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Chemical composition of lignocellulosic biomass in the wood of *Abies religiosa* across an altitudinal gradient

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Abstract Tree adaptation to environment has been extensively studied. However, little is known about the variations in structure and chemical composition of lignocellulosic biomass (LB) in relation to altitudinal gradient. We wonder, are there significant variations in the LB in the wood across an altitudinal gradient? To answer this, we carried out a study of *Abies religiosa*. Wood samples were collected from 36 trees, grown between 3000 and 3500 masl, and then subjected to gravimetric and FTIR (Fourier Transform Infrared) spectroscopic analyses. The gravimetric results showed a proportion of 54.81 ± 2.20 % cellulose, 12.37 ± 1.33 % hemicellulose and 24.68 ± 1.16 % of insoluble lignin. Using the principal components analysis with analysis of variance (ANOVA), significant differences were found at 3100 and 3200 masl in two independent components related to both hemicellulose and lignin, through gravimetry as well as the spectroscopic bands assigned to the carbonyl groups of these polymers, respectively. However, the observed changes in chemical composition of LB did not follow a linear relationship with respect to the altitudinal gradient, which suggests that complex environmental interactions could also be playing an important role. Also, there were significant differences ($p < 0.05$) in two of the empirical indexes calculated from the FTIR analysis.

Keywords Elevation gradient · FTIR spectroscopy · Sacred fir · *Oyamel* · PCA analysis

Introduction

Wood resources are the lignocellulosic materials most widely used all over the world, since they are accessible and possess interesting physical, chemical and energetic properties [1, 2]. Lignocellulosic biomass (LB) is a natural resource, a complex fraction of the structure of plants, and the most abundant source of neutral carbon in the planet. It is formed by the components of the cell wall, mostly polysaccharides of the cellulose and hemicellulose, and lignin, which is a phenolic polymer [3–5]. The composition of LB is heterogeneous and there is little information about the organization and coordination of its main components during and after biosynthesis [6], whereas the complex physiological process is little understood [7]. The physical and chemical properties of LB differ from one plant species to another [8], as does its composition, which may vary according to the plant's genotype, the method and date of cultivation, its anatomic fraction, its age or growth stage, and the storage techniques [3, 4, 9, 10]. It has also been reported that some components of LB may change as response to biotic or abiotic stress [5].

Abiotic stress is the main factor negatively affecting the development and productivity of plants; sources of stress

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include drought, ultraviolet (UV) radiation, cold and heat [11]. Responses of the cell wall in plants to abiotic stress are complex and vary according to the species and the intensity of the stress. This complicates the identification of response patterns to abiotic stress of the cell wall and of its lignocellulosic components. Although no clear response patterns to abiotic stress have been found for cellulose, it is known that lignin tends to increase in conditions of water scarcity and low temperatures, whereas hemicelluloses tend to increase when water is scarce [12].

Environmental factors such as temperature, rainfall and soil components tend to vary across an altitudinal gradient (AG) [13, 14]. The effects of altitude on plants have been studied using an altitudinal gradient [15, 16]. In recent years, the relationship between altitude and plant development across an AG has been the focus of many ecological studies [17–22]. However, few of these studies have looked at the influence of altitudinal variation on the composition of the LB from different anatomic parts of trees. Richardson reported that lignin and cellulose in leaves of *Picea rubens* and *Abies balsamea* diminished abruptly with an increase in altitude, as opposed to hemicellulose [23]. Similarly, Genet et al. studied the cell wall of *Abies georgei* roots and found a decrease in the holocellulose (cellulose and hemicellulose) at higher altitudes [24]. Therefore, the AG may have an effect on the anatomic structure and the physical and chemical properties of wood and, consequently, on its quality [25]. The study of the possible effects of altitudinal variation on the LB of wood will lead to understand the response patterns of lignocellulosic materials.

Due to their wide distribution and economic value, conifers, to which the genus *Abies* belongs, are among the most sought-after trees. The wood of *Abies religiosa* (Kuntch) Schldl. *et* Cham, also known as *abeto* (sacred fir) or *oyamel*, a conifer with wide distribution in Mexico and Guatemala, is very much appreciated due to its physical and mechanical properties [26, 27]. These trees develop under the geographic, climatic and ecological conditions characteristic of mountainous areas, within an altitudinal gradient from 2400 to 3600 meters above sea level (masl), notably along the so-called Neovolcanic Transversal Axis [28]. In the state of Veracruz in Mexico, it is found mostly at the Cofre de Perote National Park, where trees are distributed along circular bands within a gradient from 3000 to 3500 masl [26, 29].

The objective of this study was to investigate the possible significant changes in the structural composition of LB in the wood of *Abies religiosa* across an altitudinal gradient. The researching hypothesis was that, if the distribution of *Abies religiosa* along an altitudinal gradient is limited by environmental conditions, then the limits are

representing by the extreme environmental conditions. Based on this premise, it was expected that below 3000 masl (lower limit) and above 3500 masl (upper limit), a larger lignin content would be found due to stressful environmental conditions [12], as well as a larger content of hemicellulose, a more labile structural polymer related to energy reserves [30]. As for cellulose, a reduction was expected in conditions of increased lignin and hemicellulose [31].

Materials and methods

Study area

The study was carried out within the Cofre de Perote National Park (19° 31.908′ N; 97° 09. 246′ W), in the locality named Ejido El Conejo, in the municipality of Perote, Veracruz, Mexico. The climate is cool temperate subhumid, with a temperature range between −5 and 22 °C and a summer rainfall regime of 1200–1500 mm [2, 32]. In the study area, there are no weather stations. We were able to place dataloggers HOBO U23 Pro v2 (Onset Technologies) to register temperature and relative humidity (RH) only at three places across the AG (3000, 3250 and 3500 masl), and data collection was carried out during almost 15 months. With datalogger information of relative humidity and temperature, the other altitudes were estimated through linear equations.

Field sampling

The collection of wood samples from *Abies religiosa* trees was carried out along a 500-m-long AG in August 2014. The gradient was divided into six altitudinal levels with a distance of 100 m from each other (3500, 3400, 3300, 3200, 3100 and 3000 masl). At each of these levels, wood samples from six trees were collected at a trunk height of 1.3 m. A total of 36 trees were sampled.

The selection of trees was systematic, based on the following criteria: healthy, pest-free individuals with unhampered growth and with a normal diameter (ND) of around 40 cm, according to the close correlation established between age and ND [26]. Sampled trees were geo-referenced with a minimum distance of 100 m from each other. For the extraction of wood samples, a hand drill equipped with an auger bit measuring 1 inch in diameter was used. Samples were carefully taken from each tree at the opposite side of the slope, avoiding the tree bark, so as to obtain only the xylem tissue, which was placed in plastic bags. The extraction holes were then sealed with wood plugs, and samples were taken to the laboratory for pretreatment and analysis.

Pre-treatment of samples

Pre-treatment consisted in the dehydration of 20 ± 0.03 g of each sample so as to obtain a dry base (105 ± 5 °C for 24 h) in a forced convection furnace, according to Technical Association of the Pulp and Paper Industry (TAPPI) standards (T 264 cm-07, Preparation of Wood for Chemical Analysis). Samples were then ground with a Hamilton Beach grinder model 80374 and sieved so as to obtain a uniform particle size suitable for further analysis [33].

Analysis of the chemical composition of wood

The main polymeric constituents of wood (cellulose, hemicellulose and lignin) were determined through a fiber analysis based on the Van Soest gravimetric method using α-amylase [34]. From each sample unit, 0.5 g of dry wood of homogenous particle size (through a mesh 60/40, 0.274/0.516 mm) was taken and then introduced into ANKOM filter bags and submitted to the sequential digestions indicated by the method using an ANKOM-200 fiber analyzer [23, 35]. The estimated parameters were neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin insoluble in sulfuric acid at 72 %, 1 N (IL). The chemical composition analyses were performed three times for each sample. The proportion of hemicellulose was calculated as the subtraction of ADF from NDF (NDF-ADF), the proportion of cellulose as the subtraction of IL from ADF (ADF-IL), and for IL, an ash correction was applied, as the method indicates.

Attenuated total reflection-Fourier transform infrared (ATR-FTIR) analysis

The 36 wood samples were subjected to a spectroscopic analysis ATR-FTIR. A Frontier spectrophotometer (PerkinElmer) equipped with an external module for ATR was used, as per the following procedure: 32 scanning operations per each sample; spectral resolution of 4 cm^{-1}, and wavenumber rank of 4000–400 cm^{-1}. For instrumental control and data processing, Spectrum and Spekwin32 softwares were used. Measurements were carried out on all previously homogenized wood samples of *A. religiosa*, taking 0.03 g of dry base of similar particle size (less 60 mesh, 0.274 mm) from each of them. Samples were spread over the entire area of the diamond lens of the ATR, and a similar pressure was used on each measurement (80 %). For quantitative comparison of the absorbance intensity of each band, the spectrum magnitude was normalized with respect to the value of the band with the most intensity

(1027 cm^{-1}) [36]. Six spectra were obtained from each altitudinal level. However, to save time and resources, only one sample measurement was used for each tree, as the variation of the same tree sample was found to be negligible in comparison to that between different trees.

The following empirical indexes reported in the existing literature were calculated at each altitudinal level, using the absorbance intensities: cellulose crystallinity index [37]; lateral order index (LOI, B1422/B896) and total crystalline index (TCI, B1368/B2891) [38, 39]; intensity index of the hydrogen bonds (HBI, B3340/B1316) [38]; lignin/carbohydrates index (B1231/B1156) [40] and the carbonyl/carbohydrate indexes (B1735/B1368 and B1735/B1156) [41]. In all cases, B represents the band (wavenumber of FTIR spectra) selected for calculation of each index.

Statistical analysis

The relationship between the altitudinal gradient and the chemical composition of wood was evaluated considering the first variable as independent and the second variable as the dependent variable, respectively. The following dependent variables were used: (a) For the chemical analyses of wood: the proportions of cellulose, hemicellulose, lignin and other minor components, (b) For the ATR-FTIR analysis: the absorbance intensities of the bands of the FTIR spectra of wood.

Principal component analysis (PCA) in a correlation matrix, was carried out using the data matrixes compiled by the dependent variables in analyses (a) and (b). In the correlation matrix, the data were centered and scaled based on the variables means. Variables means that demonstrated high square cosines were considered for the most explanatory principal components (PCs); then, these were singled out for further analysis through one-way ANOVAs and the post hoc Tukey's honestly significant ($\alpha = 0.05$) to test for the significant differences between scores. In all cases, the dependent variables that did not fulfill the assumptions of a normal distribution were transformed into ranks suitable for parametric statistical analysis [42]. In addition, analysis of variance (ANOVA) was used to analyze the empirical indexes and some parameters in analyses (a) and (b), separately.

The PCAs were performed using the software XLSTAT (Addinsoft, Paris, France). For the ANOVAs, each wood samples from each tree was considered as a sample unit, with six replicates ($n = 6$) for each altitudinal level (6 wood samples per 6 altitudinal levels = 36). A trust rate of 99.5 % ($p < 0.05$) was analyzed using R statistical free software. Graphs were drawn with GraphPad Prism 6 software.

Results

Microclimate conditions

Through the datalogger collections, the average temperature data were: 7.50 ± 3.4, 8.41 ± 3.5 and 9.55 ± 4.0 °C, and the average relatives humidities were: 75.32 ± 22.7, 73.70 ± 22.8 and 71.97 ± 23.9 % for the altitudinal levels 3000, 3250 and 3500 masl, respectively. Also, an inverse pattern for both climatic variables versus the altitude was observed, with a linear correlation of $R^2 = 0.99$ for both variables. Using the obtained models, the temperature and RH data, estimated every 100 meters across of AG, were as follows: 9.14 °C and 74.66 % (3100); 8.73 °C and 73.99 % (3200); 8.32 °C and 73.32 % (3300); 7.91 °C and 72.65 % (3400). Then, the temperature/100 m rate was -0.41 °C, and relative humidity/100 m rate was -0.67 %.

Chemical composition analysis

On average, the chemical composition of the wood of 36 *A. religiosa* trees analyzed here was of 54.81 ± 2.20 % of cellulose, 12.37 ± 1.33 % of hemicellulose, 24.68 ± 1.16 % of insoluble lignin and 8.13 ± 2.15 % of other minor components (extracts and soluble sugars, among others). The gravimetric analysis showed no significant differences ($p > 0.05$ in all cases) in the composition of cellulose, hemicellulose, insoluble lignin and other minor components of wood in connection with the altitude when the parameters are analyzed separately (Table 1).

On the other hand, through PCA, principal components 1 and 2 explained 78.27 % of the total variance of the data set. These PCs scores were selected from the multivariate analysis of the data matrix of the four variables studied (cellulose, hemicellulose, lignin and other minor components), taken as a data set representing the lignocellulosic biomass of wood (Fig. 1a).

The first principal component (PC1) contributed to 47.6 % of the variance and consisted in the meaningful contribution of two parameters: cellulose and other minor components with high square cosines, 0.8 and 0.73,

respectively (subsection b in Fig. 1). Whereas the second principal component (PC2) explained 30.6 % of the total variance and was conformed by the meaningful contribution of hemicellulose and lignin (subsection b, in Fig. 1). After applying the ANOVA tests to PC1 and PC2 (using the coordinates of the observations for each one), statistical difference was obtained for the second component ($F = 4.558$, $p = 0.003$), observing an increase of PC2 toward the extremes of the altitudinal gradient under study and a decrease at the center (Fig. 1d), where the altitude of 3100 masl was the lowest. There was no significant statistical difference for the first component (Fig. 1c).

ATR-FTIR characterization

The FTIR average spectra for wood samples of *A. religiosa* developed along the altitudinal gradient (3500–3000 masl) were obtained (Fig. 2). For this study, a spectral region from 3500 to 800 cm^{-1} was selected, where 15 main bands were found. In general, the spectra were very similar between them, but in the spectral region from 1700 to 1600 cm^{-1}, it is possible to observe differences at the bands 3, 4 and 5. The last band corresponds to the vibration of aromatic rings and the two first bands correspond to the stretching of carbonyl groups. The highest intensity of this functional group was found at 3300 masl for band 3 and at 3000 masl for band 4, while the lowest intensity was found at 3200 masl for both bands. The band assignments of the mid-infrared region were carried out in comparison with previous studies (Table 2).

In addition, significant differences along the altitudinal gradient were found in two of the empirical indexes calculated (Table 3). Total crystalline index (TCI) ($F = 3.485$, $p = 0.013$) decreases significantly at higher altitudes. Furthermore, index 1735/1368 ($F = 2.669$, $p = 0.041$) increases toward the upper and lower ends of the altitudinal gradient and decreases at the elevation of 3200 masl.

Moreover, through the multivariate analysis PCA, three principal components were selected, which together account for 88 % of the total variation (PC1 with 67.54 %, PC2 with 12.66 % and PC3 with 7.43 %). Score plots for

Table 1 Chemical composition of the wood of *A. religiosa* along the altitudinal gradient

Altitude (masl)	Cellulose (%)	Hemicellulose (%)	Insoluble lignin (%)	Other (%)
3500	54.35 ± 2.47a	13.24 ± 1.22a	25.08 ± 1.24a	7.32 ± 2.68a
3400	55.56 ± 1.58a	12.72 ± 1.63a	25.10 ± 1.06a	6.61 ± 1.66a
3300	55.61 ± 2.57a	11.82 ± 1.18a	24.38 ± 0.81a	8.17 ± 3.25a
3200	54.71 ± 1.01a	11.85 ± 1.26a	24.78 ± 1.02a	8.64 ± 1.33a
3100	55.33 ± 1.75a	11.65 ± 0.97a	23.98 ± 0.73a	9.01 ± 1.25a
3000	53.29 ± 3.38a	12.92 ± 1.38a	24.75 ± 1.90a	9.01 ± 0.77a

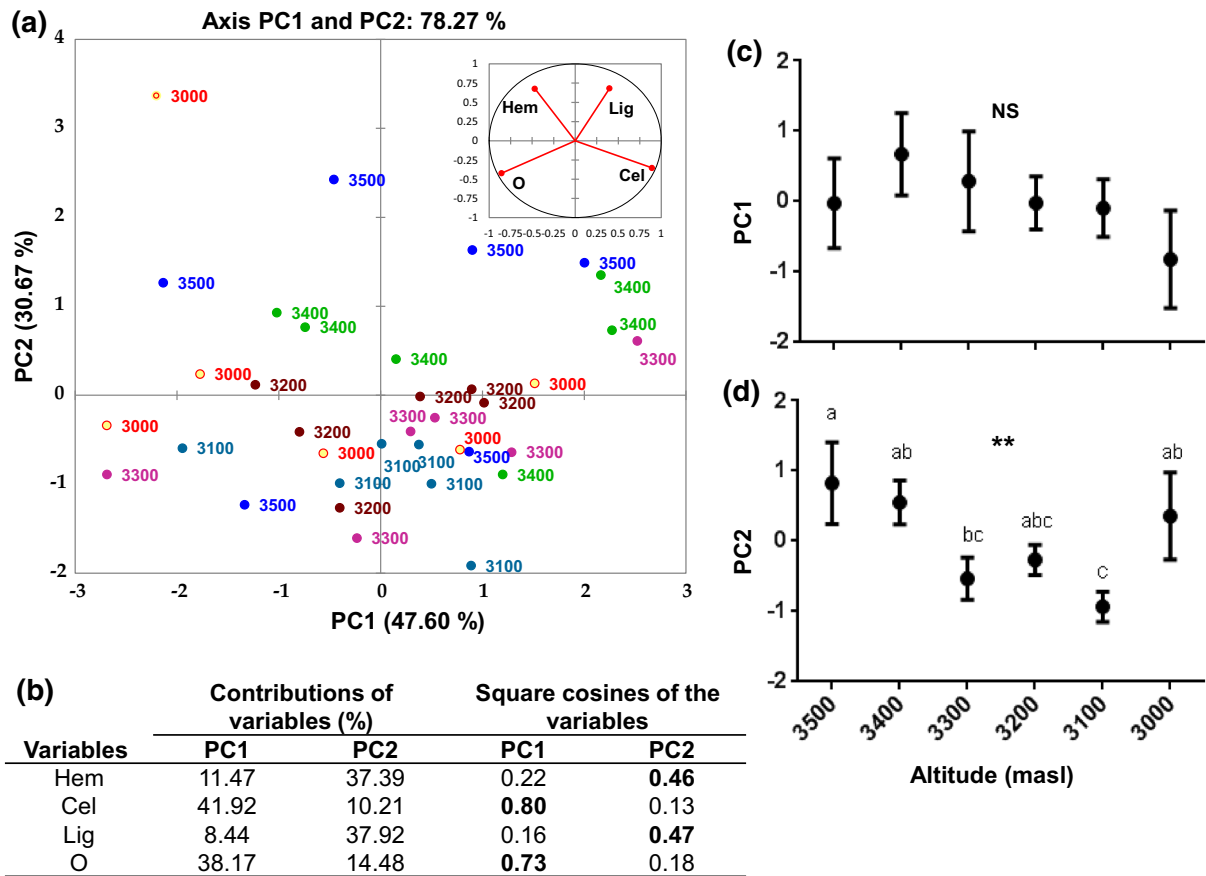Data are averages of $n = 6 \pm$ Standard deviation. Same letters denote no significant differences

(a)

Axis PC1 and PC2: 78.27 %

(b)

Variables	Contributions of variables (%)		Square cosines of the variables	
	PC1	PC2	PC1	PC2
Hem	11.47	37.39	0.22	**0.46**
Cel	41.92	10.21	**0.80**	0.13
Lig	8.44	37.92	0.16	**0.47**
O	38.17	14.48	**0.73**	0.18

Fig. 1 Principal component analysis (PCA) of chemical composition analysis of wood: **a** *Scatter diagram* of PC1 and PC2 (principal component 1 and 2) scores showing the samples and the correlations; **b** contribution scores and loadings of the studied variables; **c**, **d** loadings *graphs* of PC1 and PC2 along the altitude gradient, where the points are average values ($n = 6 \pm$ standard error), NS (non-significant) and ** (statistically significant)
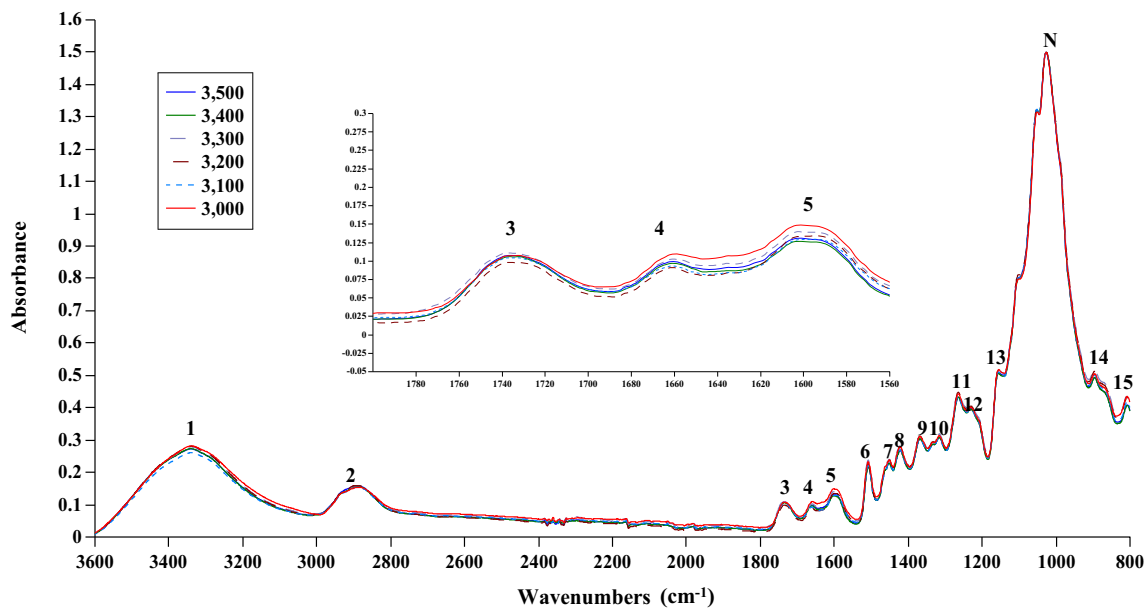

Fig. 2 Average FTIR (Fourier transform infrared) spectra of wood of *A. religiosa* at different altitudes. The altitudinal levels (masl) are represented by *different colors*. *N* is the band of normalization

Table 2 Assignment of functional groups and polymers to FTIR bands

Band number	Wavenumber (cm^{-1})	Assigned band	References	Assigned polymer
1	3340	O–H stretching	[38, 39, 43–45]	C, H, L
2	2891	C–H stretching	[39, 43, 44]	C, H, L
3	1735	C=O stretching in non-conjugated ketones and free aldehydes	[39, 43, 44, 48]	H, L
4	1660	C=O stretchings conjugated in p-substituent aromatic ketones and unconjugated carbonyls	[39, 44, 47, 48]	L
5	1600	Aromatic ring vibration	[39, 43, 44, 48]	L
6	1508	C=C Stretching in aromatic structures	[39, 43, 44, 47, 48]	L
7	1451	C–H deformation, asymmetric bending vibration of –CH$_3$ and –CH$_2$ groups	[39, 43, 44, 46–48]	L
8	1422	CH$_2$ stretching, C–O–H in plane bending vibration	[39, 49]	C
9	1368	C–H bending	[39]	C, H
10	1316	C–H deformation vibration, C–O–H in plane bending vibration	[39, 44, 49]	C, H, L
11	1264	Aromatic rings vibration of Guaiacyl units	[43, 44]	L
12	1231	C–O stretching of Guaiacyl units	[39]	L
13	1156	C–O–C asymmetric stretching	[39, 44]	C, H
14	896	Glucose ring stretching, C1–H deformation, C–H Stretching out of plane of aromatic ring	[39, 47]	C
15	808	C–H out-of-plane bending	[36]	L

Represented polymers correspond to: Cellulose (C), Hemicellulose (H) and Lignin (L)

Table 3 Empirical indexes calculated for the wood of *A. religiosa* along the altitudinal gradient

Empirical indexes	Altitude (masl)					
	3500	3400	3300	3200	3100	3000
TCI*	1.91 ± 0.025a	1.95 ± 0.015ab	1.97 ± 0.020ab	1.96 ± 0.020ab	1.98 ± 0.011ab	2.02 ± 0.020b
LOI	0.54 ± 0.006	0.55 ± 0.010	0.54 ± 0.009	0.54 ± 0.010	0.54 ± 0.007	0.55 ± 0.009
HBI	0.89 ± 0.014	0.89 ± 0.012	0.88 ± 0.014	0.89 ± 0.011	0.85 ± 0.017	0.90 ± 0.023
Ratio 1231/1156	0.078 ± 0.004	0.78 ± 0.013	0.79 ± 0.009	0.79 ± 0.002	0.78 ± 0.009	0.77 ± 0.009
Ratio* 1735/1368	0.36 ± 0.007a	0.35 ± 0.007ab	0.35 ± 0.013ab	0.32 ± 0.009b	0.35 ± 0.010ab	0.35 ± 0.006ab
Ratio 1735/1156	0.21 ± 0.004	0.21 ± 0.006	0.21 ± 0.006	0.19 ± 0.005	0.21 ± 0.006	0.21 ± 0.003

Data are averages of $n = 6 \pm$ Standard Error

Different letters indicate significant differences (Tukey's HSD, $\alpha = 0.05$)

* Statistically significant data

PC1 against PC2 and PC1 against PC3 showed differences among the samples (Fig. 3). For the first case, PC1 and PC2 can explain around 80 % of the total variance, and few differences were observed (Fig. 3a). However, for the second case, PC1 and PC3 can explain around 75 % of the total variance and higher differentiation was seen at 3200 masl (Fig. 3b).

The contribution of each band to the generation of each principal component and the square cosines are shown in Table "a" into Fig. 4. Component 3 (PC3) showed a significant difference ($F = 4.558$, $p = 0.003$) relative to the AG and the chemical composition of LB. This component comprises the band number 3 in 1735 cm^{-1} and band number 4 in 1660 cm^{-1}; both are associated to the

stretching of different carbonyl groups, increasing toward the ends of the altitudinal gradient and decreasing at an altitude of 3200 masl (Fig. 4d). There was no significant statistical difference between the first and second components (Fig. 4b, c).

Discussion

Chemical composition analysis

Although no complete report has yet been published on the composition of wood of *Abies religiosa*, it is known that lignin accounts for 25.29 % of it [50], a figure close to the
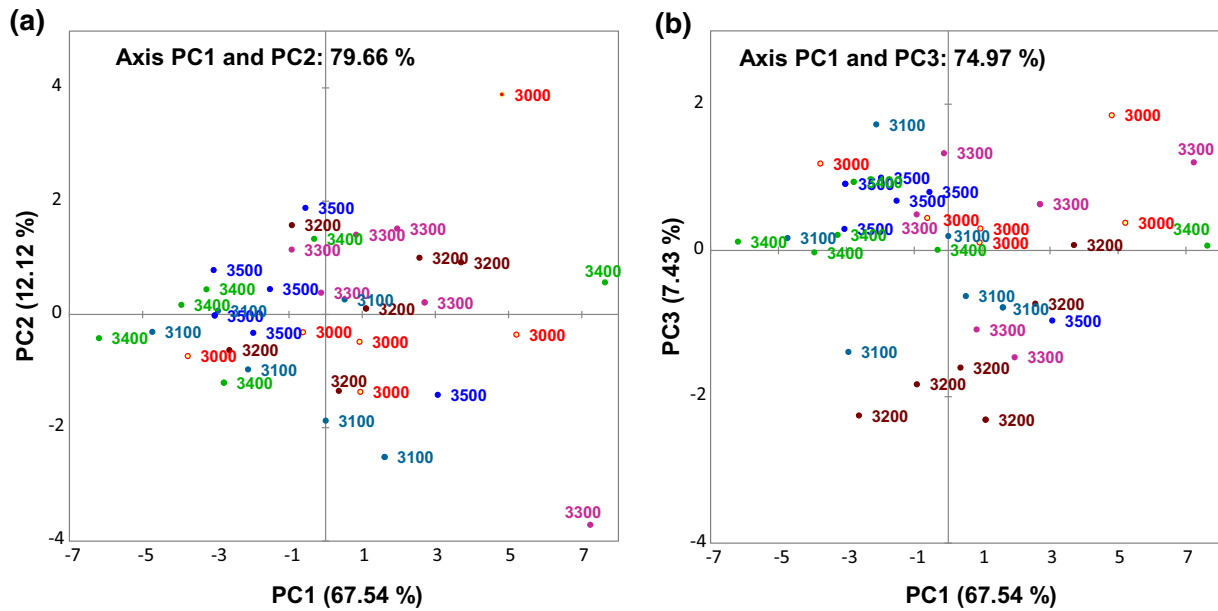
Fig. 3 Principal component analysis (PCA) of ATR-FTIR characterization: **a** *Scatter diagram* of scores PC1 and PC2 (principal component 1 and 2) **b** and between PC1 and PC3 (principal component 1 and 3). *Scatter diagrams* show the samples and the correlations between variables

(a)

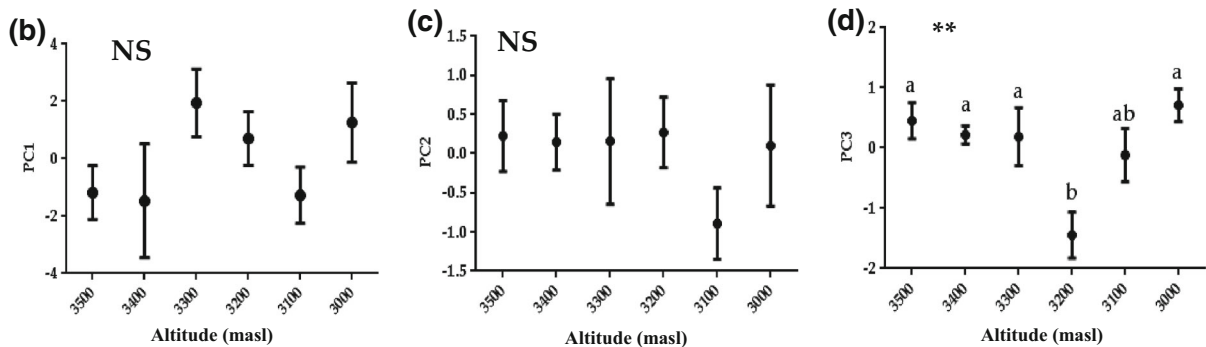| Variables	Contributions of variables (%)			Square cosines of the variables		
	PC1	PC2	PC3	PC1	PC2	PC3
B3340	1.34	42.94	0.00	0.14	**0.78**	0.00
B2891	3.74	20.44	2.68	**0.38**	0.37	0.03
B1735	2.86	0.15	46.66	0.29	0.00	**0.52**
B1660	3.62	3.57	33.97	0.37	0.06	**0.38**
B1600	7.74	0.16	0.33	**0.78**	0.00	0.00
B1508	7.80	3.85	0.00	**0.79**	0.07	0.00
B1451	9.28	0.06	1.47	**0.94**	0.00	0.02
B1422	9.01	0.04	3.57	**0.91**	0.00	0.04
B1368	8.91	2.65	1.26	**0.90**	0.05	0.01
B1316	7.00	9.24	1.30	**0.71**	0.17	0.01
B1264	8.36	3.07	1.99	**0.85**	0.06	0.02
B1231	8.41	3.76	0.02	**0.85**	0.07	0.00
B1156	8.24	2.72	3.14	**0.84**	0.05	0.03
B896	6.12	2.74	2.07	**0.62**	0.05	0.02
B808	7.57	4.60	1.55	**0.77**	0.08	0.02

Fig. 4 Principal component analysis (PCA) of ATR-FTIR characterization: **a** Contribution and *square* cosines of the study variables; **b**–**d** *graphs* of the loadings of PC1, PC2 and PC3 (Principal Component 1, 2 and 3) along the altitude gradient, where the points are average values ($n = 6 \pm$ standard error), NS (nonsignificant) and ** (statistically significant)

24.68 % that was found here through the Van Soest gravimetric method. However, there is no information on the cellulose and hemicellulose components for this species. Nevertheless, the composition for *Abies* sp. has been recorded as 48.2 % cellulose, 20.5 % hemicellulose, 26.5 % lignin and 4.8 % other minor components [51]. Comparing these data with our results, we found higher average cellulose content (54.81 %) as well as of other minor components (8.13 %). In contrast, a lower content of hemicellulose (12.37 %) and a lignin value close to 24.68 % were obtained. These differences may be explained by the variations between species of *Abies* and their respective original habitat. As far as we know, our study is the first report on wood composition of *A. religiosa* from a Mexican forest. The PCA analysis revealed a significant decrease in the principal component constituted of both, hemicellulose and lignin at 3100 followed by 3300 masl. In contrast, an increase of this principal component was observed, mainly toward the upper end (3500 masl) of the AG, followed by the corresponding data at 3400 and 3000 masl. These results agree partially with our hypothesis in relationship with the effect of environmental stress on the proportion of hemicellulose and lignin in the wood of *A. religiosa* trees along the AG.

Chemical changes in the cell wall components related to environmental factors have been reported before [12, 52, 53]. It is known that these changes will vary according to the species and the anatomical fraction studied (roots, leaves and wood) [23, 24]. Also, the hemicellulose tends to rise in response to cooler temperatures along altitudinal gradients, as well as to dryness [12, 30]. Meanwhile, the lignin content in plants is related to low temperatures, but very little is known about the effects of deficit water on lignin content [52]. In our study, we found that the temperature and the RH decrease with altitude along the AG. According to these data, our results suggest one possible response mechanism of hemicellulose and lignin to environmental stress conditions along an altitude gradient in *Abies religiosa*, since the cell wall is usually the first line of defense to various types of stress [54]. Furthermore, our results seem to indicate that at 3100 and 3300 masl, the hemicellulose and lignin contents decrease significantly, which could mean less environmental stress conditions in these altitudinal levels that affect the development of *A. religiosa* cell wall. As Whittaker has pointed out, all species present optimum growth and higher competitiveness at some point in the environmental gradient [55]. Tree growth conditions determine the chemical composition of tissues and, in less stressful environments, tend to help the production of less costly energy substrates (such as cellulose) over more costly ones (such as hemicellulose and lignin) [31, 56, 57]. This energy savings may lead to the benefit

of other physiological processes, such as growth and reproduction [58].

ATR-FTIR characterization

This is the first known report on the FTIR spectrum of wood of *A. religiosa*. It is very similar to the spectra reported for woods from the *Abies* genus as expected [59–61]. Otherwise, the region in spectrum with the highest variation along the altitudinal gradient is in the range of 1640–1760 cm^{-1} (Fig. 2), which comprises two bands: 1660 and 1735 cm^{-1}, both assigned to carbonyl functional groups. The first band is related to lignin carbonyls, while the second is assigned to lignin and hemicellulose [39, 44]. Changes in these carbonyl bands from wood samples are related to chemical variations mainly from lignin, as reported in another studies [59, 62–64].

Moreover, the band 1660 cm^{-1} showed the biggest change along the altitudinal gradient, increasing mostly at the lower end (3000 masl) and decreasing at 3200 and 3100 masl. Also, the PC3 of the FTIR spectra consisted of the bands 1735 and 1660 cm^{-1} described above. It also differed significantly, decreasing mostly toward 3200 masl, which suggests that the carbonyl functional groups vary along the altitudinal gradient possibly by environmental conditions from this altitudinal level, and these functional groups are, in turn, within hemicellulose and lignin polymers. The variations in carbonyl groups related with hemicelluloses and lignin found by the FTIR spectroscopic analysis using PCA agree with the findings in the analysis of fibers, and partly with our hypothesis.

Among the empirical indexes calculated from FTIR spectroscopy, only two showed statistically significant differences. The TCI indicates that the crystallinity of the cellulose in wood of *A. religiosa* decreases at higher altitudes, where temperature and relative humidity are lower, which may mean less recalcitrance (i.e., greater degradation) in the wood, then the physicochemical properties could be changes; nevertheless, further studies in this sense would be necessary. Finally, the rate 1735/1368 indicates that the a carbonyl/ carbohydrate relationship in the wood of *A. religiosa* decreasing at 3200 masl, a trend also found in carbonyl groups and similar to the result from the PCA analysis of the FTIR spectroscopy.

Conclusions

Multivariate analysis in conjunction with ANOVA showed significant differences in some polymers of lignocellulosic biomass in the wood of *A. religiosa* along the altitudinal gradient. Through the gravimetric analysis data, we found a significant difference in the principal

component composed by the concentration of both polymers: hemicellulose and lignin, since it reaches its lower value at 3100 masl. In addition, other principal component, developed with the IR absorption bands assigned to the carbonyl groups of hemicellulose and lignin, was significantly different at 3200 masl, where it had its lower value. From these results, it seems that the amount of both polymers is affected mainly in the altitudinal range 3100–3200 masl. However, significant changes in the chemical composition of lignocellulosic biomass in the wood of *A. religiosa* did not follow a linear relationship with respect to the altitudinal gradient, which suggests that complex environmental interactions could also be playing an important role. In this sense, further studies would be valuable to understand, at least in part, which environmental factors have an influence on the chemical composition of the wood of *A. religiosa*.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The authors declare that the research complied with all ethical standards.

Human and animal rights statement This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Kačík F, Šmíra P, Kačíková D, Reinprecht L, Nasswettrova A (2014) Chemical changes in fir wood from old buildings due to ageing. Cellul Chem Technol 48:79–88

2. Dahlquist E (2013) Biomass as energy source: resources, systems and applications (sustainable energy developments). CRC Press/Taylor & Francis, London, pp 137–138

3. Guerriero G, Hausman JF, Strauss J, Ertan H, Siddiqu KS (2016) Lignocellulosic biomass: biosynthesis, degradation, and industrial utilization. Eng Life Sci 16:1–16

4. Sluiter JB, Ruiz RO, Scarlata CJ, Sluiter AD, Templeton DW (2010) Compositional analysis of lignocellulosic feedstocks. 1. Review and description of methods. J Agric Food Chem 58:9043–9053

5. Burton RA, Fincher GB (2014) Plant cell wall engineering: applications in biofuel production and improved human health. Curr Opin Biotechnol 26:79–84

6. Van Acker R, Vanholme R, Storme V, Mortimer JC, Dupree P, Boerjan W (2013) Lignin biosynthesis perturbations affect secondary cell wall composition and saccharification yield in *Arabidopsis thaliana*. Biotechnol Biofuels 6:46

7. Guerriero G, Sergeant K, Hausman JF (2014) Wood biosynthesis and typologies: a molecular rhapsody. Tree Physiol 34:839–855

8. Tao G, Lestander TA, Geladi P, Xiong S (2012) Biomass properties in association with plant species and assortments I: a synthesis based on literature data of energy properties. Renew Sustain Energy Rev 16:3481–3506

9. Brandt A, Gräsvik J, Hallett JP, Welton T (2013) Deconstruction of lignocellulosic biomass with ionic liquids. Green Chem 15:550–583

10. Hames BR (2009) Biomass compositional analysis for energy applications. In: Jr Mielenz (ed) Biofuels, methods in molecular biology. Humana Press, Totowa, pp 145–167

11. Rodziewicz P, Swarcewicz B, Chmielewska K, Wojakowska A, Stobiecki M (2014) Influence of abiotic stresses on plant proteome and metabolome changes. Acta Physiol Plant 36:1–19

12. Gall H, Philippe F, Domon JM, Gillet F, Pelloux J, Rayon C (2015) Cell wall metabolism in response to abiotic stress. Plants 4:112–166

13. Osborne PL (2012) Tropical ecosystems and ecological concepts. Cambridge University Press, Cambridge

14. Serrada Hierro R (2008) Apuntes de selvicultura. In: Serrada Hierro R (ed) Apuntes de selvicultura universidad politécnica de madrid (in Spanish). EUI Técnica Forestal, Madrid, pp 83–131

15. Suter L, Ruegg M, Zemp N, Hennig L, Widmer A (2014) Gene regulatory variation mediates flowering responses to vernalization along an altitudinal gradient in *Arabidopsis thaliana*. Plant Physiol 166:1928–1942

16. Galván-Hernández DM, Lozada-García JA, Flores-Estévez N, Galindo-González J, Vázquez-Torres SM (2015) Altitudinal gradient effect on morphometric variation and leaf symmetry of *Platanus mexicana* Moric. Rev Chapingo Ser Ciencias For y del Ambient 21:171–183

17. Garten CT (2011) Comparison of forest soil carbon dynamics at five sites along a latitudinal gradient. Geoderma 167–168:30–40

18. Coomes DA, Allen RB (2007) Effects of size, competition and altitude on tree growth. J Ecol 95:1084–1097

19. Hoch G, Körner C (2012) Global patterns of mobile carbon stores in trees at the high-elevation tree line. Glob Ecol Biogeogr 21:861–871

20. Tsui CC, Tsai CC, Chen ZS (2013) Soil organic carbon stocks in relation to elevation gradients in volcanic ash soils of Taiwan. Geoderma 209–210:119–127

21. Xu G, Jiang H, Zhang Y, Korpelainen H, Li C (2013) Effect of warming on extracted soil carbon pools of *Abies faxoniana* forest at two elevations. For Ecol Manage 310:357–365

22. Michalet R, Schöb C, Lortie CJ, Brooker RW, Callaway RM (2014) Partitioning net interactions among plants along altitudinal gradients to study community responses to climate change. Funct Ecol 28:75–86

23. Richardson AD (2004) Foliar chemistry of balsam fir and red spruce in relation to elevation and the canopy light gradient in the mountains of the northeastern United States. Plant Soil 260:291–299

24. Genet M, Li M, Luo T, Fourcaud T, Clément-Vidal A, Stokes A (2011) Linking carbon supply to root cell-wall chemistry and mechanics at high altitudes in *Abies georgei*. Ann Bot 107:311–320

25. Battipaglia G, De Micco V, Sass-Klaassen U, Tognetti R, Makela A (2014) Special issue: WSE symposium: Wood growth under environmental changes: the need for a multidisciplinary approach. Tree Physiol 34:787–791

26. Sánchez-Velásquez LR, Pineda-López MR, Martínez AH (1991) Distribución y estructura de la población de *Abies religiosa* (H.B.K.) Schl. *et* Cham., en el Cofre de Perote, Estado de Veracruz, México (in Spanish). Acta Botánica Mex 16:45–55

27. Pineda-López MR, Sanchez-Velásquez LR, Vazquez-Domínguez G, Rojo-Alboreca A (2013) The effects of land use change on carbon content in the aerial biomass of an *Abies religiosa* (Kunth Schltdl. *et* Cham.) forest in central Veracruz, Mexico. For Syst 22:82–93

28. Rzedowski J (1978) Vegetación de México (in Spanish). Limusa, Mexico

29. Pineda-López MR, Ortega-Solis R, Sánchez-Velásquez LR, Ortiz-Ceballos G, Vázquez-Domínguez G (2013) Population structure of *Abies religiosa* (Kunth) Schltdl. *et* Cham., in the ejido El Conejo of the national park Cofre de Perote, Veracruz, Mexico (in Spanish). Rev Chapingo Ser Ciencias For y del Ambient 19:375–385

30. Hoch G (2007) Cell wall hemicelluloses as mobile carbon stores in non-reproductive plant tissues. Funct Ecol 21:823–834

31. Chapin S, Matson PA, Vitousek P (2012) Plant carbon budgets. In: Chapin S, Matson PA, Vitousek P (eds) Principles of terrestrial ecosystem ecology. Springer, New York, pp 157–181

32. Lara-González R, Sánchez-Velásquez LR, Corral-Aguirre J (2009) Regeneration of *Abies religiosa* in canopy gaps versus understory, Cofre de Perote national park, México (in Spanish). Agrociencia 43:739–747

33. TAPPI T 264 cm-07 (2007) Preparation of wood for chemical analysis. Technical Association of the Pulp & Paper Industry, Peachtree Corners

34. Goering HK, Van Soest PJ (1970) Forage fiber analyses (apparatus, reagents, procedures and some applications). USDA-ARS agricultural handbook 379, US Government Printing Office, Washington, DC, p 20

35. Stewart CE, Moturi P, Follett RF, Halvorson AD (2015) Lignin biochemistry and soil N determine crop residue decomposition and soil priming. Biogeochemistry 124:335–351

36. Mclean JP, Jin G, Brennan M, Nieuwoudt MK, Harris PJ (2014) Using NIR and ATR-FTIR spectroscopy to rapidly detect compression wood in *Pinus radiata*. Can J For Res 44:820–830

37. Nelson ML, O'Connor RT (1964) Relation of certain infrared bands to cellulose crystallinity and crystal lattice type. Part II. A new infrared ratio for estimation of crystallinity in celluloses I and II. J Appl Polym Sci 8:1325–1341

38. Poletto M, Zattera AJ, Santana RMC (2012) Structural differences between wood species: evidence from chemical composition, FTIR spectroscopy, and thermogravimetric analysis. J Appl Polym Sci 126:E337–E344

39. Chen C, Luo J, Qin W, Tong Z (2014) Elemental analysis, chemical composition, cellulose crystallinity, and FT-IR spectra of *Toona sinensis* wood. Monatsh Chem 145:175–185

40. Euring D, Löfke C, Teichmann T, Polle A (2012) Nitrogen fertilization has differential effects on N allocation and lignin in two Populus species with contrasting ecology. Trees Struct Funct 26:1933–1942

41. Popescu MC, Popescu CM, Lisa G, Sakata Y (2011) Evaluation of morphological and chemical aspects of different wood species by spectroscopy and thermal methods. J Mol Struct 988:65–72

42. Conover WJ, Iman RL (1981) Rank transformations as a bridge between parametric and nonparametric statistics. Am Stat 35:124–129

43. Lupoi JS, Singh S, Parthasarathi R, Simmonsa BA, Henry RJ (2015) Recent innovations in analytical methods for the qualitative and quantitative assessment of lignin. Renew Sustain Energy Rev 49:871–906

44. Xu F, Yu J, Tesso T, Dowell F, Wang D (2013) Qualitative and quantitative analysis of lignocellulosic biomass using infrared techniques: a mini-review. Appl Energy 104:801–809

45. So CL, Eberhardt TL (2013) A mid-IR multivariate analysis study on the gross calorific value in longleaf pine: impact on correlations with lignin and extractive contents. Wood Sci Technol 47:993–1003

46. Santoni I, Callone E, Sandak A, Sandak J, Dirè S (2015) Solid state NMR and IR characterization of wood polymer structure in relation to tree provenance. Carbohydr Polym 117:710–721

47. Chen H, Ferrari C, Angiuli M, Yao J, Raspi C, Bramanti E (2010) Qualitative and quantitative analysis of wood samples by Fourier transform infrared spectroscopy and multivariate analysis. Carbohydr Polym 82:772–778

48. Sammons RJ, Harper DP, Labbé N, Bozell JJ, Elder T, Rials TG (2013) Characterization of organosolv lignins using thermal and FT-IR spectroscopic analysis. Bioresources 8:2752–2767

49. Maréchal Y, Chanzy H (2000) The hydrogen bond network in I (β) cellulose as observed by infrared spectrometry. J Mol Struct 523:183–196

50. Espinoza-Herrera R (1996) Aspectos químicos de la Madera de *Abies religiosa* var. Típica (Oyamel) (in Spanish). Ciencia y Tecnología de la madera 10:11–17

51. Avendaño-Hernández DM, Acosta-Mireles M, Carrillo-Anzures F, Etchevers-Barra JD (2009) Estimación de biomasa y carbono en un bosque de *Abies religiosa* (in Spanish). Rev Fitotec Mex 32:233–238

52. Moura JCMS, Bonine CAV, Viana JdOF, Dornelas MC, Mazzafera P (2010) Abiotic and biotic stresses and changes in the lignin content and composition in plants. J Integr Plant Biol 52:360–376

53. Tenhaken R (2014) Cell wall remodeling under abiotic stress. Front Plant Sci 5:771

54. Largo-Gosens A, Hernández-Altamirano M, García-Calvo L, Alonso-Simón A, Álvarez J, Acebes JL (2014) Fourier transform mid infrared spectroscopy applications for monitoring the structural plasticity of plant cell walls. Front Plant Sci 5:1–16

55. Whittaker RH (1978) Direct gradient analysis: techniques. In: Whittaker RH (ed) Handbook of vegetation science 5. Ordination and classification of communities. Dr Junk W. Springer, The Hague, pp 9–51

56. Laureano RG, Lazo YO, Linares JC, Luque A, Martínez F, Seco JI, Merino J (2008) The cost of stress resistance: construction and maintenance costs of leaves and roots in two populations of *Quercus ilex*. Tree Physiol 28:1721–1728

57. Liu N, Guan LL, Sun FF, Wen DZ (2014) Alterations of chemical composition, construction cost and payback time in needles of Masson pine (*Pinus massoniana* L.) trees grown under pollution. J Plant Res 127:491–501

58. Grubb PJ (2015) Trade-offs in interspecific comparisons in plant ecology and how plants overcome proposed constraints. Plant Ecol Divers 0874:1–31

59. Rosu D, Teaca CA, Bodirlau R, Rosu L (2010) FTIR and color change of the modified wood as a result of artificial light irradiation. J Photochem Photobiol B Biol 99:144–149

60. Emmanuel V, Odile B, Céline R (2015) FTIR spectroscopy of woods: a new approach to study the weathering of the carving face of a sculpture. Spectrochim Acta Part A Mol Biomol Spectrosc 136:1255–1259

61. Emandi A, Vasiliu CI, Budrugeac P, Stamatin I (2011) Quantitative investigation of wood composition by integrated FT-IR and thermogravimetric methods. Cellul Chem Technol 45:579–584

62. Lupoi JS, Singh S, Simmons BA, Henry RJ (2013) Assessment of lignocellulosic biomass using analytical spectroscopy: an evolution to high-throughput techniques. Bioenergy Res 7:1–23

63. Popescu CM, Popescu MC, Vasile C (2011) Structural analysis of photodegraded lime wood by means of FT-IR and 2D IR correlation spectroscopy. Int J Biol Macromol 48:667–675

64. Calienno L, Lo Monaco A, Pelosi C, Picchio R (2014) Colour and chemical changes on photodegraded beech wood with or without red heartwood. Wood Sci Technol 48:1167–1180