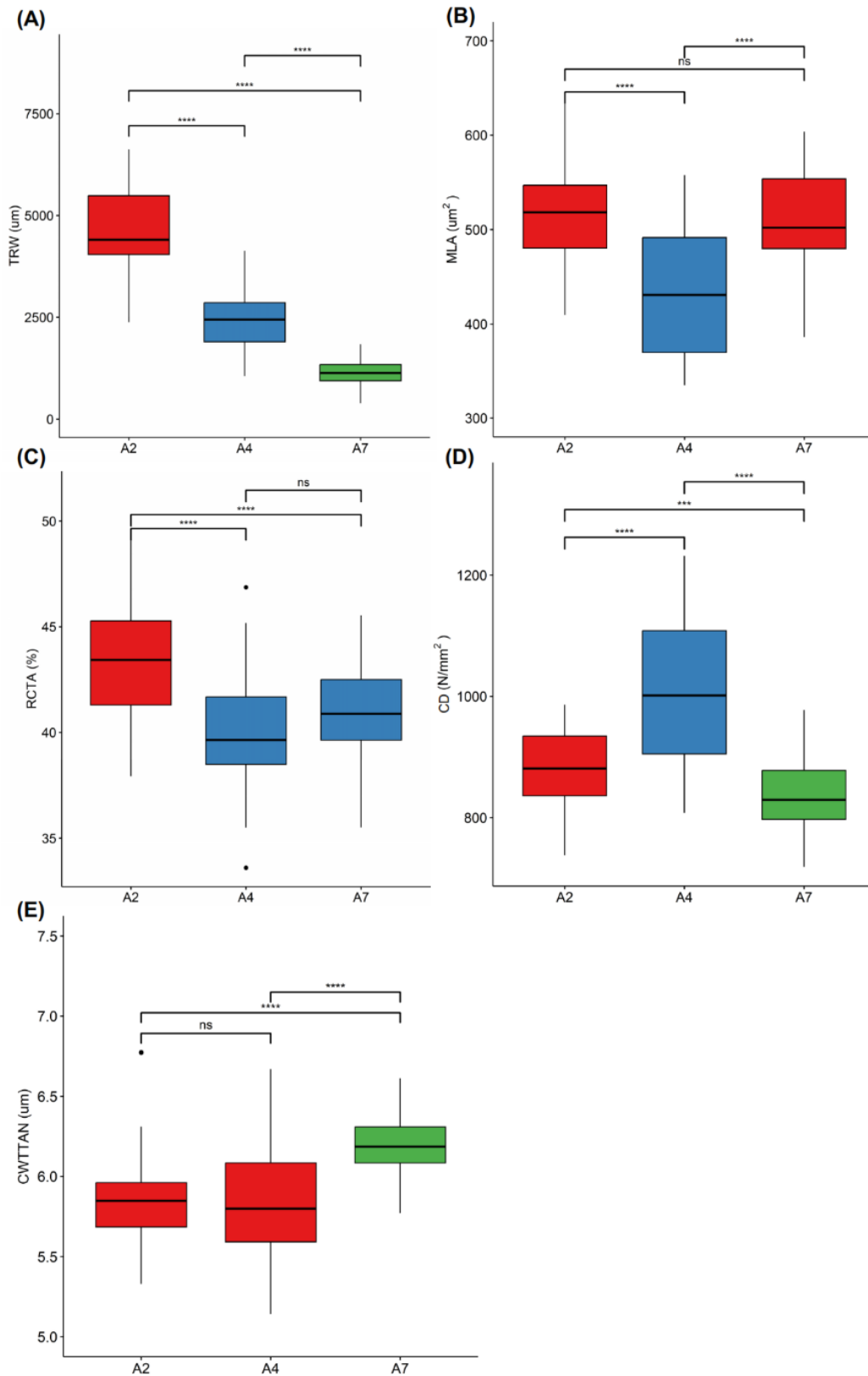


Fig. 2. Differences among the sites Suceava (A2), Vrancea (A4) and Gorj (A7) in (A) tree ring width (MRW), (B) mean vessel area (MVA), (C) relative conductive area (RCTA), (D) cell density (CD) and (E) tangential cell wall thickness (CWTTAN) in silver fir analyzed by rm-ANOVA or the Friedman test. The significance of differences in tree ring characteristics between sites is marked by ns-not significant. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , and \*\*\*\* $p < 0.0001$ .

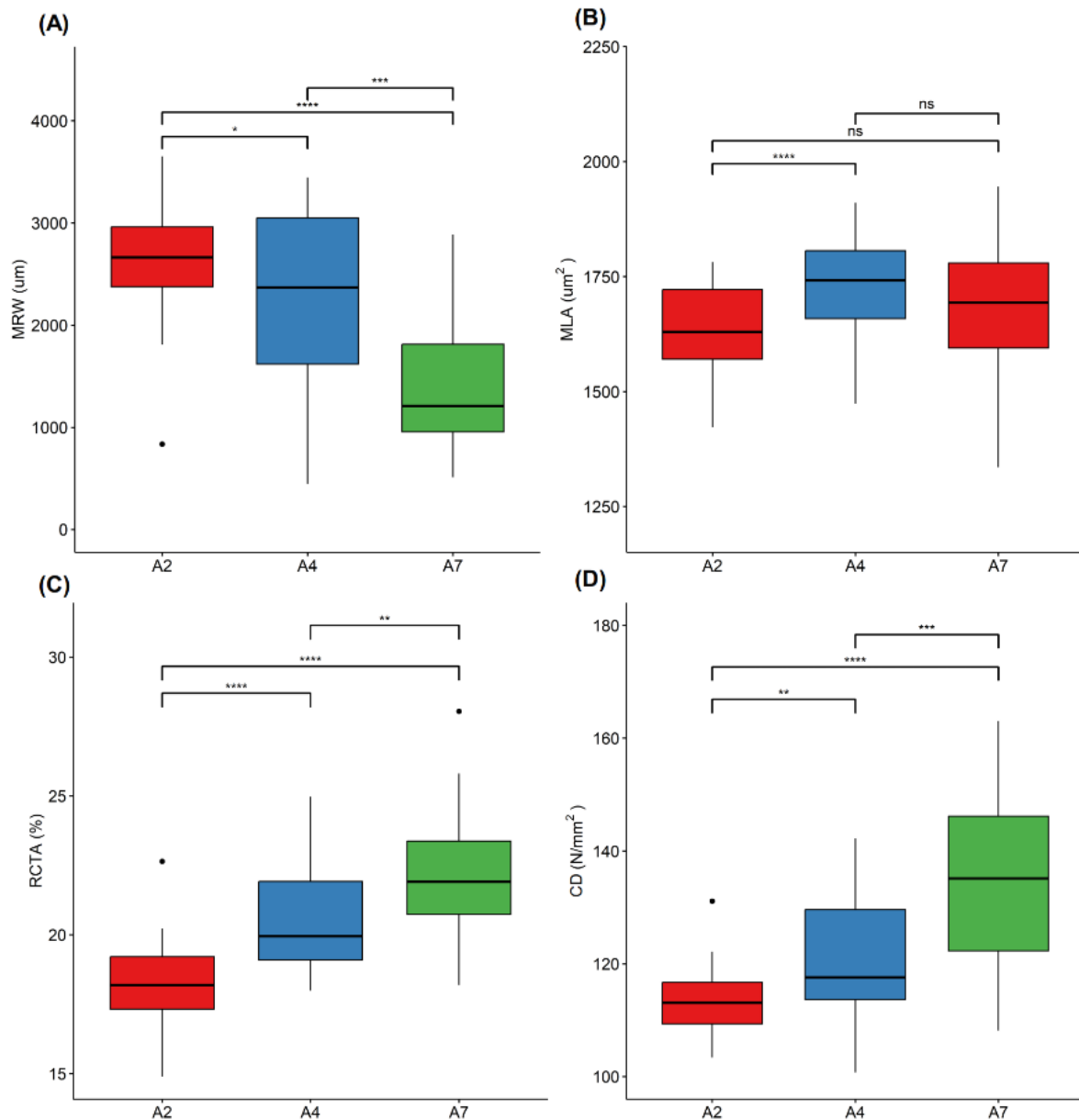


Fig. 3. Differences among the sites Suceava (A2), Vrancea (A4) and Gorj (A7) in (A) mean ring width (MRW), (B) mean vessel area (MVA), (C) relative conductive area (RCTA) and vessel density (CD) in European beech analyzed by *rm*-ANOVA or the Friedman test. The significance of differences in tree ring characteristics between sites is marked by *ns*-not significant. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , and \*\*\*\* $p < 0.0001$ .

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