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Changes of wood anatomical characters of selected species of *Araucaria* during artificial charring: implications for palaeontology

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ABSTRACT

Charcoal is widely accepted as evidence of the occurrence of palaeo-wildfire. Although fossil charcoal remains have been used in many studies, investigation into the anatomical changes occurring during charring are few. The present study analyses changes in selected anatomical characters during artificial charring of modern wood of three species of the genus *Araucaria* (i.e. *Araucaria angustifolia*, *Araucaria bidwillii* and *Araucaria columnaris*). Wood samples of the studied species were charred under controlled conditions at varying temperatures. Measurements of anatomical features of uncharred wood and artificial charcoal were statistically analysed. The anatomical changes were statistically correlated with charring temperatures and most of the parameters showed marked decreases with increasing charring temperature. Compared to the intrinsic variability in anatomical features, both within and between growth rings of an individual plant, the changes induced by temperature account only for a comparatively small percentage of the observed variability. Regarding *Araucaria* charcoal, it seems possible that at least general taxonomic and palaeoenvironmental implications can be drawn from such material. However, it is not clear so far whether these results and interpretations based on only three taxa, can be generalized for the entire family and anatomically similar fossil taxa or not.

Keywords: anatomy, Araucariaceae, *Araucaria angustifolia*, *Araucaria bidwillii*, *Araucaria columnaris*, charcoal, wood

Introduction

Fossil plants represent excellent proxies for reconstructing and understanding environmental changes through time (e.g. Beerling 2007). A number of anatomical and morphological characters of plants can be used to reconstruct selected aspects of local and global climate change (Gastaldo *et al*. 1996; Chaloner & McElwain 1997). In many cases the information which can be gathered from fossil plant remains strongly depends on the preservation of plant remains (e.g. Uhl 2006). One mode in which plant remains can be preserved for very long geological, as well as short time scales, is charcoal, as charred plant material is almost unsusceptible to most forms of chemical and biological degradation (Scott 2000).

The ignition and spread of wildfires is controlled by a number of climatic and environmental parameters, and fires are important sources of disturbance in many ecosystems (Bowman *et al*. 2009; Scott *et al*. 2014). It is known through the fossil record of charcoal that fires occurred more or less regularly, ever since the invasion of the continents by the first embryophytic plants during the Silurian (Glasspool *et al*. 2004; Scott *et al*. 2014).

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Charcoal is easily and frequently preserved in the fossil record and represents direct evidence for the occurrence of wildfires during Earth history (e.g. Scott 1990; 2000; 2010; Scott *et al*. 2014). Qualitative characters of the original plant anatomy remain mostly unmodified in charcoal (cf. Scott 2000; Schweingruber 2007), and even quantitative parameters (e.g. as cell diameter, cell-wall thickness, diameter of tracheids and cross-field pitting, rays high and width) of charred wood have repeatedly been used for taxonomical and palaeoecological interpretations (e.g. Prior & Gasson 1993; Süss & Schultka 2001). However, the latter is at least questionable, as it has repeatedly been demonstrated that a number of anatomical parameters may change during charring.

Such modifications of wood anatomy induced by charring can include cell wall homogenization (e.g. McGinnes *et al*., 1971; Prior & Alvin 1983; Scott & Jones 1991a; b), formation of diagonal cracks crossing tracheid bordered pits (e.g. Jones 1993), changes of tracheid dimensions (e.g. Cutter *et al.* 1980) and expansion (and even bursting) of rays (e.g. Harris 1958). Other authors reported changes of the dimensions of intervessel pits (e.g. Elder *et al*. 1979), as well as changes in the appearance of cross-field pits (e.g. Gerards *et al.* 2007) during charring.

To help to fill out the gap of information about the potential changes on anatomical structures that might be affected by fire, the present study analyses quantitative alterations occurring in *Araucaria* wood during artificial charring and investigates the influence of temperature on taxonomically significant anatomical details. Three species of the genus *Araucaria* have been chosen as living models for the fossil wood genus *Agathoxylon*, which is anatomically similar to the wood of modern Araucariaceae. *Agathoxylon* has a long stratigraphic record, dating as far back as the Devonian, it is globally distributed, but wood assignable to this genus has been produced by a variety of extinct gymnosperm groups systematically not closely related to Araucariaceae (e.g. Dutra & Stranz 2003; Philippe 2011; Rößler *et al.* 2014). From an anatomical point of view, most species traditionally assigned to *Agathoxylon* fall well within the xylological variability of one or two modern species of the Araucariaceae (e.g. Seward & Ford 1906; Greguss 1955; Gondran *et al*. 1997; Dutra & Stranz 2003; Philippe 2011).

Materials and methods

Wood samples were collected from trees growing in different areas of Rio Grande do Sul, Brazil. Samples of native *Araucaria angustifolia* (Bertol.) Kuntze were collected in the municipality of São José dos Ausentes (28º47'06.56"S 49º58'50.85"W). Samples of *Araucaria bidwillii* Hooker (native to Australia) were collected in the municipality of Novos Cabrais (29º47'3.48"S 52º58'14.59"W) and samples of *Araucaria columnaris* (Forst.) Hooker (native to New Caledonia) in the municipality of Colinas (29º32'28.84"S 51º50'28.35"W) (Fig. 1). The later two taxa have been introduced into Rio Grande do Sul for horticultural purposes in the 20th century.

The samples have been obtained in the form of "trunk-discs" located at 1.50 m height above ground. The discs were 8.0 cm thick, with a diameter varying between 18.0 and 35.0 cm. From each of these discs, cubic samples of 1.0 cm^3 were cut out, weighed and then placed in a porcelain pot (Chiarotti A-37), covered with sand (10 g, Merck®) and charred in a muffle furnace (SP Labor SP-1200) at temperatures varying from 50 ºC to 1000 ºC, for 1 hour, with temperature increments of 50 ºC. In total, samples from each species were charred at 20 different temperatures, and 6 replicate samples of each taxon were charred at each temperature.

After charring, the samples were cooled down to room temperature in a desiccator and the charcoal was reweighed with a precision balance (Polimate PL2000). Charcoal weight loss is expressed as percentage of the weight of the original, uncharred wood cubes.

The colour of the samples was analysed for each charring temperature with naked eye, before and after charring. After charring the pieces were fractured (under a stereomicroscope) to reveal the three structural planes of the wood for anatomical analyses (transverse, tangential and radial section), before they were mounted on aluminium stubs using double-sided adhesive tape and analysed under a Scanning Electron Microscope (SEM - Zeiss EVO LS15) at the Parque Científico e Tecnológico do Vale do Taquari (Tecnovates) at UNIVATES.

Measurements of uncharred wood and charcoal were done in accordance with recommendations of the IAWA list of microscopic features for softwood identification (IAWA Committee 2004). The determination of quantitative anatomical features was based on 25 measurements of 7 parameters: (a) tangential tracheid diameter [μm], (b) intracellular width of tracheids in cross section (lumen diameter) [μm], (c) ray width [μm], (d) ray height [μm], (e) pit diameter on cross-field [μm], (f) pit diameter in radial section [μm] and (g) pit diameter in tangential section [μm]. These qualitative anatomical structures of wood are the same as those used in the description of the wood of Araucariaceae reported by previous authors (e.g. Greguss 1955; Esteban *et al*. 2002; Booi et al. 2014). All measurements were taken from calibrated images with the aid of the software package *ImageJ* (Rasband 1997-2014).

Statistical analyses were performed using the software package PAST (Hammer *et al*. 2001). Correlations between anatomical changes and charring temperatures were tested with bivariate linear, polynomial, quadratic and exponential regressions. Although for some variables polynomial regressions of higher order (4-5) produced good results, it appeared that bivariate linear regression commonly provided more reliable results. This was probably due to the relatively small number of measurements which resulted

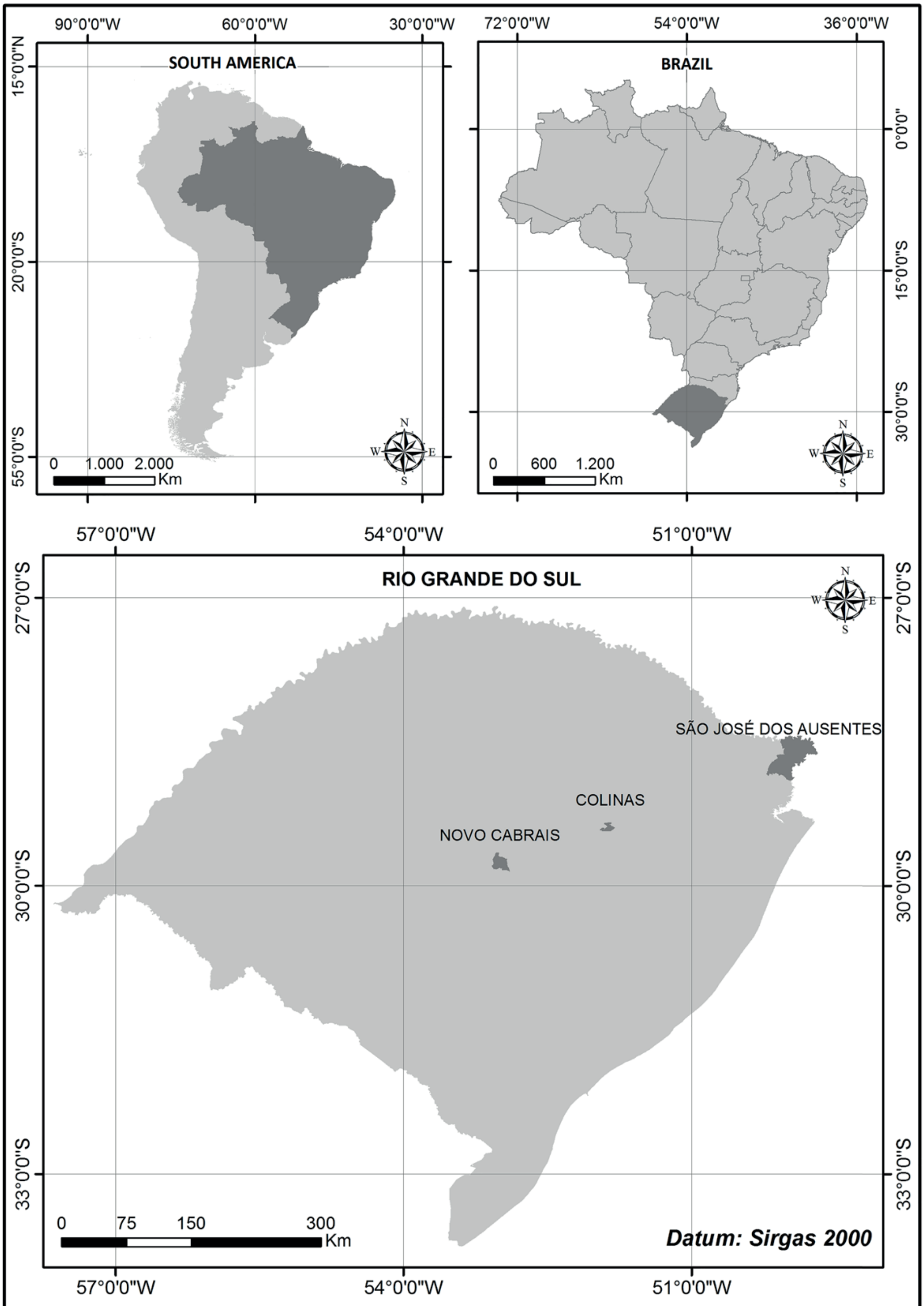


Figure 1. Map of Brazil, Rio Grande do Sul, and the localities where the *Araucaria* samples have been collected: *Araucaria angustifolia* (native) – São José dos Ausentes (28°47'06.56"S 49°58'50.85"W), *Araucaria bidwillii* (planted) – Novos Cabrais (29°47'3.48"S 52°58'14.59"W) and *Araucaria columnaris* (planted) – Colinas (29°32'28.84"S 51°50'28.35"W).

in a considerable scatter of the data, most likely caused by the intrinsic variability of anatomical parameters within and between growth rings.

Results

Anatomical description of the studied taxa

Araucaria angustifolia exhibits growth rings of variable width. In cross section rays are inconspicuous, with thin and unpitted horizontal walls. Walls of tracheids are smooth and without spiral thickenings. Tangential tracheid walls, predominantly in latewood, exhibit bordered pits. These are few in number, mostly detached, or in rows of two or three. Theoutline of the pit borders are rather indistinct. The apertures are circular or short-elliptic, but most frequently long-elliptic or lenticular. On radial tracheid walls bordered pits occur in 1-3 rows. Here pit apertures are predominantly elliptic, in the widest earlywood tracheids they are sometimes circular. The pits in the cross fields are fairly large and vary in number. When few, they are circular; when more and touching, they usually occupy the entire area of the field. Their number ranges from 1 to 9; when 9, they are arranged in threes in superposed rows. Their apertures are lenticular or slit-like, in the earlywood sometimes elliptic. Apertures of horizontal or nearly horizontal orientation have only been encountered in pits that have arisen from the fusion of two pits radially next to each other (Greguss 1955).

Araucaria bidwillii specimens studied here, have growth rings which are quite indistinct; their delimitation is marked only by the thicker walls of the latewood tracheids. Tracheids in cross section are mostly rounded, occasionally tetra- or polygonal. Horizontal walls of ray cells are smooth, without pitting. Rays are comparatively narrow and not more than 1 – 6 – 8 cells high. Tangential walls of tracheids are generally smooth, very rarely with 1 – 2 solitary, bordered pits. Horizontal and tangential walls of rays are smooth. In cross fields 1 – 4 (5) pits occur, mostly in horizontal rows; in the marginal cells 4 – 6, irregularly arranged, pits occur. The pits on tracheid walls are sometimes arranged in longitudinal rows (cf. Greguss 1955).

Araucaria columnaris has very indistinct growth rings, marked solely by the late terminal tracheids being slightly smaller. Tracheids in cross section are commonly rounded, tetra- or pentagonal, occasionally irregular in shape. Wood parenchyma cells are absent. On tracheid walls bordered pits are irregularly arranged, sometimes in two, very exceptionally in three, rows and alternating. In cross section ray cells look barrel-shaped. In radial section tracheid walls are smooth, occasionally with uniseriate, more frequently with biseriate alternating pitting. Pits in three rows are quite rare, occurring only in earlywood tracheids. Parenchyma was not observable in radial sections. In crossfields 4-12 pits with clearly defined borders and slit-like or short-elliptic, slightly inclined or vertical apertures can be observed, which do not extend to anywhere near the pit annulus (cf. Greguss 1955).

Anatomical comparison with literature data

To test whether our method for the analysis of wood anatomical parameters is suitable for taxonomic purposes, we compared our measurements of uncharred wood samples from all three taxa with data from the literature (e.g. Greguss 1955; Esteban *et al.* 2002) (Tab. 1). All parameters presented here are in agreement with data previously published by various authors for the three species investigated. This demonstrates that our preparation and measurement methods are suitable for the purpose of this study.

Changes of size and form

The cubic wood samples remained intact in size and form up to 150 °C. After 200 °C the width and height of the pieces decreased, the cubes becoming increasingly smaller in all dimensions. All specimens underwent such shrinkage with increasing charring temperatures. Above 600 °C, samples became more and more fragile and frequently fragmented if touched even slightly. At 850 °C the samples of *Araucaria bidwillii* and *Araucaria columnaris* were completely destroyed, with only ash remaining. The same occurred with *Araucaria angustifolia* at 900 °C.

Colour

The samples changed colour from light to dark brown below 250 °C and then to black at 300 °C as observed visually (Fig. 2). Brittleness increased with temperature, the largest changes occurring between 250 °C and 300 °C for *Araucaria angustifolia* and *Araucaria bidwillii*, and between 300 °C and 350 °C for *Araucaria columnaris* wood, based on visual analysis. Also a silky sheen developed between these temperatures.

Weight loss

Weight loss during experimental charring in the muffle furnace was around 10 % up to 150 °C and varied from 20 % in *Araucaria columnaris* to approximately 60 % in *Araucaria angustifolia* and *Araucaria bidwillii* at 300 °C. Weight loss increased significantly at 300-350 °C in all species. After charring at 500 °C, all species lost approximately 80 % of their initial weight, and 90 % at 650 °C (Fig. 3).

Cell wall

Homogenization of the cell wall, which is, besides other parameters, frequently used for identification of fossil charcoal (e.g. Scott 2000), occurs at 300 °C in *Araucaria angustifolia* (Fig. 4), *Araucaria bidwillii* (Fig. 5) and *Araucaria columnaris* (Fig. 6).

Table 1. Comparative overview on the measurements taken from the three species of *Araucaria*: A – Average of 25 measurements from uncharred wood. B – Values published by Greguss (1955). C – Values published by Esteban *et al*. (2002).

		Tangential tracheids diameter (µm)	Lume diameter (µm)	ray width (µm)	ray height (µm)	pits diameter on cross-field (µm)	pits diameter in radial section (µm)	pits diameter in tangential section (µm)
Araucaria angustifolia	A	31.81	27.06	20.24	107.09	7.52	12.83	10.32
	B	-	-	-	-	7 - 9	-	-
	C	-	-	-	-	6 - 11	10 - 15	≥5
Araucaria bidwillii	A	37.86	30.88	19.55	189.79	10.71	13.44	9.85
	B	-	35 – 40	-	-	7 - 11	15 - 20	-
	C	-	-	-	-	6 - 15	10 - 15	-
Araucaria columnaris	A	13.22	20.91	21.53	119.05	8.17	12.05	9.35
	B	10 - 12	20 – 22	-	-	9 - 11	16 - 17	12 – 13
	C	-	-	-	-	6- 15	10 - 15	≥5

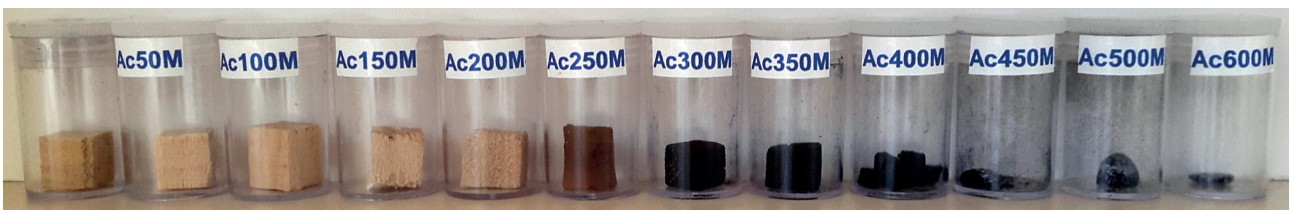


Figure 2. Colour changes observed in *Araucaria columnaris* samples during charring. Temperatures are indicated on the top; uncharred material, measuring 1.0 cm³, is the first from the left. Please see the PDF file for colour reference.

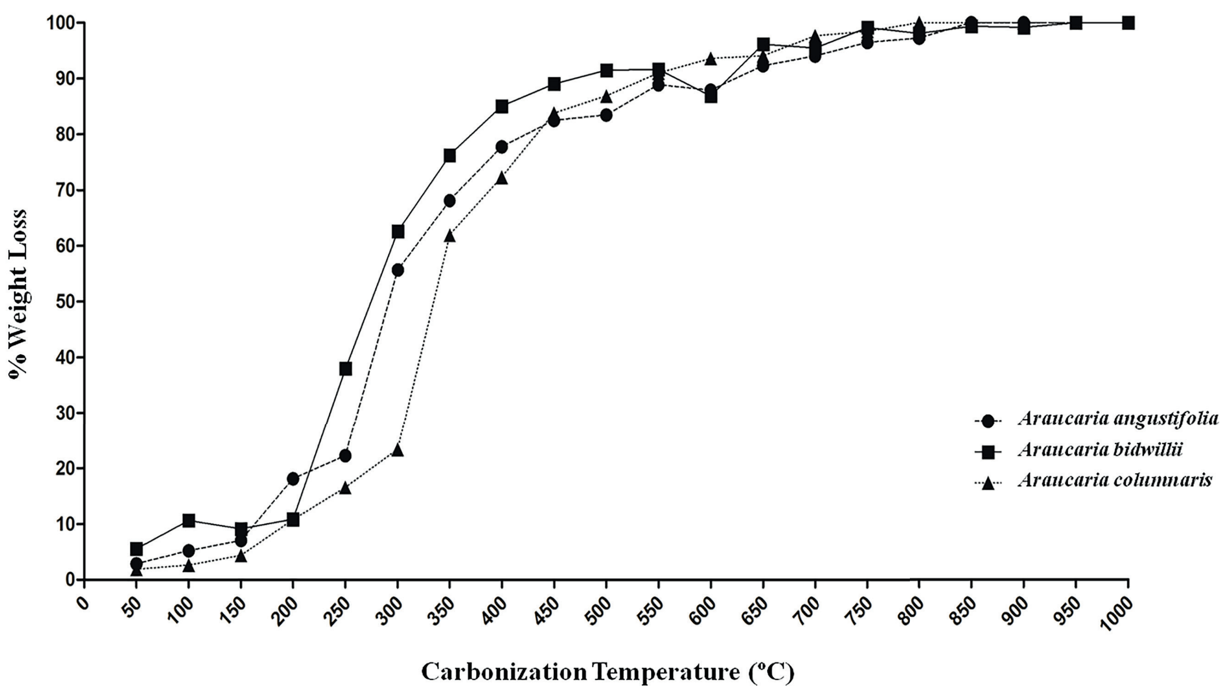


Figure 3. Percentage weight loss of samples from *Araucaria angustifolia*, *Araucaria bidwillii* and *Araucaria columnaris* after charring for 60 minutes at the 20 temperatures used in this study. For original data see Table S1 in supplementary material.

Correlation between temperature and anatomical changes

The results of the statistical analysis are presented in three charts, one for each species, with separate graphics for each of the seven parameters analysed here.

For Araucaria angustifolia (Fig. 4) p values indicate that the temperature has a statistically significant influence on the measured parameters. All measurements for this species have a negative correlation with temperature, except width of

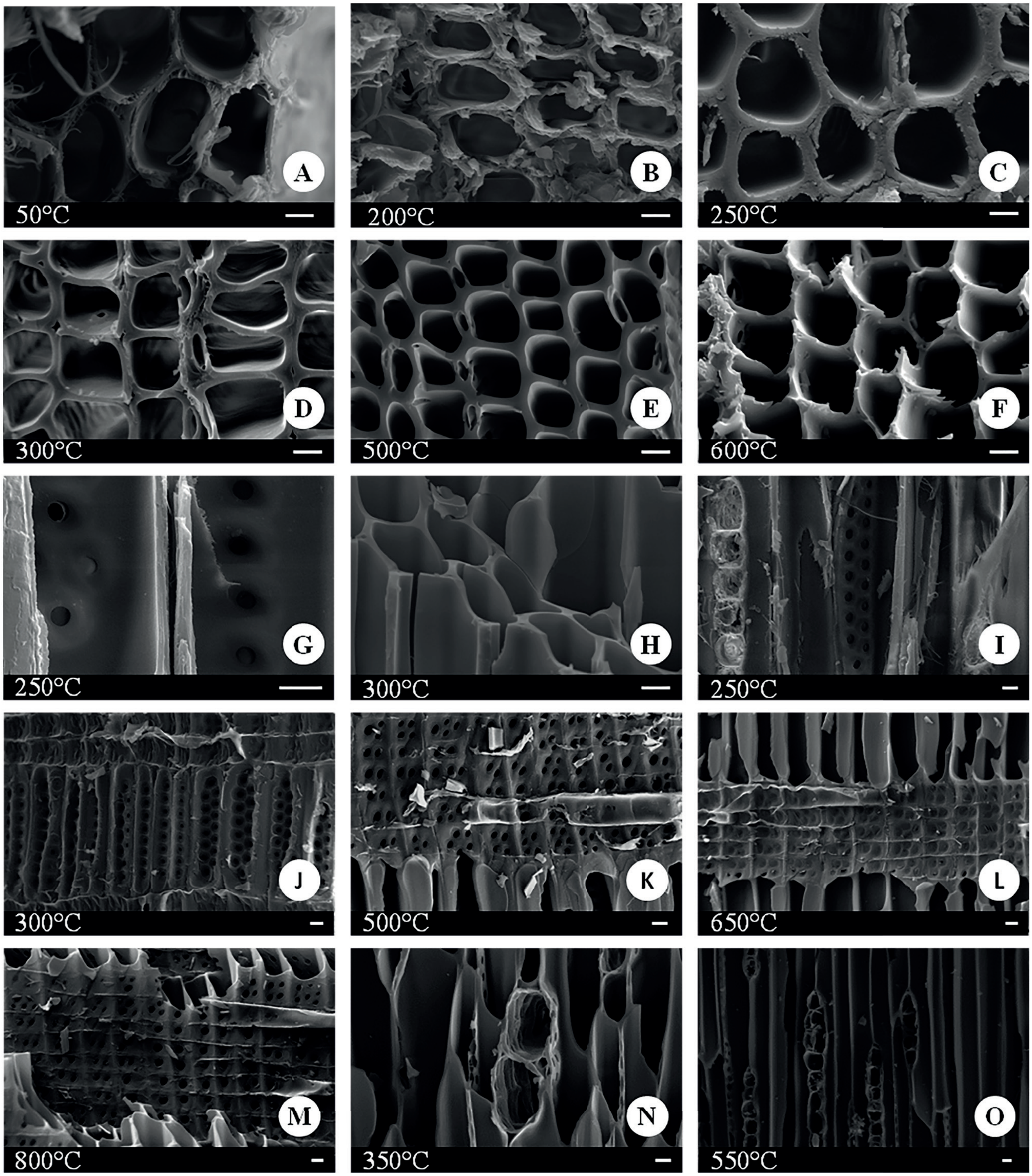


Figure 4. Anatomical details of *Araucaria angustifolia*. Scale bar = 10 µm. **A**-**F** – transversal section in different temperatures, from 300 °C upwards the cell wall is homogenized. **G** – detail from not yet homogenized tracheid cell wall in radial section. **H** – homogenized tracheid cell wall in tangential section. **I** – detail of the pits in tangential section. **J** – pits in radial section. **K**-**M** – cross-field pitting, showing the preservation of the features in different temperatures. **N** – ray with detail of the pitting inside the ray and on the tracheid walls. **O** – rays after charring at 550 °C.

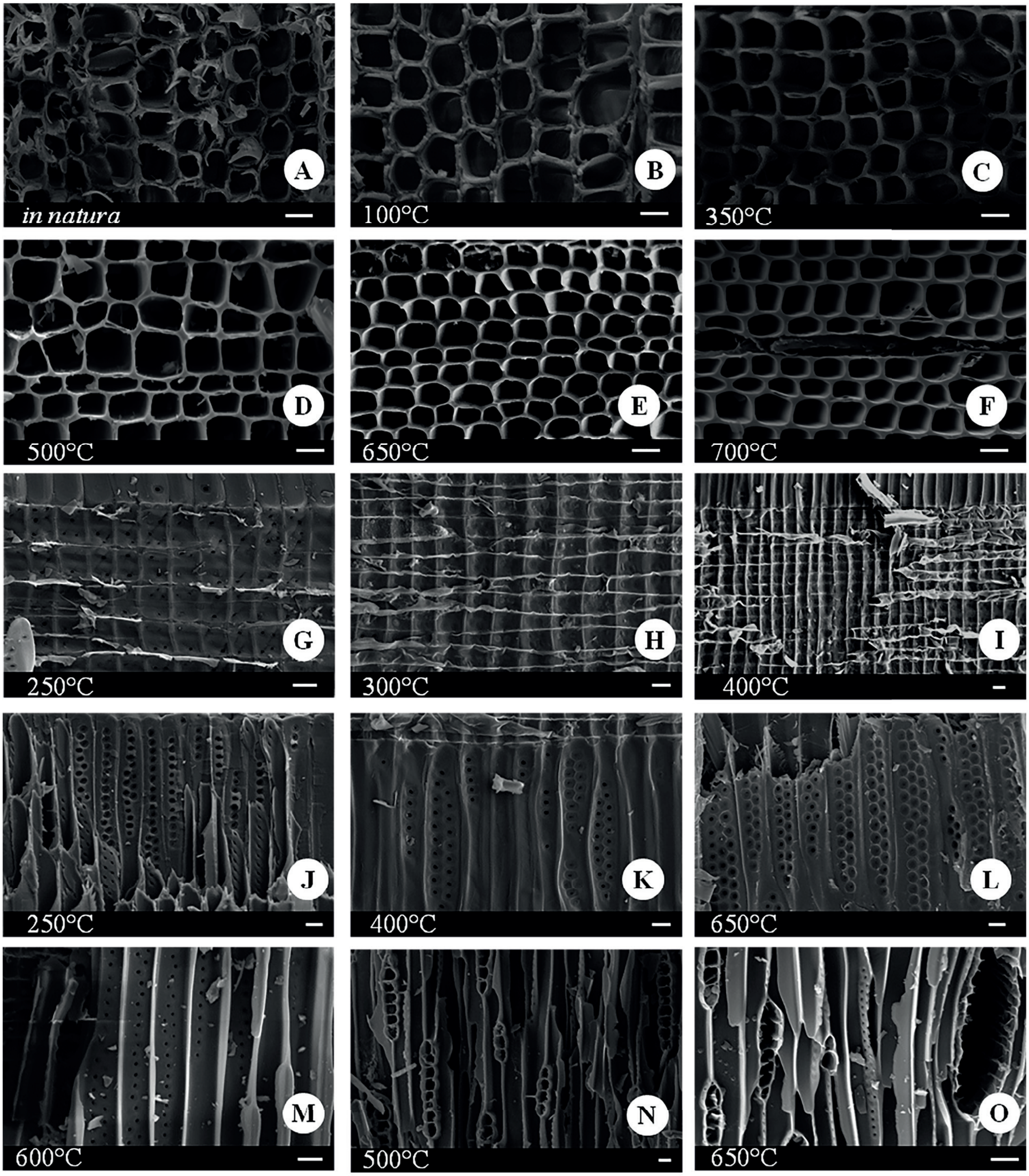


Figure 5. Anatomical details of *Araucaria bidwillii*. Scale bar = 20 µm. **A-F** – transversal section in different temperatures, above 300 °C the cell wall is homogenized. **G-I** – cross-field with cross-field pitting in different temperatures, showing that this feature is preserved. **J-L** – radial section, with tracheids with two to three rows of pits, in J the tracheid cell walls are not homogenized, but in K and L they are. **M** – radial section, showing the pits with apertures and the cell walls well homogenized. **N, O** – tangential section, with rays preserved in high temperatures, in o showing one burst ray.

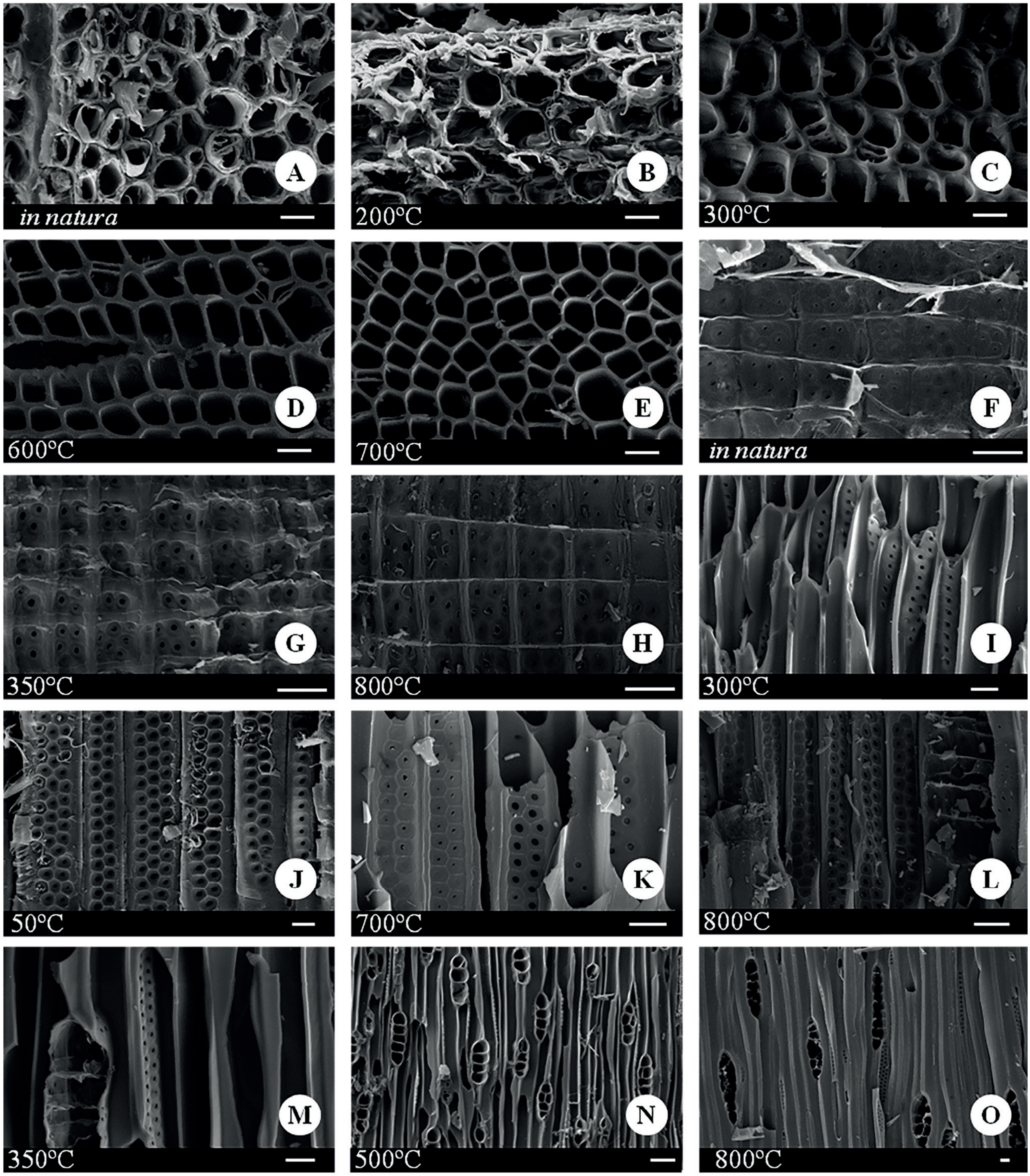
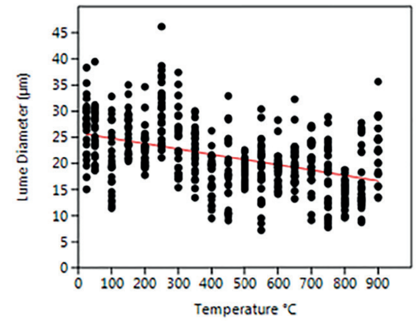


Figure 6. Anatomical details of *Araucaria columnaris*. Scale bar = 20 µm. **A-E** – transversal section at different temperatures, above 300 °C the cell wall is homogenized. **F-H** – cross-field with cross-field pitting in different temperatures, showing that this feature is preserved. **I** – tangential section, tracheids with pitting and cell wall homogenized. **J-L** – radial section, with tracheids with bi- to triseriate pits. **M** – radial section, showing a ray with pits inside the ray and on the tracheid. **N, O** – tangential section, with rays preserved in high temperatures, in o showing burst rays.

Araucaria angustifolia

Variable	Slope	Error	Intercept	Error2	r	p	r^2	$\%r^2$
Lume Diameter (µm)	-0.01022	0.00097	25.836	0.51017	-0.437	1.62E-23	0.191	19.053
Traqueid Diameter (µm)	-0.00967	0.0011	28.044	0.58152	-0.374	3.50E-17	0.140	13.958
Cross-field pitting (µm)	-0.00267	0.0004	9.7093	0.21226	-0.292	8.78E-11	0.085	8.520
Tangential Pitting (µm)	-0.00373	0.00047	11.779	0.24765	-0.342	1.63E-14	0.117	11.727
Radial Pitting (µm)	-0.00371	0.00038	13.244	0.19853	-0.412	7.04E-21	0.170	16.968
Ray Height (µm)	-0.01601	0.00674	122.08	3.5521	-0.109	0.01796	0.012	1.178
Ray Width (µm)	0.00735	0.00094	15.621	0.49548	0.3382	3.59E-14	0.114	11.435



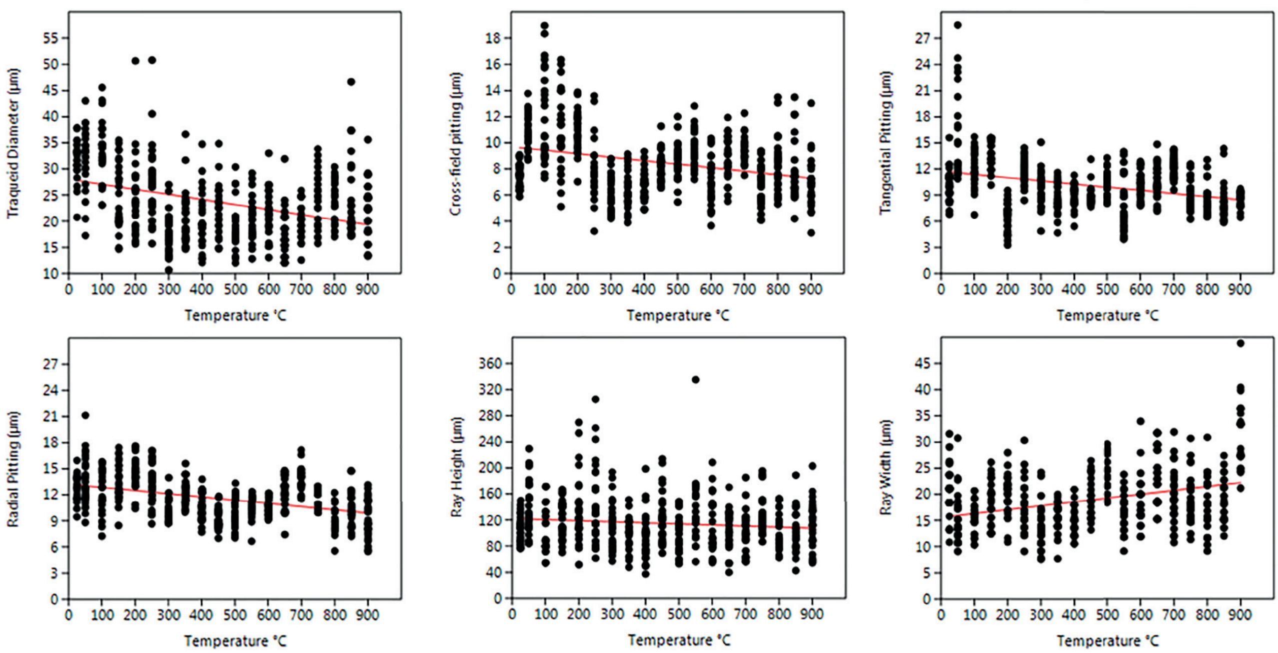


Figure 7. Bivariate linear regression analysis for *Araucaria angustifolia* charred at different temperatures. The values of r, r^2 and $\%r^2$ for 7 anatomical features are shown in individual graphs. Measurements were replicated 25 times in fresh wood and the 20 charcoal fragments produced at the different charring temperatures. The line represents the linear trendline. For original data see Table S2 in supplementary material.

rays. As the charring temperature increases, the dimensions of the anatomical parameters decrease, the opposite occurs with the width of the rays, which increases with increasing temperature, resulting in a positive correlation (Fig. 7).

For *Araucaria bidwillii* (Fig. 5), the values of p are below 0.05 for all parameters measured, indicating that the temperature has a statistically significant influence on the variables. The only exception is the width of the rays, with p = 0.70 and the correlation with variable is positive and not statistically significant. For the other parameters, the correlations are negative (Fig. 8).

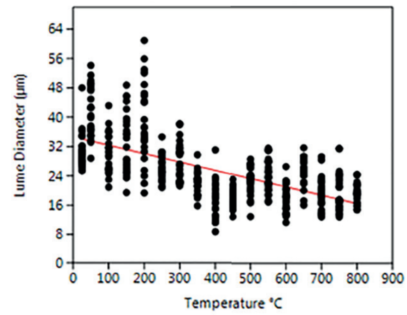
In *Araucaria columnaris* (Fig. 6) p values are below 0.05 for all parameters measured, indicating that also in the species temperature has a statistically significant influence on the variables. The only exception is the height of rays with p = 0.816, where the correlation is positive and not statistically significant (Fig. 9).

The r^2 values have the highest values in Araucaria angustifolia (19.1 %) and *Araucaria bidwillii* (39.9 %) for the lumen diameter, and in *Araucaria columnaris* for the ray width (34.1 %). Table 2 presents all p, r, and r^2 values from bivariate linear regressions for the three species.

Discussion

There is a wealth of studies on experimental charcoal production (e.g. Kollmann & Sachs 1967; McGinnes *et al*. 1971; Beall *et al*. 1974; Correia *et al*. 1974; Slocum *et al*. 1978; Prior & Alvin 1983; Scott 1989; Jones *et al*. 1991; Scott & Jones 1991a; b; Prior & Gasson 1993; Guo & Bustin 1998; Bustin & Guo 1999; Erçin & Yürum 2003; Gerards & Gerrienne 2004; Gerards *et al*. 2007; Scott & Glasspool 2005; Kim & Hanna 2006; McParland *et al*. 2007; Braadbaart &

Araucaria bidwillii								
Variable	Slope	Error	Intercept	Error2	r	p	r²	%r²
Lume Diameter (µm)	-0.02287	0.00137	34.640	0.64033	-0.6316	1.08E-48	0.399	39.889
Traqueid Diameter (µm)	-0.01471	0.00137	35.803	0.64096	-0.4638	4.72E-24	0.215	21.508
Cross-field pitting (µm)	-0.00361	0.00036	9.197	0.16743	-0.4410	1.20E-21	0.194	19.445
Tangential Pitting (µm)	-0.00352	0.00044	10.025	0.20822	-0.3602	1.83E-14	0.130	12.974
Radial Pitting (µm)	-0.00456	0.00051	12.686	0.23952	-0.3979	1.41E-17	0.158	15.836
Ray Height (µm)	-0.07860	0.01121	177.160	5.2573	-0.3228	9.27E-12	0.104	10.417
Ray Width (µm)	0.00058	0.00153	20.236	0.71799	0.0185	0.70331	0.000	0.034



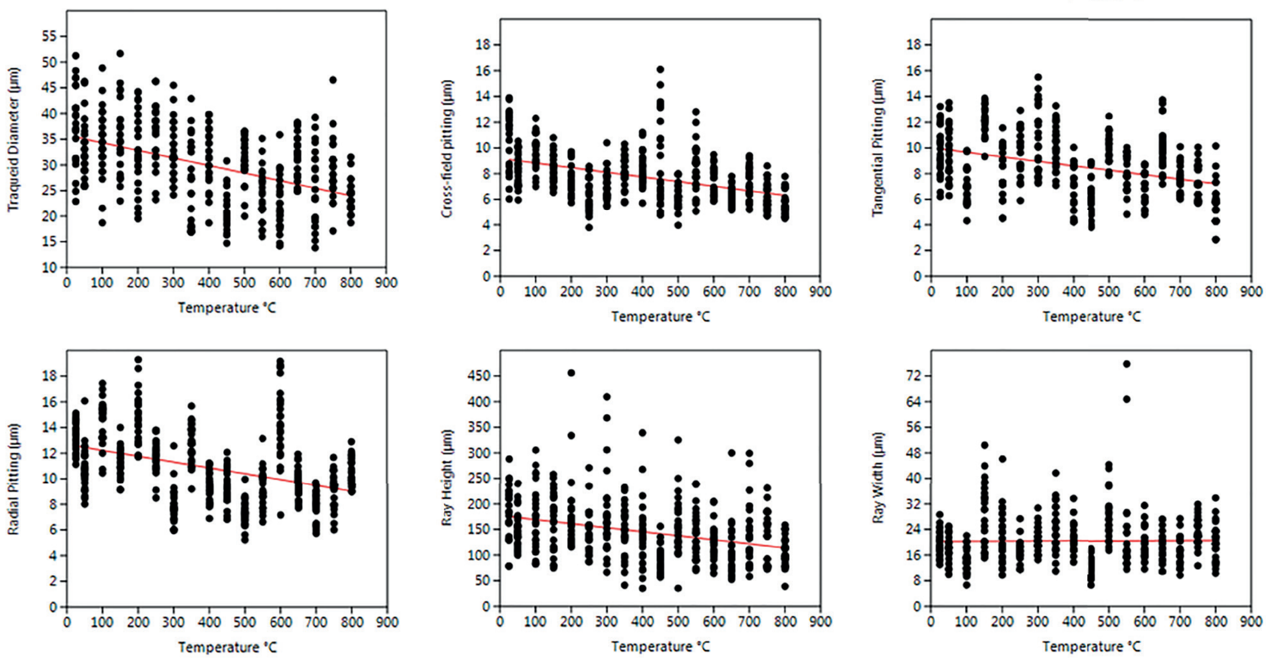


Figure 8. Bivariate linear regression analysis for *Araucaria bidwillii* charred at different temperatures. The values of r, r² and %r² for 7 anatomical features are shown in individual graphs. Measurements were replicated 25 times in the fresh wood and the 20 charcoal fragments produced at the different charring temperatures. The line represents the linear trendline. For original data see Table S3 in supplementary material.

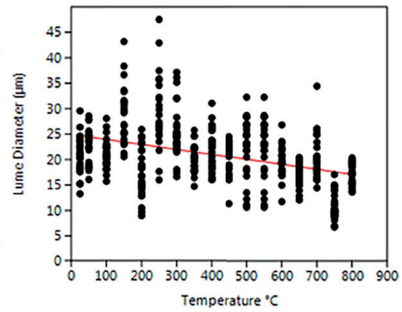
Poole 2008; Kwon *et al*. 2009; Gonçalves *et al*. 2012; 2014; Muñiz *et al*. 2012a; b; Nisgoski *et al*. 2014; Afonso *et al*. 2015). Although these studies present a wide taxonomic coverage, only a few have so far utilized Araucariaceae wood samples (i.e. Gerards *et al*. 2007: *Araucaria araucana*; Muñiz *et al*. 2012b: *Araucaria angustifolia*).

Previous studies have demonstrated that during charring of wood, physical and chemical processes occur, which lead to changes in the dimensions of cellular structures, but generally the overall anatomical microstructure of the wood remains largely unchanged in a wide variety of different taxa (e.g. Schweingruber 2007). As our new data show, this is also true for the three species of *Araucaria* analysed in the present study. Qualitative anatomical data did not change significantly during charring and are in good agreement with data from uncharred wood observed here or published earlier (e.g. Greguss 1955; Esteban *et al*. 2002). Thus, it seems to be possible to discriminate between different *Araucaria* species based on charcoalified wood, insofar as such qualitative wood anatomical characters can be used for such a purpose (cf. Greguss 1955; Esteban *et al*. 2002). There are, however, some qualitative as well as quantitative changes of anatomical changes which occur during charring of the three species.

In addition, anatomical changes occur, depending on taxonomic affiliation, chemical and physical features of the wood, size of a charred specimen and intensity and dynamics of combustion/heating. Although experiencing weight loss (70–80 %), fragment shrinking (7–13 % in length and 12–25 % radially and tangentially), the taxonomic affiliation can usually be determined after charring (e.g. Schweingruber 1978). The same can be observed in the present study for the species of *Araucaria*, as even after heating at elevated temperatures, the overall anatomical structure has not changed significantly, and the charred samples still exhibit taxon-specific anatomical details. Nevertheless, it is possible to deduce a statistically significant effect of charring temperatures on several quantitative parameters of the wood of all three species of *Araucaria*

Araucaria columnaris

Variable	Slope	Error	Intercept	Error2	r	p	r²	%r²
Lume Diameter (µm)	-0.00990	0.00111	24.969	0.51877	-0.399	1.08E-17	0.159	15.939
Traqueid Diameter (µm)	-0.01704	0.00245	35.877	1.15	-0.32	1.38E-11	0.103	10.253
Cross-field pitting (µm)	-0.00261	0.00032	8.329	0.1501	-0.369	3.97E-15	0.136	13.590
Tangential Pitting (µm)	-0.00379	0.00040	10.449	0.18564	-0.422	8.05E-20	0.178	17.843
Radial Pitting (µm)	-0.00386	0.00044	11.496	0.20589	-0.393	3.54E-17	0.155	15.472
Ray Height (µm)	0.00296	0.01271	183.12	5.9637	0.0113	0.816	0.000	0.013
Ray Width (µm)	0.02830	0.00191	23.006	0.89819	0.5836	3.78E-40	0.341	34.055



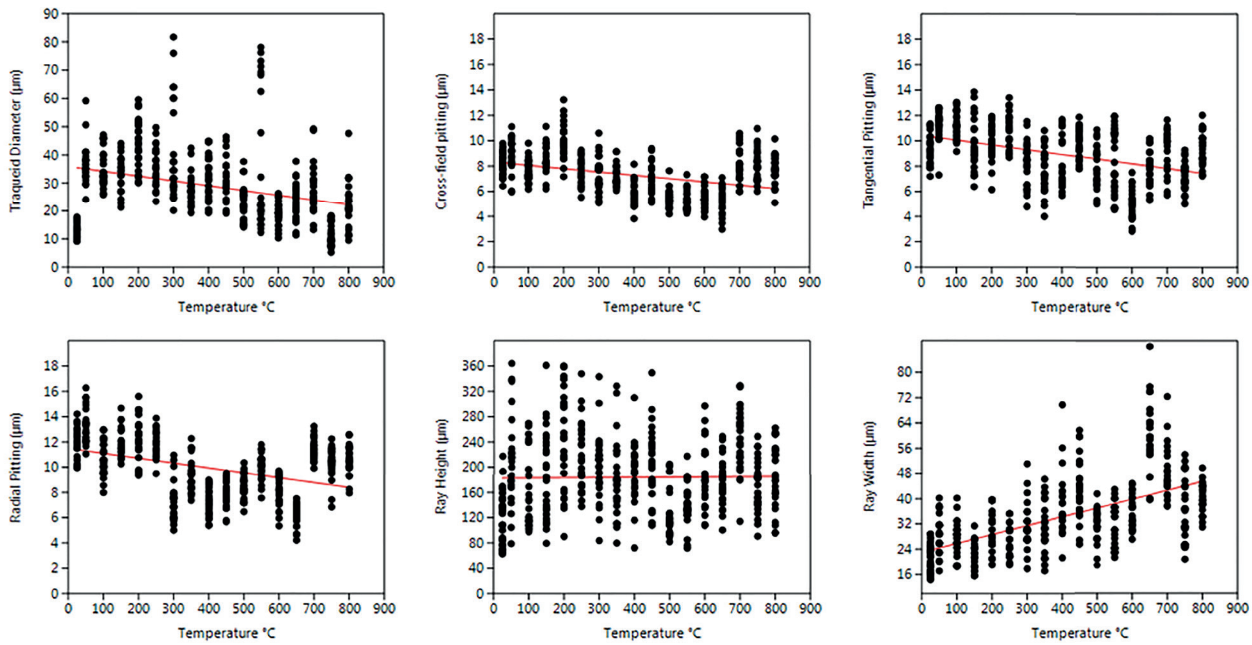


Figure 9. Bivariate linear regression analysis for *Araucaria columnaris* charred at different temperatures. The values of r, r^2 and $\%r^2$ for 7 anatomical features are shown in individual graphs. Measurements were replicated 25 times in the fresh wood and the 20 charcoal fragments produced at the different charring temperatures. The line represents the linear trendline. For original data see Table S4 in supplementary material.

analysed here. In all cases, except the widening of rays, increasing charring temperatures led to a dimensional decrease of measured anatomical details. This observation suggests that such changes should be considered carefully whenever quantitative data from fossil charcoal are used for taxonomic or palaeoecological purposes (e.g. Prior & Gasson 1993; Süss & Schultka 2001). Especially taxonomic interpretations or even erection of new taxa which are solely based on quantitative differences between different pieces of (charred and/or uncharred) wood seem to be highly problematic in the light of our data. The charring of wood leads to differential degradation of wood components and their recombination into a carbon-enriched structure during thermal degradation from hemicelluloses, followed by cellulose and finally lignin (e.g. Slocum *et al.* 1978; Wiedemann *et al.* 1988). During charring, hemicelluloses degrade between 170-300 ºC, cellulose above 240 °C and lignin above 280 °C (e.g. Beall & Eickner 1970; Fengel & Wegener 1989; Zeriouh & Belkbir 1995; Byrne & Nagle 1997). However, the pyrogenic decomposition of wood is not evident until the temperature reaches about 275 °C (e.g. Kollmann & Sachs 1967) and thus charring of wood can usually not be observed below 300 °C (e.g. Kwon *et al.* 2009). During charring wood experiences several changes: (a) colour – darkening of the wood, which at the end of the process becomes black; (b) physical properties – considerable mass losses, contraction, possible anatomical distortions resulting from charring and loss of many volatile substances; (c) chemical properties – continuous and gradual conversion of the three main chemical components of wood (cellulose, hemicellulose and lignin) forming a new product, rich in carbon and chemically altered (e.g. Shafizadeh 1982; Boon *et al* 1994; Braadbaart & Poole 2008).

Significant weight losses result from the fact that the principal chemical constituents of cell walls are pyrolized (Wiedemann *et al.* 1988; Treusch *et al.* 2004; Paris *et al.* 2005). The data observed by us demonstrate that at 300 °C the charring process is still incomplete for the three taxa

Table 2. Values of p, r and r^2 for the anatomical features analysed in this study for all three species of *Araucaria*.

Anatomical feature [µm]	*Araucaria angustifolia*			*Araucaria bidwillii*			*Araucaria columnaris*		
	p	r	r^2	p	r	r^2	p	r	r^2
Lumen diameter	1.62E-23	-0.437	0.191	1.08E-48	-0.6316	0.399	1.08E-17	-0.399	0.159
Tracheid diameter	3.50E-17	-0.374	0.140	4.72E-24	-0.4638	0.215	1.38E-11	-0.32	0.103
Cross-field pitting	8.78E-11	-0.292	0.085	1.20E-21	-0.4410	0.194	3.97E-15	-0.369	0.136
Tangential pitting	1.63E-14	-0.342	0.117	1.83E-14	-0.3602	0.130	8.05E-20	-0.422	0.178
Radial pitting	7.04E-21	-0.412	0.170	1.41E-17	-0.3979	0.158	3.54E-17	-0.393	0.155
Ray height	0.01796	-0.109	0.012	9.27E-12	-0.3228	0.104	0.816	0.0113	0.000
Ray width	3.59E-14	0.3382	0.114	0.70331	0.0185	0.000	3.78E-40	0.5836	0.341

analysed, as weight loss still increased with higher charring temperatures. Not surprisingly Lara *et al.* (2017) observed similar weight losses for the three taxa investigated here, by means of thermogravimetric analysis.

In many cases the qualitative features of the original wood remain intact, whereas quantitative parameters such as cell size and wall thickness change. Quantitative features are particularly critical when attempting to distinguish between closely related species or when drawing ecological inferences (e.g. Prior & Gasson 1993; Gonçalves 2012). Thus, it seems problematic to use charcoal for delimitation between different species of closely related taxa, as such taxa often differ only slightly in the dimensions of anatomical features (e.g. Jones *et al.* 1991; McParland *et al.* 2007).

A number of previous studies reported that the disappearance of discrete cell-wall layers as well as the development of black colour and a silky sheen depended on the heating rate and the charring temperature and in general such homogenized cell walls together with the black colour and the silky sheen are frequently used for the identification of fossil charcoal (e.g. Scott 2000; 2010). Our results agree very well with these previous studies, as the cell walls lost the distinctive layering at around 300 °C. From this temperature onwards, there was no distinction between the middle lamella and the cell wall layers but the charcoal retained all other anatomical features. Additionally, a black colour and silky sheen developed between 300 °C and 350 °C, which is also in agreement with data from the literature (cf. Scott 2000; 2010 and citations therein).

Although our analysis demonstrates that different parameters changed statistically significant with increasing charring temperatures, it became evident, that most of the variability observed for the three *Araucaria* species comes from the variability of the parameters within and between growth rings (thus is largely controlled by climatic and ecological factors at the growing locality). In many cases only up to 35 % of the overall variability of measured parameters can be explained by the charring temperature.

For six anatomical features the tendency is negative, only ray width is positively correlated in *Araucaria angustifolia* and *Araucaria columnaris*. In high temperatures, the rays burst (Fig. 5), and when these features are measured, the values are higher at higher temperatures than at lower temperatures. One reason for this could be that the rays were bursting due to fast heating of remnant water within the rays.

All in all, our data suggest that, despite some quantitative changes occurring during charring, it seems possible to use charcoal to differentiate between the three *Araucaria* species analysed here. The question remains, whether this can also be done for the dozens and hundreds of fossil wood taxa assignable to *Agathoxylon*. Although a study on only three taxa of *Araucaria* is far from comprehensive with regard to the entire family Araucariaceae, it seems very likely that this could also be possible for this taxon, as the results obtained for the three *Araucaria* species are in agreement with previous data from a large number of systematically not really closely related taxa. Thus, it seems at least possible that these observations can be generalized, although one should always keep in mind that so far, the database for Araucariaceae (and other palaeontologically relevant families) is still far from comprehensive and potential exceptions from previous observations are never impossible.

Conclusions

Our data are in good agreement with previous charring studies on systematically unrelated taxa.

Despite some minor changes, qualitative anatomical features of the wood, commonly used for taxonomic purposes, remain unchanged during charring.

With increasing charring temperatures, the wood experienced an increasing weight loss.

Small quantitative changes of the dimensions of several anatomical parameters occur, and these changes depend on charring temperatures.

These changes are, however, smaller than the intrinsic variability of these parameters within and between growth rings in our samples.

Above a charring temperature of 300 °C cell walls of all three taxa became homogenized, colour changed to black and a silky sheen developed, comparable to observations from previous studies on different taxa.

Despite the changes, it seems possible to use charcoal from the three *Araucaria* species analysed here to differentiate between these three taxa.

It is likely that fossil charcoal assignable to *Agathoxylon* can also be used to differentiate between different taxa, as long as the source taxa exhibit anatomical differences which can be observed in fossil charcoal.

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