Variability of ray anatomy of *Larix gmelinii* along a forest productivity gradient in Siberia

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Abstract

Key message This study provides new data and an alternative framework to the debate of tree carbon economy in a context of increasing stress.

Abstract For long-living trees, the resilience in times of stress is directly linked to the amount of accessible reserves. Despite the simplicity of this principle, the understanding of how carbon reserves limit growth and/or induce mortality under global change is still debated. In this study, we quantify how anatomical properties of rays—one of the main container for carbon reserves in tree stems—vary among sites, individuals, and annual rings of *Larix gmelinii* growing in contrasting sites in Siberia to verify if (1) the ray proportion and anatomical structure is linked to the environment, and/or (2) to changes in other wood tissues. Our observations have highlighted that ray proportion mainly varies among individuals, but little among sites and consecutive annual rings. We also observed that ray size and density scale to the wood structure with a relatively constant ratio of ~2.5 rays per tracheid, independent of site conditions. These results suggest that the functional connection between the anatomy of rays and tracheid is unaffected by environment and highlight the importance of

considering allometric relations in ecological comparisons. Comparative studies of long-term trajectory of ray proportion of living and dead trees might unravel observed variability among individuals validating the link between long-term depleted reserves and mortality.

Keywords Tree ring · Tree-ring anatomy · Rays · Ray density · Ray height · Ray volume · Forest decline · Carbon starvation

Introduction

Increasing observations of drought-induced forest decline and dieback (Allen et al. 2010) are raising fundamental questions about the ability of trees to cope with intensified heat and drought stress. These questions obtain even more relevance when considering that future climate is expected to be warmer and characterized by more extreme events (IPCC 2013). Trees, being long-living organisms, have developed mechanisms to deal with the natural climatic variability. During periods of prolonged water deficit and heat stress, trees typically aim at reducing water consumption by closing stomata (Chaves et al. 2002). This short-term response, which has a negative influence on carbon assimilation, is usually mitigated by the ability of the tree to store and mobilize previously accumulated carbon compounds as sugars and lipids, thus guaranteeing the maintenance and restoration of fundamental metabolic processes during and after the unfavorable period (McDowell et al. 2008; Wiley and Helliker 2012). However, a long-term increasing frequency and magnitude of unfavorable events as heat and drought might deplete the trees reserves intensifying the sensitivity to drought (McDowell 2011). Thus, tree survival and recovery in

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future harsher climate might also depend on the capacity to store and remobilize available reserves (Sala et al. 2010; McDowell 2011). Despite the awareness of the importance of carbon reserves to overcome stress periods, the way a tree allocates carbon for its functioning (i.e., maintenance, growth defense, ion uptake and transport, reproduction, storage) is poorly understood, and even basic mechanisms such as the control of carbon allocation to storage versus growth are still debated (Korner 2003; Ryan 2011; Sala et al. 2012; Wiley and Helliker 2012).

To unravel the mechanism and temporal dynamics of storage, remobilization and use of carbon in relation to environmental stressors (mostly drought or cold), recent tree physiological research has been mainly focusing on identifying priorities in the allocation of reserves (Sala et al. 2012). Particular focus has been given to the quantification (e.g., Hoch et al. 2003; Galiano et al. 2011; Gruber et al. 2012) and dating (Carbone et al. 2013; Richardson et al. 2013) of stored carbon compounds in stressed versus control trees. However, a major limitation of these tree physiological investigations is the limited observational time-window, which, with few exceptions, cannot supply a long-term perspective (e.g., years or decades) of the dynamics of reserve allocation and use (Ryan 2011; Sala et al. 2012; Wiley and Helliker 2012).

Tree-ring anatomy is an emerging methodological approach based on dendrochronology and quantitative wood anatomy that allows complementing the short-term physiological view by a retrospective tree or stand life-span perspective on plant anatomical and structural adjustments in relation to environmental changes (Fonti and Jansen 2012). Many studies measuring xylem cell characteristics across time series of annual rings have already demonstrated a common signal encoding environmental information (see Fonti et al. 2010 for a review). So far, due to the important role of vessels and tracheids in shaping the hydraulic properties of water uplift from the soil to the leaves (Tyree and Zimmermann 2002), these investigations have mainly focused on the characteristics of the water conducting tissue. Examples of identified associations between water conducting cells and the environmental condition have been found in both hardwood and softwood species across a large array of biomes from tropical (Verheyden et al. 2005), to subtropical (Oladi et al. 2014), Mediterranean (DeSoto et al. 2011; Olano et al. 2013a; Hetzer et al. 2014), temperate (Bryukhanova and Fonti 2013) and boreal (Kirdyanov et al. 2003; Fonti et al. 2013). In contrast, despite the important role of the parenchyma tissue for storage and for regulating physiological processes occurring in the xylem and/or in connection to the phloem (Spicer 2014), only very few studies have investigated its environmental sensitivity (Eckstein and Schweininger 2009).

Parenchyma is a living tissue in woody plants that is important for the storage of water and carbon compounds (Braun 1970; Gartner 1995). This tissue is the living part of the sapwood and, besides providing defence against pathogens (Hudgins et al. 2006), wounding (Arbellay et al. 2010, 2012), refilling of cavitated conduits (Salleo et al. 2009), and formation of heartwood (Bamber 1976), it plays an important metabolic and biological function also by actively regulating the replenishment, storage and use of carbon reserves and water inside the tree stem. Parenchyma cells in the xylem of conifers are predominantly found in rays, although rays can additionally include tracheids cells at their longitudinal tips (Gartner et al. 2000). Rays provide communication, storage, and radial transport between the xylem (mainly storage and sink), the cambium (sink) and the phloem (source) (Romberger et al. 2004). In conifers, ray parenchyma in the xylem can be considered as one of the main container (the repository) for storing carbon compounds (Spicer 2014) and thus might be of particular interest to retrospectively explore whether changes in ray abundance within the same species or the same individual can reflect trees' (maximal) storage capacity in response to environmental stress (von Arx et al. 2012). Assuming that active storage improves the chances of survival in response to an increased threat of carbon starvation (Wiley and Helliker 2012), we could expect higher ray proportion under increased stressful conditions. However, since (1) increased storage can also be achieved through higher concentrations within the same container and the variation of stored and mobilized carbohydrates during the season (Regier et al. 2010; Simard et al. 2013) might equal those over long-term variations, (2) in absolute term the amount of non-structural carbohydrates in sapwood contains 25–41 % of whole tree reserves (Jacquet et al. 2014) although concentrations in the phloem can be an order of magnitude greater than those in the wood (Gruber et al. 2013); (3), the plasticity of the ray volume might be low due to the longevity of the tissue in the sapwood; it is still unclear if and to which extent short- and long-term environmental variation can affect the volume of the containers within the xylem (Eckstein 2013).

Moreover, since the ray network (size and density) needs to fit tracheid size to provide functional integration (e.g., hydraulic conductivity, freezing tolerance, embolism repair, heartwood formation, see review in Spicer 2014), we would expect a higher density of smaller rays in smaller trees featuring smaller tracheids, which might affect the evaluation of the ecological influence on the ray properties (Lev-Yadun and Aloni 1995). This tapering or widening in the dimension of tracheids along the stem axis and consequently between trees of different size is explained by a different duration the cell spends in the expansion phase (Anfodillo et al. 2013) due to decreasing gradient of auxin

concentration from leaves to root (Aloni 2014; Uggla et al. 1996).

This study aims at providing a quantification of the variability of the anatomical properties of rays among sites, individuals, and annual rings to verify (1) if ray abundance is linked with the amount of storage as imposed by the environmental conditions, and/or (2) if the changes in ray dimensions and density are associated with changes in other wood tissues. The study area has been explicitly selected at high latitude to highlight tree's carbon economy in a temperature-limited environment to minimize interference with drought responses. By using tree-ring anatomy, we compared several ray characteristics across a gradient of forest productivity in the permafrost region of Central Siberia, and we related the results to tracheid size.

Materials and methods

Study area and site characteristics

The study was carried out in the boreal forest of Central Siberia, a relatively harsh environment for tree growth. The study area was located in the proximity of the Tura field station of the VN Sukachev Institute of Forest (Evenkia, Krasnoyarsk region, 64°18' N, 100°11' E). The climate is continental characterized by extremely cold winter with relatively warm summer. According to the instrumental records of the meteorological station in Tura (1929–2010, 150 m asl), the mean annual air temperature of the region is −9.0 °C, with January being the coldest (−35.9 °C) and July the warmest month (16.5 °C). Annual precipitation is 357 mm, with 171 mm (48 %) falling during the summer (June–August). The region is located in the continuous permafrost zone predominantly covered by Dahurian Larch (*Larix gmelinii*). This deciduous conifer naturally grows at the thermal limit of forest distribution of the Asian part of Russia where the resources of soil and mineral nutrition are limited (Abaimov and Koropachinskii 1984). In this environment, *Larix gmelinii* generates massive mono-specific forests. In the region, the growing season for this species is usually very short starting at the second part of May and ending at the beginning of September (Bryukhanova et al. 2013). These harsh growing conditions combined with the needs to mobilize large amount of stored carbon every spring to build new foliage, makes the selected set-up an ideal study case for exploring environmental effects on variability in ray parenchyma traits. In addition, larch wood is characterized by the presence of only uniseriate rays, i.e., stacked cells to form narrow plates just one cell wide and with tracheids at their extremities.

The three sites were selected along a gradient of forest productivity (in terms of the size and height of mature

trees, see Table 1), which is mainly determined by the thermal regime of the layer of seasonally thawing soil. The most productive site (*High*) has been identified on the river bank of the Kochechum River, where due to direct solar radiation and the low thermal insulation of a very thin layer of soil vegetation, the conditions for tree growth are comparably favorable for the region (Kirdyanov et al. 2013). The less productive site (*Low*) is located about 5 km away and about 200 m higher in elevation. The site lies on a north-facing slope (10°) where tree growth is extremely limited by the persistence of permafrost close to the upper soil layers. The third site (*Mid*) represents an intermediate level of productivity and is located in the riparian zone of a temporary creek close (ca 30 m away) from the most productive site on the river bank. The differences in site productivity have been verified in terms of radial growth rates. Ring widths of trees from the low productive site (0.10 mm) were only about 8 % of the ones observed in the most productive site (1.31 mm) and 13 % of the intermediate one (0.80 mm).

Sampling and anatomical measurements

Wood material for stand characterization was collected in 2010 and 2011 from cores (for *High* and *Mid*) and discs (for *Low*) taken at breast height from a total of 68 randomly selected dominant trees distributed among the sites. Each core and disc has been polished with sand paper to perform tree-ring width measurements and cross-dating.

Anatomical measurements have been performed on wood cross-section and tangential section from a selection of trees per site. In total, 20 cross-sections from 20 different trees of about 0.25 µm thickness covering the annual rings from 2000 to 2010, and 39 tangential sections for the years 2005 and 2011 were prepared using a sledge microtome. For anatomical measurements, the micro-sections were stained with astra blue and safranin and permanently fixed in Canada balsam following standard dendrochronological procedures (Gärtner and Schweingruber 2013). Stained slides were then photographed through the photo-tube of a microscope (Olympus BX41, Tokio Japan) with a 40× magnification using a mounted digital camera (Canon 650D, Tokyo, Japan) to get high-resolution images (0.945 pixels/µm). Subsequently, overlapping (15–25 %) images were stitched using the PTGui v8 software (New House Internet Services BV, The Netherlands). The measurements on the cross-sections were restricted to a 5-mm-wide stripe. Measurements on the tangential section were considering square-shaped subset image areas of 2 × 2 mm. Rays, identified on both section planes (cross and tangential) as single objects of one or more rays but excluding resin ducts, were manually outlined to obtain

Table 1 *Larix gmelinii* stand characteristics of the three selected sites in Siberia (mean $\pm$ standard deviation)

Site productivity	Low	Mid	High
Number of trees surveyed	31	18	19
Tree height (m)	6.1 ± 1.7	9 ± 1	17 ± 2
DBH (cm)	5.4 ± 2.1	10 ± 1	22 ± 3
Age at DBH	~ 100	~ 50	~ 50
Radial width of the last ten annual rings (mm)	0.10 ± 0.05	0.80 ± 0.43	1.33 ± 0.81
Correlation between the full tree-ring width series (Rbt)	0.60	0.58	0.65
Depth of soil active layer	40	40	160
Moss layer thickness (cm)	20	30–40	1–2

High high productivity, *Mid* medium productivity, *Low* low productivity site, *DBH* stem diameter at 1.3 m height, *rbt* mean correlation between trees

anatomical characterization of rays (number, density, height and area). On each section, both cross and tangential, the percentage of ray surface was calculated as the ratio of the total ray area to the wood area considered. Tracheid characterization (tangential width and length in tangential section and lumen area in cross-section) was also measured. Tracheid length has been manually measured on tracheids clearly showing both their ending tips on the images. Lumen area has been measured for earlywood cells only and then we calculated the 75th percentile for a better weighting of cells that have a higher contribution to water conductivity, while measurements on tangential sections were performed on both earlywood and latewood cells. Tracheid measurements on cross-section were performed for all individuals for the years 2005 and 2011 and for five individuals per site for measurements on tangential sections. Cell anatomical measurements were performed on the images of the micro-sections using the image analysis tools ROXAS (von Arx and Dietz 2005; von Arx and Carrer 2014; Fig. 1) and Image-Pro Plus software (Media Cybernetics Inc, USA).

Statistical analysis

Factors influencing tree-ring growth, ray and tracheid parameters were identified using linear mixed-effect model. The best model was identified by defining the fixed effects of each initial models, and then by selecting the best random structures based on AICc using the maximum likelihood method (Zuur 2009). Tree identity was included as a random factor, while site and year were considered as fixed effects. Each initial model and all the possible subset models were then compared based on ML criterion and AICc to obtain the optimal model. In case several models showed similar AICc values <2 , these were refitted by the REML method to obtain estimates and significances of factor effects, and the simplest model with significant fixed effects was chosen according to the principle of parsimony (Burnham and Anderson 2002). Homoscedasticity and normality of residuals were checked graphically. Statistical analyses were performed in R version 3.1.0 (R Development Core Team 2014) including the packages “lmerTest” and “MuMIn”.

Fig. 1 Example of **a** cross- and **b** tangential micro-sections used for the measurement of ray parenchyma. In both sections, each single ray was outlined manually. The blue objects show some of the outlined rays. Resin ducts in rays were excluded from the ray area measurements

Partitioning of variance components between and within sites was performed using a generalized linear model (GLM) with two-way ANOVA.

Results

Variability in ray proportion

In this study, ray quantification resulted from measurements of an average of 153 ± 97 (mean $\pm$ standard error) rays on each cross-section ($n = 20$) and 137 ± 24 on each tangential section for each selected sample ($n = 39$) (Table 2). In general, the measurement of ray surface performed on tangential section (range between 2 to 10 % of ray area) was more variable than on the cross-section likely due to a more accurate rays' surface quantification on the tangential section. Although the linear mixed-effect model indicated clearly significant differences in the annual radial growth and cell lumen area among the sites (Fig. 2; Table 3, $p < 0.01$), the site differences in % ray surface were found statistically significant ($p < 0.05$) only in the measurements performed on tangential section (Figs. 2, 3). Average ray surface of sites ranged between a minimum of 4.60 % at the low productivity site and 7.12 % at the high productive one (data refer to measurement on tangential sections). However, the full range of ray surface from all tangential sections ranged from a minimum of 3.01 % to a maximum of 11.18 % (Fig. 3), indicating a high inter-tree variability. This result has been confirmed by the quantification of the partitioning of the

variance, which has shown that the difference among the sites only little contributed to overall variability (7 and 23 % for cross- and tangential section, respectively), whereas the contribution of the within-site variability was considerably larger (62 and 63 %, respectively).

Considering the variability in ray surface along the series of tree rings (period 2000–2010), thus considering only the measurements from the cross-sections (Fig. 4), we observed an average coefficient of variation of 10.7, 13.0 and 15.1 % for Low, Mid and High, respectively. The correlation between all site time series of percentage ray surface (Rbt) was 0.00, and ranged between -0.15 for Low and 0.20 for High, indicating no common signal between and within the sites.

Ray and tracheid anatomical structure

Earlywood tracheid lumen area of the low productive site was significantly smaller than for the other two sites. On average, the 75th percentile of the tracheid lumen area was $547 \pm 38 \mu\text{m}^2$, $1121 \pm 63 \mu\text{m}^2$, $965 \pm 59 \mu\text{m}^2$, for Low, Mid and High, respectively (mean $\pm$ standard error, Fig. 2). Similar significant site differences have been provided by the linear mixed-effect model for ray height ($p < 0.01$) and density ($p < 0.05$), as measured on the tangential section (Fig. 3). Average ray height was smaller at the lower site ($133 \pm 1.8 \mu\text{m}$), corresponding to 37 and 45 % of the height observed in the Mid ($212 \pm 2.8 \mu\text{m}$) and High ($242 \pm 2.9 \mu\text{m}$) sites, respectively (Table 2). Site average ray density was $40.1 \pm 1.9 \text{ mm}^{-2}$, $33.3 \pm 1.7 \text{ mm}^{-2}$ and $30.9 \pm 1.1 \text{ mm}^{-2}$, for Low, Mid

Table 2 Number of trees, micro-sections, annual rings and anatomical characteristics measured in both the cross-section and tangential section (mean $\pm$ standard error) of the *Larix gmelinii* site in Siberia

Site	Cross-section			Tangential section		
	Low	Mid	High	Low	Mid	High
No. of trees	5	10	5	5	7	8
No. of sections	5	10	5	10	14	15
Annual rings (years)	2000–2010	2000–2010	2000–2010	2005, 2011	2005, 2011	2005, 2011
Tree-ring width (mm)	0.09 ± 0.04	0.76 ± 0.47	1.32 ± 0.70	0.08 ± 0.16	0.54 ± 0.44	1.31 ± 0.56
Total no. of rays	404	1884	774	1617	1714	2011
Ray proportion (%)	5.70 ± 0.24	6.78 ± 0.14	6.44 ± 0.12	4.60 ± 0.36	6.24 ± 0.36	6.64 ± 0.44
Single ray area (μm^2)	–	–	–	1133 ± 355	1879 ± 35	2383 ± 45
Ray height (μm)	–	–	–	133 ± 1.76	212 ± 2.78	242 ± 2.86
Ray density (mm^{-2})	–	–	–	40.1 ± 1.9	33.3 ± 1.7	30.9 ± 1.1
Cell lumen area (μm^2)	547 ± 38	1121 ± 63	965 ± 59	–	–	–
Tracheid tangential width (μm)	–	–	–	28.0 ± 1.4	30.2 ± 2.4	30.4 ± 1.1
Tracheid length (μm)	–	–	–	2273 ± 174	2506 ± 192	2643 ± 224

Cell lumen area was measured only for the years with tangential sections and quantified as a 75 % percentile. Tracheid tangential width and length were measured on five individuals per site as means including both early- and latewood cells

High high productivity, Mid medium productivity, Low low productivity site

Fig. 2 Summary plot of ring width, cell lumen area and proportion of ray surface as measured on the cross-sections and grouped by site. The *box plot* indicates the distribution of tree average values; the *red points with error bars* indicate within-tree values (mean $\pm$ standard error of single annual ring measurements, years 2000–2010 or ring

width and ray surface, and 2005 and 2011 for cell lumen area). *High* high productivity, *Mid* medium productivity, *Low* low productivity. *Different letters* indicate significant differences at $p < 0.05$ based on linear mixed-effect models (for Ring width without considering the Year effect)

Table 3 Results of linear mixed-effect models (estimates $\pm$ SE) of the analyzed anatomical parameters

Variables	Ring width (mm)	Cell lumen area (μm^2)	Cross-sectional ray surface (%)	Tangential ray surface (%)	Ray density (n mm^{-2})	Ray height (μm)	Rays per tracheid
Intercept	11.5 ± 27.1	$-25,611 \pm 17,156$	$81 \pm 29^{**}$	$4.6 \pm 0.6^{***}$	$40 \pm 2^{***}$	$137 \pm 14^{***}$	$2.5 \pm 0.1^{***}$
High	$139.2 \pm 35.2^{***}$	$420 \pm 120^{**}$	–	$2.2 \pm 0.8^*$	$-9 \pm 3^{**}$	$99 \pm 18^{***}$	–
Mid	2.7 ± 30.5	$562 \pm 125^{***}$	–	1.6 ± 0.8	$-7 \pm 3^*$	$78 \pm 19^{***}$	–
Low	0	0	–	0	0	0	–
Year	-0.005 ± 0.01	13.0 ± 8.5	$-0.04 \pm 0.01^*$	–	–	–	–
High $\times$ year	$-0.068 \pm 0.02^{***}$	–	–	–	–	–	–
Mid $\times$ year	-0.001 ± 0.02	–	–	–	–	–	–
Low $\times$ year	0	–	–	–	–	–	–

High high productivity, *Mid* medium productivity, *Low* low productivity site

Significant relationships at 0.05, 0.01 and 0.001 probability levels are marked with *, **, ***, respectively

and High, respectively. There was a positive relationship between ray area and the tracheid area of both measured on tangential sections ($r = 0.65$, $p < 0.01$). Considering the average tangential tracheid surface (by multiplying tracheid length and width, Table 2), the tracheid density (i.e., the number of tracheid per mm^2 of tangential section) was 16, 14 and 13, for Low, Mid and High, respectively. Given the measured ray density (Table 2), it results in a ratio of 2.46, 2.42 and 2.58 rays per tracheid for Low, Mid and

High, respectively, with no statistical differences among the sites (Fig. 5, $p > 0.05$).

Discussion

Trees growing in contrasting habitats or facing changes in their interaction with the environment are expected to show physiological and structural adjustments to best cope with

Fig. 3 Summary plot of annual ray surface, ray density and ray height as measured on the tangential sections (years 2005 and 2011 for each tree) and grouped by site. *High* high productivity, *Mid*

medium productivity, *Low* low productivity sites. *Blue filled dot* indicates single section values. *Different letters* indicate significant differences at $p < 0.05$ based on linear mixed-effect models

Fig. 4 Percentage of ray surface as measured along the annual rings (from 2000 to 2010, *left plot*) and *box plot* summary grouped by site (*right plot*). *Green* high productivity, *orange* medium productivity, *blue* low productivity sites. *Thick lines* (*left plot*) and *filled dot* (*right plot*) indicate year average. *Different letters* indicate significant differences at $p < 0.05$

the specific environmental conditions, a pattern that is true among (Baas and Miller 1985; Carlquist 1985), but also within species (Larcher 1995; Meinzer et al. 2011). These adjustments often include changes in wood structure, as wood structure determines essential functions related to tree performance and survival (Gartner 1995). For a deciduous

species growing in a cold environment as the ones selected in this study, we expected that available storage space in the stem (i.e., the proportion of ray parenchyma) well reflects the need for storage at the time of ray formation, and that the size and density of rays scale with the size and density of the other surrounding wood tissues (e.g. tracheid).

Fig. 5 Summary plot of number of ray per tracheid. *High* high productivity, *Mid* medium productivity, *Low* low productivity sites. *Red filled dot* indicates individual measurements. *Different letters* indicate significant differences at $p < 0.05$

Variability of ray surface to the environment

Indications of positive responses of ray abundance have been observed in relation to injuries (Lev-Yadun and Aloni 1992; Arbellay et al. 2010, 2012) confirming that the tissue is capable to immediately and plastically respond to environmental stimuli with locally up to 115 % increases in ray number within few years from the damage (Arbellay et al. 2012). In our study, despite the highly significant reduction in growth ($p < 0.01$), ray abundance between sites was found to be statistically different ($p < 0.05$) only for the measurement on the tangential section. Nevertheless, our results indicate that ray surface tends to increase with increasing productivity with, on average, 13 % (cross-section) and 44 % (tangential section) more abundance in the most productive site compared to the less productive one (see Table 2; Fig. 2 for ray surface measured on cross-section, and Fig. 3 on tangential section). The proportions measured in this study (4.60–6.64 %) match with the lower range of observations performed for various coniferous wood (4.9–11.7 %, Myer 1922). Even when considering a possible bias in measurement of ray surface due to the fact that rays tended to contain one or two ray tracheids on either side of the ray, the observed declining ray height and density with declining site productivity (Table 2) would leave even less room for ray parenchyma and thus further consolidate our results. Our observations are in agreement with pioneer studies showing similar increase in ray proportion with improved growth conditions due to favorable topography (Myer 1922), or light conditions (Hartig et al.

1894). Our findings showing an increased ray proportion with increasing site productivity indicate, contrarily to our initial hypothesis, for an absence of increased xylem carbon container with increasing stressful conditions and suggest that the xylem ray volume is rather linked to the growth ability of the tree, i.e., related with tree vigor and/or leaf area. However, ray abundance in response to elevated CO_2 concentration is contrasting, being negative in 20-year-old Scots pine (Kilpelainen et al. 2007), unaffected in young Scots pine (Ziche and Overdieck 2004), and positive in European beech saplings (Overdieck et al. 2007). Any significant difference in abundance of ray parenchyma has been found between different pine provenances from differently drought-exposed sites in Spain, although with differences in the proportion of ray tracheids, suggesting greater water storage capacity in the trunk of the more drought-exposed trees (Esteban et al. 2010; Martin et al. 2010).

Absence of common year-to-year variability

The quantifications performed along the series of annual rings (Fig. 4) showed that ray proportion, although with some year-to-year variation (coefficient of variation of sites between 10 and 15 %), has no common signal ($R_{bt} < 0.2$). Although some of the year-to-year variation might be caused by the low representativeness of the measurement, which seems to be more unstable when measurements are performed on the cross-section compared to the tangential section, results from a 10-year period indicate an absence of response to any common inter-annual climate variation. Explorations along tree rings of *Picea abies* have shown similar weak responses with ray height and ray cell number (Wimmer and Grabner 2000), while some characteristics related to the formation of ray parenchyma in *Juniperus thurifera* from dry environment have been associated with climatic conditions occurring at critical stages of tree-ring formation (Olano et al. 2013b). The absence of coherent year-to-year variability among trees from the same site or between site chronologies could be either obscured by individual variability (especially with respect to our sampling depth) and/or related to the fact that the parenchyma cells within the rays usually stay alive while being part of the sapwood, (up to 30 annual rings in our trees), making rays rather inert to changes in volume.

Other kinds of variability: individual and size related

Part of the quantified variation in ray abundance was not directly related to the environment. When considering the components of variability, we observed these to be larger between the individuals where ray-abundant trees

displayed up to three times more ray surface than ray-poor trees from the same site. These differences between individuals might be related to the leaf area as observed by (Gartner et al. 2000) in *Pseudotsuga menziesii*. In terms of proportion of explained variance, the variability between the sites was responsible for only 8 % (in cross-sections) to 22 % (in tangential sections), while it was ~60 % within the sites. In addition, in our study, we also observed that the lower ray percentage of the less productive site is mainly related to a smaller ray size but with a higher density. Similar increase in ray density and decrease in size have been observed in juvenile compared to the mature earlywood of *Pinus halepensis* and *Pinus pinea* (Lev-Yadun 1998). These relationships suggest that the lower ray values (proportion, area and height) are mostly due to a general downscaling related to smaller wood cells rather than from a lower density of rays per surface unit. In fact, wood is a complex biological structure designed to conduct water from the roots to the leaves, to provide mechanical support of the plant body, and to store biochemicals and thus the three main tissue types providing these functions—vascular tissue, fibers and parenchyma—are closely interconnected and their functioning cannot be independent from the properties of the other tissues. Our results support the hypothesis that each single tracheid should share a surface with at least one ray cell (we quantified this ratio to be 2.5) to be directly integrated with the phloem and cambium. This would allow the living tissue (rays) to always actively regulate functional processes (i.e., hardwood formation, embolism repair,) in each single tracheid (Spicer 2014). The variability in ray proportion seems therefore to be mainly driven by difference in ray height and only little in ray density, since this feature has to be integrated in the wood structure and thus has to be more conservative.

Conclusions and perspectives

The quantification of the variability of ray surface among sites, individuals, and annual rings suggest that ray abundance primarily reflects individual-related properties (e.g., size, vitality, social status) rather than the environmental conditions in our study system. First, the size and density of rays have to fit the structure of the other tissues they are connected with to guarantee the necessary functional connectivity. The dimension of an individual ray, as other anatomical features, is linked to the location within the tree and its size, and thus when accounting for size differences, the site effect tend to be annulled while the number of rays per tracheid is kept relatively constant (~2.5 in our trees). In addition, our results also indicate that the size of the container in the xylem does not necessarily reflect the amount of stored carbohydrates since we did not observe a higher ray proportion under increased stressful conditions.

This result is thus rather consistent with reports linking more vigorous individuals to greater parenchyma proportion compared to less vigorous ones (Gartner et al. 2000).

Our results are however divergent with regard to the ability to use ray abundance as proxy for environmental conditions. On the one hand, the absence of common response of ray proportion to climatic variability is not promising for high-frequency climatic reconstruction. However, on the other hand, there is part of unexplained variability between individuals and partially between sites that might be associated with the changing balance between carbon supply and demand to the environmental conditions the tree experiences in the long term. Trees in more stressful conditions acquire less carbon but their demands might not decrease with the same amount. The response might also be different depending on the stress factor. For example in a cold environment, a direct inhibition of growth will limit carbon consumption but less assimilation (Korner 2003), while under dry conditions the absence of water would lead to reduced assimilation. These considerations suggest that medium- and low-frequency variation of ray abundance can still provide keys for understanding the dynamic of long-term carbon storage economy of trees (Eckstein 2013; Olano et al. 2013b). In this regard, it would be of particular interest to retrospectively investigate if there are coherent environmental indications explaining the strong inter-tree variability, which might be either related to trees internal factors, such as for example genetic make-up or vigor. Further studies retrospectively quantifying trajectory or ray abundance in living and dead individuals (sensu Hereş et al. 2014) might be helpful to test if, for example, the reduction in ray abundance over a tree's life can be used as an early warning indicator for depleting carbon reserves and/or declining tree vitality. Positive results might supply a complementary tool for a more thorough understanding of the response of trees to global climatic change through retrospective analysis of tree's carbon economy. However, we agree with (Lev-Yadun and Aloni 1995) that tree size and ontogeny have to be taken into account to avoid the ecological hypothesis to just be a sampling artifact. More knowledge of allometric relation and on long-term variation in ray parenchyma over many tree rings is thus welcome (Lei et al. 1996; Meinzer et al. 2011).

Author contribution statement PF and GvA have designed the study; AK has sampled the wood material; MT has performed the tree-ring width and anatomical survey under the guidance of PF, GvA and MB; and PF has written the manuscript with the support of all coauthors.

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Conflict of interest The authors confirm that there is no conflict of interest.

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