Carbon in heartwood, sapwood and bark along stem profile in three Mediterranean *Pinus* species

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ABSTRACT

Context: Understanding biological processes in forests is necessary to orientate ecosystem management towards potential C sequestration. To achieve this, information is required about changes in forest biomass C pools, including the stem components (bark, sapwood and heartwood).

Aims: This study aimed to determine whether there are differences in C concentration in axial and radial directions within stem biomass in *Pinus nigra*, *Pinus pinaster* and *Pinus sylvestris*.

Methods: Wood samples from a permanent plantation in northern Spain were examined for C concentration and wood bulk density:

Results: The results showed that C concentration was higher in heartwood than in sapwood in the three species. *Pinus* spp. sapwood C concentration increased along the stem, while the C concentration in heartwood tissue showed the opposite behavior. In bark, *Pinus pinaster* showed a decreasing trend, in contrast to *Pinus nigra* and *Pinus sylvestris,* where higher values were found at the base and top of the stem. Finally, wood bulk density decreased in heartwood, sapwood and bark areas when stem height increased. Estimating C content taking account different anatomical parts and heights is important in considering the specificy of the different parts because of their potential commercial or ecological use in the forest ecosystems.

Key words: *Pinus nigra/ Pinus pinaster/ Pinus sylvestris/* Radial and axial Carbon concentration/ Anatomical parts

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1 INTRODUCTION

Wood production under sustainable forest management strategies has positive environmental effects since forests fix large amounts of carbon dioxide. Stem tree carbon fixation can be calculated easily by using biomass equations or biomass expansion factors (BEFs) and multiplying each value by generic C concentration of 50.0 cg g^{-1} of dry matter according to Kollmann (1959) and the Intergovernmental Panel on Climate Change (IPCC) recommendations (Penman et al., 2003). Although this concentration has been widely assumed, other reports support that C concentration of wood varies depending on the species, stem anatomical parts, growth, site conditions, stand characteristics and management practices (Elias and Potvin, 2003). Previous studies have shown that the concentration could vary from 48 to 54.4 cg g^{-1} d.m. in temperate pines (Matthews, 1993). Lamlon and Savidge (2003) found that the heartwood of different softwood species from North America showed a C concentration between 47.2 and 55.2 cg g^{-1} d.m.. Differences could be expected because of the radial and vertical variation in wood characteristics. Anatomically, stem is composed of heartwood, sapwood and bark that show different properties and chemical composition. Secondary compounds therefore tend to accumulate in heartwood, while storage products (starch), soluble sugars, amino-acids and mineral elements are removed from senescing sapwood rings (Meerts, 2002).

In recent decades, a large amount of new data on mineral element concentrations in sapwood and heartwood anatomical parts has been published (Augusto et al., 2008). Studies to ascertain the chemical properties along the stem and in the different parts have also been carried out to attempt to improve yields in pulp industry (Barahona, 2005). However, there is a lack of specific studies on the variation in C concentration within and along the stem. Improved knowledge of C concentration along the stem is relevant to adequate quantification of C at tree and stand levels and to reduce uncertainties in biomass C estimation (Zhang et al., 2009).

In this paper, C concentration patterns in heartwood, sapwood and bark were explored along the stem in three different species. Our specific objectives were: (1) to assess variation ranges and mean values of C concentration in heartwood, sapwood and bark, (2) to test whether this concentration was constant along the stem height, (3) to test whether the heartwood/sapwood/bark C concentration varied depending on the species considered.

2 MATERIAL AND METHODS

2.1 Study area

The study area is situated in northern Spain. This area represents a homogeneous transitional sector between the mountainous and crop areas, with small mountains and valleys. Altitude ranges from 800 to 1000 m a.s.l. (Figure 1). The climate is Mediterranean, with a long, cold winter and a warm, dry summer (a mean temperature of 10.7 ºC and mean annual rainfall of 630 mm).

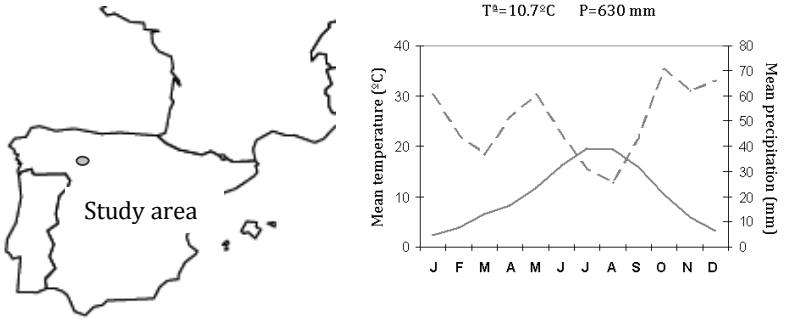

Figure 1. Location and climatic diagram of the study area in Northern Spain. Note: Tª is mean annual temperature (ºC) and P is the mean annual amount of precipitation (mm).

The present study is located in *Pinus* spp. plantations established mostly during the 1960's. These are young- and middle-aged plantations (around 30-60 years old) of Black pine (*Pinus nigra)*, Maritime pine (*Pinus pinaster)* and Scots pine (*Pinus sylvestris*). Scattered patches of Heather (*Erica* spp.) and Rock roses (*Cistus* spp.) can be also found. The soils can be classified as Ultisols and Alfisols.

2.2 Sampling and analytical method procedures

Nine permanent plots [30 x 30 m] were measured, covering different site qualities and densities to represent the whole study area (Table 1). They were located in middle-aged stands. In each plot, the diameter at breast height (dbh) and the total height of all trees were recorded. Thirty-four trees were sampled to obtain wood samples. Twelve trees from *Pinus pinaster*, 12 from *Pinus sylvestris* and 10 from *Pinus nigra* were used (Table 2).

Trees were felled as close to the ground as possible in autumn 2004. Cross-sectional disks, 5 cm thick, were collected at several points along the stem. Disks were taken from each section, starting at stump height, at a height of 0.3 and 0.8 m, at breast height (1.30 m) and at 1 m intervals along the stem up to the end. To obtain the test material, the disks were divided

into heartwood, sapwood and bark parts. The delimitations of the different parts were made by visual observation of the wood cross-section because the heartwood showed a distinctive brown color compared to the lighter-colored sapwood. Samples from the different parts and from heights along the stem were extracted by a jig saw and dried. Each sample of dry wood (50 g approx.) was directly ground into 0.2 mm powder by two types of mills. Larger samples were first ground in an Ultra Centrifugal Mill (10 mm) and then a subsample was ground in a Mixed Mill, with a final fineness of about 5 µm. The C concentration (cg g^{-1} of dry matter [cg g^{-1} d.m.]) was analyzed using the automated C/N analyzer (CHN-2000 LECO; analytical error: C: ±0.07%). A total of 1006 samples were analyzed to determine the C concentration. The number of samples taken from each stem depended directly on the height and the formation of heartwood. Consequently, for example, *Pinus nigra* trees sampled did not present enough heartwood area to get wood samples. Along the stem, from stump height up to height=6.3 m, the average sapwood/heartwood ratio varied in the different species [*Pinus nigra*: 15.4-1.1; *Pinus pinaster*; 5.0-1.2; *Pinus sylvestris*: 8.6-0.6].

Table 1. Main plot characteristics. *X,Y* UTM coordinates of the plots; *d* mean dbh (diameter at breast height) of the plot in cm; *QMD* quadratic mean diameter; *Hdom* dominant height; *N* number of trees per hectare; *BA* basal area of the stand.

Plot	X Location	Y Location	d (cm)	QMD (cm)	Hdom (m)	N (stem ha^{-1})	BA (m^2 ha^{-1})	Trees sampled[a]
Pinus nigra								
Site 1	369688	4713892	16.3	16.7	11.1	1833	40.0	4
Site 2	346814	4723449	19.3	19.7	7.5	689	21.0	2
Site 3	388565	4728054	19.1	19.5	14.4	1367	40.8	4
Pinus pinaster								
Site 4	384998	4712559	31.9	32.1	15.2	478	38.6	5
Site 5	390702	4704958	18.0	23.7	12.7	667	29.3	3
Site 6	392609	4708014	25.5	26.1	13.1	689	36.7	4
Pinus sylvestris								
Site 7	347970	4728484	24.0	24.3	15.9	733	34.0	4
Site 8	372411	4715563	20.5	20.8	13.2	1100	37.4	4
Site 9	352392	4724462	23.6	24.1	6.5	678	31.0	4

[a] Trees harvested to carry out this study.

Table 2. Mean characteristics of harvested trees. *d* dbh of the harvested tree; *h* total height; *hc* crown insertion height; *Age* age of harvested tree.

		d (cm)				h (m)				hc (m)				Age (years)			
	n	mean	std	min	max	mean	std	min	max	mean	std	min	max	mean	std	min	max
Pinus nigra	10	19.5	4.5	10.7	24.1	11.3	1.8	7.1	13.7	3.6	1.4	1.8	6.1	34	5	27	39
Pinus pinaster	12	27.2	5.1	17.1	34.9	12.7	1.6	11.0	16.1	5.1	1.4	3.1	7.1	42	8	29	50
Pinus sylvestris	12	21.0	3.9	13.4	26.5	13.4	2.0	8.6	15.7	6.7	2.3	4.5	11.2	42	6	36	53

Bulk density (g cm^{-3}) was calculated for the different parts, heights and species, as well as the ratio between oven-dry weight and fresh volume. Three different trees by species, whose diameter was close to plot quadratic mean diameter, were chosen to estimate the average wood bulk density by parts and along the stem. Disks obtained, as well as the previous case, were cut into smaller pieces to calculate the dry weight and the fresh volume. Disks from the different parts were oven-dried at 75±2°C up to constant weight and fresh volume was obtained using the water displacement method (measured to the nearest 0.001 g; McDonald et al. 1995).

The stem C content by volume unit (Mg m^{-3}) was calculated through C concentration and wood bulk density along the stem and the total C mass (Mg) was calculated by multiplying C content by volume unit and by the volume of this section (Figure 2). The volume of the section was calculated as the volume that exists between two subsequent samples. In the case of bark components, C concentration was multiplied by the proportion of bark in the cross-section at the different heights. Stem wood volume (V, m^3 $tree^{-1}$) and heartwood volume (HV, m^3 $tree^{-1}$) were determined using Smalian's method (i.e., each portion considered a cone-trunk) for each intermediate section. Sapwood volume (SV, m^3 $tree^{-1}$) and bark volume (BV, m^3 $tree^{-1}$) were derived by difference between those and the stem wood volume without bark. The total C mass (Mg) was quantified in the different anatomical parts, heights and species. These variables allowed us to calculate a weighted mean C concentration by species (Bert and Danjon, 2006) to exclude the effect of biomass allocation.

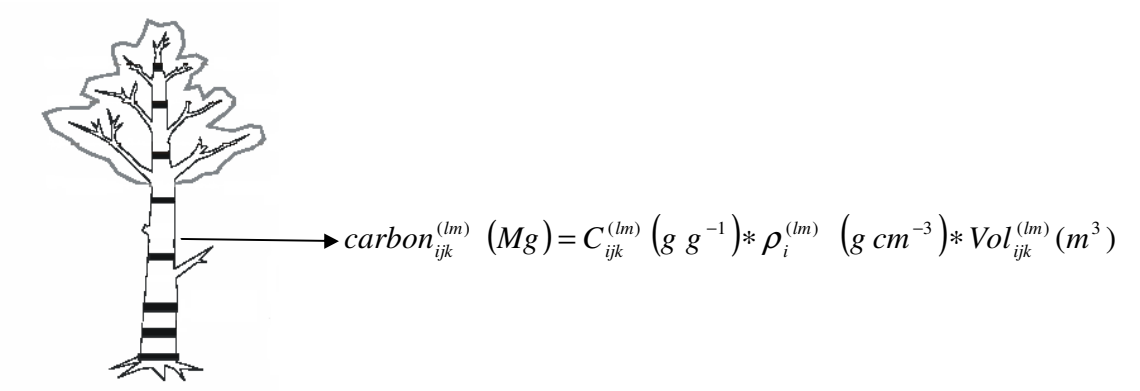

Figure 2. Total carbon estimation by stem. Note: $carbon_{ijk}^{(lm)}$ is the total carbon content (in Mg) of the tree ***k*** of the plot ***j*** and of the specie ***i*** of the anatomical part ***l*** at height *m*, C_{ijk} is the carbon concentration (in g g^{-1}), $\rho_i^{(lm)}$ is the bulk density (in g cm^{-3}) of the specie ***i*** of the anatomical part ***l*** at height ***m*** and $Vol_{ijk}^{(lm)}$ is the stem volume (in m^3) of the tree ***k*** of the plot ***j*** and of the specie ***i*** of the anatomical part ***l*** at height *m*.

2.3 Statistical methods

Descriptive analyses of the C concentration at different heights and parts from the different species were carried out. A mixed model was fitted to asses the influence of the different factors such as anatomical part (with three levels: Heartwood, Sapwood and Bark) and species (with three levels: *Pinus nigra, Pinus pinaster* and *Pinus sylvestris)* along the stem on the C concentration. The following model was fitted [Eq. 1]:

$$Y_{ijk}^{(lm)} = (\alpha_0 + \alpha_i^{(l)}) + (\beta_{o1} + \beta_{i1}^{(l)}) * h_{ijk}^{(lm)} + (\beta_{o2} + \beta_{i2}^{(l)}) * (h_{ijk}^{(lm)})^2 + \delta_{ij} + \varepsilon_{ijk}^{(lm)} \qquad \text{[Eq. 1]}$$

where, $Y_{ijk}^{(lm)}$= carbon concentration (cg g^{-1} d.m.) of tree ***k*** in plot ***j*** and of specie ***i*** of anatomical part ***l*** at cross sectional disk ***m***; α_0 is the general intercept ; $\alpha_i^{(l)}$ is the effect of anatomical part ***l*** on the intercept for specie ***i***; β_{01} is the general linear effect of height on the carbon concentration; $\beta_{i1}^{(l)}$ is the effect of anatomical part ***l*** on the general linear coefficient of the height for specie ***i***.; $h_{ijk}^{(lm)}$ is the height (cm) to a simple power of tree ***k*** in plot ***j*** and of specie ***i*** of anatomical part ***l*** where the sample is extracted; β_{02} is the general quadratic effect of height on the carbon concentration; $\beta_{i2}^{(l)}$ is the effect of anatomical part ***l*** on the general quadratic coefficient of the height for specie ***i***.; $(h_{ijk}^{(lm)})^2$ is the height to the square of tree ***k*** in plot ***j*** and of specie ***i*** of anatomical part ***l*** where the sample is extracted; δ_{ij} is the random effect of plot ***j*** of species ***i*** (j=1,2,3) where $\delta_{ij} \to N(0, \sigma_\delta^2)$; and $\varepsilon_{ijk}^{(lm)}$ is the random error of part ***l*** at cross sectional disk ***m*** in tree ***k*** in plot ***j*** and in species ***i***, where $\varepsilon_{ijk}^{(lm)} \to N_{51}(\bar{0}, \Sigma)$. Σ is an un@AR(1) variance-covariance matrix (unstructured for anatomical parts and first-order autoregressive parameter for heights). With this variance structure, we consider the next three assumptions:

1. For every fixed height we suppose that the variance-covariance matrix between anatomical parts is a general symmetrical 3x3 matrix, $A = \begin{pmatrix} \sigma_1^2 & \sigma_{12} & \sigma_{13} \\ \sigma_{12} & \sigma_2^2 & \sigma_{23} \\ \sigma_{13} & \sigma_{23} & \sigma_3^2 \end{pmatrix}$, with six variance parameters.

2. For every anatomical part ***l***, we suppose that the variance-covariance matrix between heights is a first-order autoregressive 17x17 matrix with the variance σ_l^2 for this part in the first diagonal, $\rho\sigma_l^2$ in the second diagonal, $\rho^2\sigma_l^2$ in the third diagonal and so on to

$\rho^{16}\sigma_l^2$ in the last diagonal. Therefore, we have a new variance parameter: the correlation coefficient ρ between consecutive heights.

3. The observations of different anatomical parts at different heights are independent.

To estimate the eight variance parameters we use restricted maximum likelihood method (REML).

The adequacy of the model was analyzed by the simultaneous test of the equation parameters between actual and predicted values [Eq. 2]. This test was used to ascertain if the model was biased or not. The simultaneous F-test of c_{10}=0 and c_{11}=1 was a good, intuitive and reasonable test. Presumably, the intuition underlying this test was that if the model was a good one, the regression between actual and predicted should be a 45º line and demonstrate that the model was unbiased (Huang et al., 2003).

$$actual = c_{10} + c_{11} predicted \qquad \text{[Eq. 2]}$$

where *actual* was the value of C concentration and *predicted* was the value obtained by using the model, while c_{10} and c_{11} were the parameters to be adjusted. Finally, the efficiency of the model was tested by the calculation of the pseudo-determination coefficient (pseudo R^2) of the regression between actual and predicted.

Least square means (lsmeans) of the different parts and species were considered significant at $p<0.05$ at different heights. Differences among parts in the different species and differences among species in the different parts were analyzed at height equal to 1.30 m. This height was chosen because it would be a reference point in forest inventories. However, the tendency of C concentration along the stem was also analyzed by individual contrasts of the coefficients. This analysis allowed us to know species and anatomical part patterns. The model was fitted with PROC MIXED of SAS software (SAS Institute Inc., 2010). The analysis of the R-Student residuals was also carried out by plots. PROC UNIVARIATE (SAS Institute Inc., 2010) was used to check the normality, independence and homocedasticity of the R-Student residuals.

3 RESULTS

The carbon concentration in heartwood ranged from 44.0 to 58.9 cg g^{-1} d.m. in *Pinus pinaster*. It was similar to *Pinus sylvestris* (from 44.2 to 59.7 cg g^{-1} d.m.). In sapwood, while

the maximum values obtained were similar among species (around 48 cg g^{-1} d.m.), the minimal values were smaller than 43 cg g^{-1} d.m. in *Pinus pinaster*, than 41 in *Pinus nigra* and smaller than 40 cg g^{-1} d.m. in *Pinus sylvestris.* The carbon concentration in bark varied greatly between the different species. In the case of *Pinus nigra*, values ranged from 41.5 cg g^{-1} d.m. to 54.6 cg g^{-1} d.m., from 46.6 cg g^{-1} d.m. to 51.6 cg g^{-1} d.m. in *Pinus pinaster* and from 41.8 cg g^{-1} d.m. to 59.8 cg g^{-1} d.m. in *Pinus sylvestris***.**

The mixed model showed that the height at simple and square powers, the double interaction between species and anatomical parts and the third interaction among species, anatomical parts and heights were highly significant at α <0.05 (Table 3). The C concentration at each height thus depended on the species and the anatomical part considered. The regression line between actual and predicted values, which analyzed the adequacy of the model, showed that the independent term is not significantly different from zero and the slope is not significantly different from one. The simultaneous test of the parameters of the equation between real and predicted values (Pr> F=0.7326) indicated that there was no bias in the carbon model. The pseudo R^2 statistics of the regression between actual and predicted values (pseudo R^2=0.4891) showed a good performance of the fit.

Table 3. Type 3 tests for fixed effects in the mixed model of carbon concentration. *NumDF* and *DenDF* numerator and denominator degrees of freedom, respectively. *F-Value* value of the F-statistic; *Pr>F* p-value associated with the previous F-statistic.

Effect	NumDF	DenDF	F-Value	Pr > F
Height	1	968	17.88	<.0001
Height^2	1	968	6.37	0.0118
Species x Anatomical part	7	968	29.96	<.0001
Height x Species x Anatomical part	7	968	6.08	<.0001
Height^2 x Species x Anatomical part	7	968	3.34	0.0016

Significant differences between species have been found (Table 4) in the C concentration of the different anatomical parts at height=1.3 m. While in *Pinus nigra* and *Pinus sylvestris*, significant differences were found in C concentration in heartwood, sapwood and bark tissues, in *Pinus pinaster* heartwood and bark did not show this characteristic. In comparison with the heartwood component, the carbon concentration of the bark was higher in *Pinus pinaster* and lower in *Pinus sylvestris*.

Table 4. Level of significance means of Carbon concentration, standard error and the lower (Lci) and upper (Uci) confidence interval limits, in cg g^{-1} of dry matter (d.m.), for the different species and anatomical parts at 1.30 m height. For each species, means of anatomical parts without any common lower-case letters are different at the 0.05 level of significance. For each anatomical part, means of species without any common upper-case letters are different at the 0.05 level of significance.

Species	Anatomical part	n	Mean carbon concentration (cg g^{-1} d.m.)	Standard error	Lc_i	Uc_i
Pinus nigra						
	Sapwood	112	46.5 a A	0.4	45.8	47.3
	Bark	106	49.9 b A	0.5	48.9	50.9
Pinus pinaster						
	Heartwood	87	49.5 b A	0.9	47.6	51.3
	Sapwood	156	45.8 a AB	0.4	45.1	46.5
	Bark	137	50.1 b A	0.5	49.1	51.0
Pinus sylvestris						
	Heartwood	76	52.3 c B	1.0	50.4	54.2
	Sapwood	166	45.3 a B	0.4	44.5	46.0
	Bark	166	48.5 b B	0.5	47.6	49.4

Table 4 also presents the significant differences between anatomical parts in the different species at a height of 1.30 m. In heartwood, the C concentration was different in the two species considered (*Pinus pinaster* and *Pinus sylvestris*). On the other hand, in sapwood, the C concentration obtained in *Pinus nigra* was different from *Pinus sylvestri*s, but both of them were similar to the C concentration of *Pinus pinaster*. Finally, in bark, *Pinus sylvestris* was different with respect to the other two *Pinus* species, but there were no significant differences between *Pinus nigra* and *Pinus pinaster*.

Carbon concentration C (cg g^{-1} d.m.) against height h (m) showed different patterns in the three species considered in the three anatomical parts studied (Table 5, Figure 3). In heartwood, the C concentration decreased with increased stem height in the two species (*Pinus pinaster* and *Pinus sylvestris*). On the other hand, the C concentration in sapwood increased along the stem, although in *Pinus pinaster* a slight decrease was found near the base of the stem. Finally, the C concentration estimated in bark depended on the species considered. While a decreasing trend was found in *Pinus pinaster*, the values were higher at the base and top of the stem and at their lowest in the middle in *Pinus nigra* and *Pinus sylvestris*.

Table 5. Linear components of the heartwood, sapwood and bark mixed models for the different *Pinus* species. α_0, β_1 and β_2 are the parameters of the models for the different species and anatomical parts; *se* is their standard error.

		α_0	se	β_1	se	β_2	se
Pinus nigra	sapwood	464	0.4548	0.1379	0.1546	-0.00328	0.01461
	bark	508	0.6137	-0.7358	0.2413	0.05157	0.02336
Pinus pinaster	heartwood	514	11.065	-16.999	0.6082	0.1630	0.07520
	sapwood	459	0.4163	-0.08462	0.1215	0.01464	0.01030
	bark	504	0.5642	-0.2536	0.1876	0.01115	0.01591
Pinus sylvestris	heartwood	530	11.708	-0.5075	0.6719	-0.00353	0.09193
	sapwood	453	0.4160	-0.02049	0.1149	0.01011	0.008960
	bark	498	0.5449	-10.436	0.1702	0.07004	0.01325

Comparing species, in *Pinus nigra*, there were significant differences between sapwood and bark parts at a height of less than 4.3 m. In *Pinus pinaster*, sapwood was different from heartwood and bark parts up to 2.3 and 11.3 m, respectively, while there were no significant differences between bark and heartwood parts along the stem. Finally, in *Pinus sylvestris*, there were significant differences among the three parts up to a height of 4.3 m, the heartwood being completely different along the stem with respect the other two parts. To provide an average C concentration for the species studied considering the different anatomical parts and the variation along the stem, a weighted mean C concentration was calculated for each species. This concentration was 46.4±1.7 cg g^{-1} (*Pinus nigra*), 46.8±1.6 (*Pinus pinaster*) and 45.9±1.5 cg g^{-1} (*Pinus sylvestris*).

The study results reveal that the mean sapwood wood bulk density decreased when stem height increased, in all three species considered (Figure 4). At stump level, the bulk density had the maximum values (above 0.4 g cm^{-3} in *Pinus nigra* and *Pinus pinaster*). At 1.3 m, values were close to 0.35 g cm^{-3} in the three species. However, at heights more than 4 m, the bulk density fell to below 0.3 g cm^{-3} because of the fresh volume in a small dry weight value.

The C content by volume unit (Mg m^{-3}) by species and parts are shown in Figure 5. Sapwood values obtained in the three species of *Pinus* were very similar. In bark, the trend found in *Pinus sylvestris* showed the smallest values along the stem, while the pattern was the opposite in heartwood.

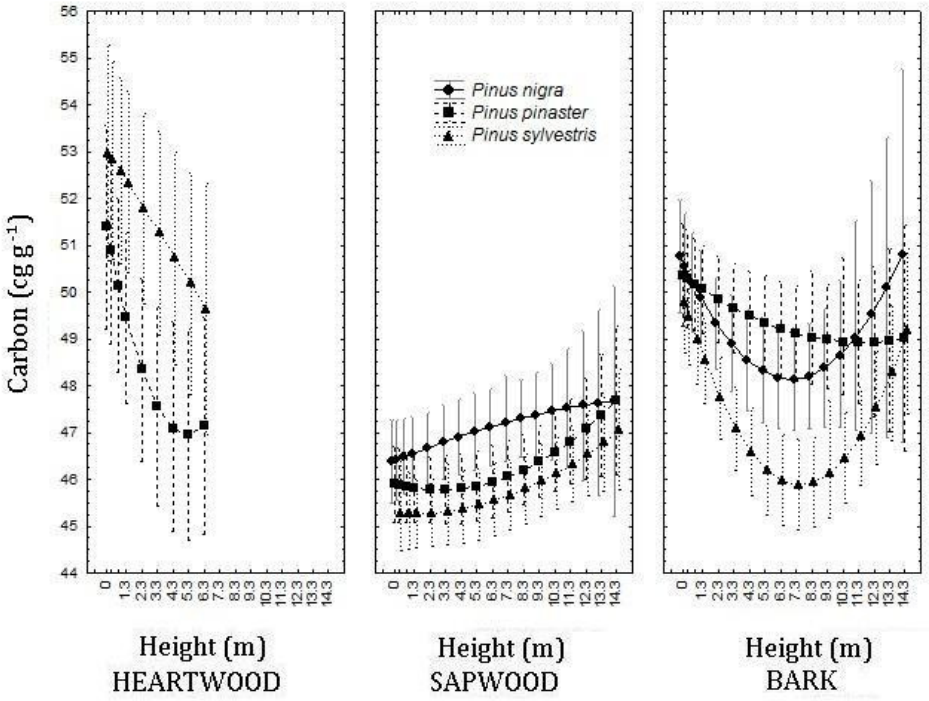

Figure 3. Carbon content (cg g^{-1} d.m.) along stem height (h [m]) in the different species and anatomical parts and their confidence interval limits.

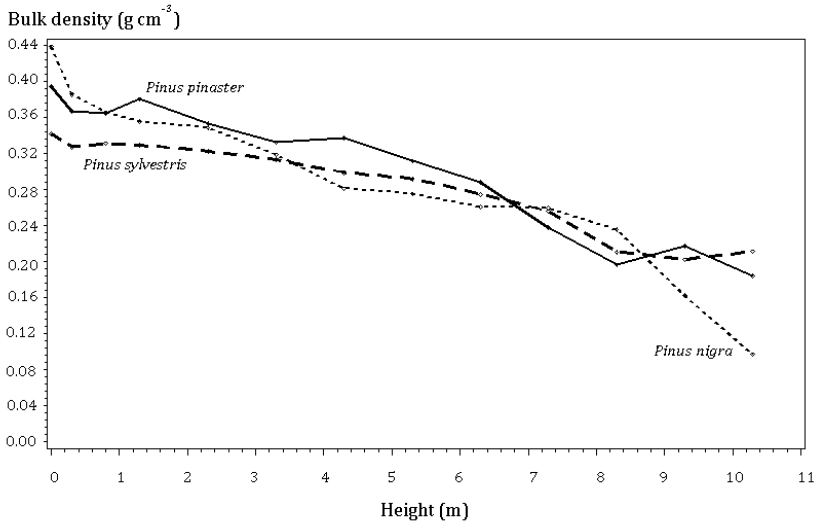

Figure 4. Sapwood bulk density along the stem height by species.

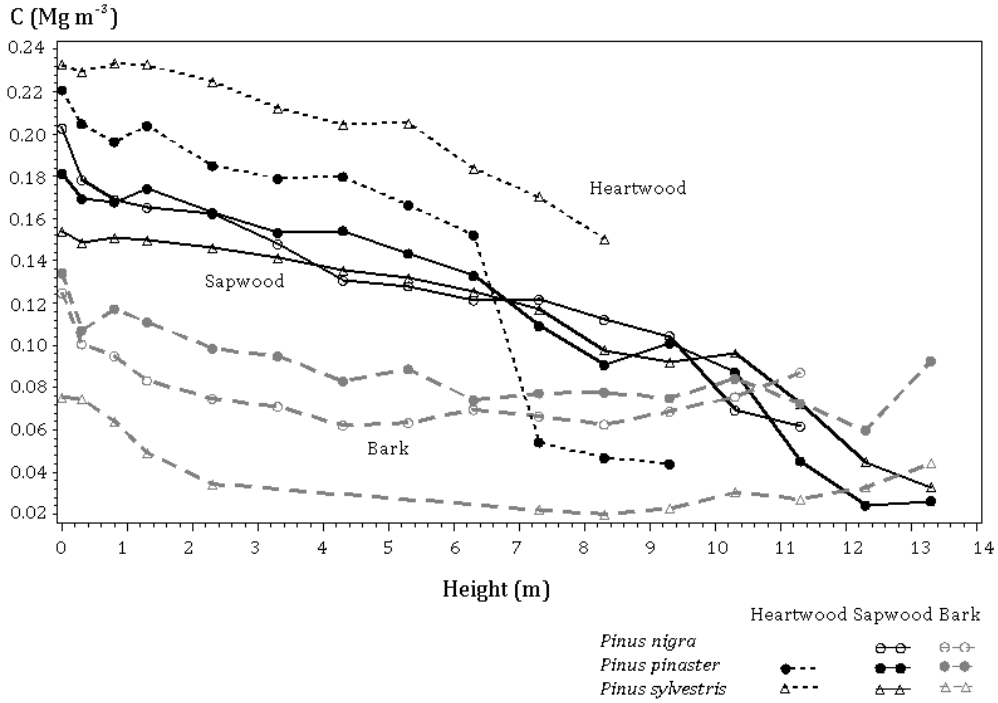

Figure 5. Carbon content by volume unit (Mg m^{-3}) by anatomical parts in the species studied.

4 DISCUSSION

Our study focused on the different C concentrations in the different anatomical parts along the stem in three *Pinus* species. The differences in C concentration found in heartwood, sapwood and bark could be due to the differences in physical and chemical properties in these tissues. The physical differences were due to the lack of physiological activity in heartwood and by the structural and chemical changes that occur during the formation of heartwood. Heartwood is a biologically dead anatomical part, without biologically alive cells (Climent et al., 1998). During heartwood formation, cells change their dimensions and functions, chemical transformations occur by development of tyloses in the vessels of many species (Hillis, 1987) and the biosynthesis of non-structural compounds leads to an important accumulation of extractives. Differences among tissues have been found by previous researchers (Augusto et al., 2008; Bert and Danjon, 2006; Fukatsu et al., 2008) and higher concentrations of extractives (resins, tannins, etc.) in heartwood than in sapwood have been shown by previous researchers (Campbell et al., 1990; Climent et al., 1998; Bergstrom, 2003; Fukatsu et al., 2008). As well as this, higher levels of extractives, lignin and tannins in bark in comparison to the other anatomical parts were found by Bert and Danjon (2006) in 50-year-old plantations of *Pinus pinaster* in France. This difference could be due to phenolic

constituents, with a C composition that could range from 40 to 88 cg g^{-1} d.m., although most C concentrations are higher than 60 cg g^{-1} d.m.. All these components increase the C concentration in heartwood and bark areas. In addition, nutrients (such as nitrogen, phosphorous and others) are translocated to younger-growing tissues. This can also play a role in changing the C concentration of mature tissues.

Our results show that C concentration depends on the species studied. Among pine species, *Pinus pinaster* and *Pinus sylvestris* also show a higher C concentration in heartwood than in sapwood at a height of 1.30 m (Tab. 4). This may also be due to the presence of resinous components. Other studies have shown similar results (Elias and Potvin, 2003; Lamlom and Savidge, 2003; Tamura et al., 2006). For example, in *Pinus canariensis*, Climent et al. (1998) found that resinification starts in latewood tracheids, observed at the heartwood boundary, especially in those sections where heartwood formation is at an early stage. However, a limitation of our study was its strong dependence on the trees available for study in obtaining the results found for heartwood C presence; heartwood formation had just started in *Pinus sylvestris* and *Pinus pinaster*, in contrast to *Pinus nigra*. The most common age at which transformation from heartwood to sapwood occurs is reported to be 14 to 18 years (Hillis, 1987). However, it could be 60 to 100 years in beech (*Fagus sylvatica*) or European ash (*Fraxinus excelsior*), as reported by Dadswell and Hillis (1962). In addition, the sapwood/heartwood ratio varies with many factors, including species, age, climate, growth rate, foliage area, site quality and tree vitality, and has been the subject of several reviews (Yang and Hazenberg, 1991). The pine plantations we studied were from 30 to 60 years old. By increasing the sample size with older trees, our findings could be improved.

Differences in heartwood, sapwood and bark C concentration were found along the stem with different patterns. Vertical variations were found in previous works (Bert and Danjon, 2006; Campbell et al., 1990). Our bark variations agree with those obtained in *Pinus pinaster* by Bert and Danjon (2006). Our results showed a trend for increasing carbon concentration along the stem in sapwood and decreasing it in heartwood. The results for the heartwood could be due to the fact that older cross-sections had larger heartwood areas and greater C concentrations than younger sections, perhaps due to the higher amount of extractives and lignin accumulated in older parts. This pattern was found by Campbell et al. (1990) in *Pinus contorta*. However, in sapwood tissue, C concentration increased when stem height increased. Younger parts of sapwood tissue showed more C than older parts. Higher

concentrations of nutrients have been found in younger trees than in mature trees (Augusto et al., 2008). This could be due to the greater cell activity near the crown. Finally, the triple interaction showed significant differences among species and anatomical parts along the stem. The different composition of the anatomical parts and the specific concentrations of lignin, resins and extractives could affect structural differences at different heights. The vertical gradients in C concentration could result from variations of these factors according to stem height. Previous studies like Barahona (2005) showed significant differences in cellulose content at different heights, without a unique trend. However, more similar patterns were found by Bert and Danjon (2006) than are found in this study.

Although prior studies suggest that wood bulk density is higher when stem height increases (Barahona, 2005), our findings showed a substantial variation in the species studied with lower wood bulk density in the top of the stem. Higher values were found in heartwood bulk density than in sapwood bulk density. This is similar to Nogueira et al. (2008). Previous research points out that heartwood is reputed to be heavier, stronger and more resistant to decay than sapwood. Because of wood bulk density values, C content by volume unit was also higher in heartwood than in sapwood in our study. Consequently, at the same relative height, for each cubic meter, the C content in sapwood was lower than in heartwood. This agrees with other reports indicating that species with higher bulk density values, although they have lower C content per unit mass, will nevertheless contain the greater quantify of C per unit volume (Lamlom and Savidge, 2003). However, wood demands and C fixation must be balanced in the different forest composition, because the heartwood of some species is of value for the wood industry (like *Pinus canariensis*) or the winery industry (*Quercus* spp.). However, in other industries, heartwood is not so desirable because resins and other components hinder the sawmill work.

Forest growth models or inventory data can be combined with tree biomass functions and carbon concentration data to estimate carbon stocks in tree biomass. Some authors consider that the use of 50.0 cg g^{-1} d.m. could introduce over- or underestimates of C biomass into the calculation (Bert and Danjon, 2006; Janssens et al., 1999), indicating the need to sample tree components in each stand for carbon, mineral or mass studies. In this respect, in the current context of the Carbon Market, our detailed approach to determining C tree content could make a significant difference in global Carbon Credit transactions. This would be more important if the price of carbon credits rose above the current level. In our study, the weighted

C obtained is similar in the three *Pinus* species, and smaller than 50 cg g^{-1} d.m., the generic C concentration widely promulgated. Janssens et al. (1999) obtained a mean value of 48.9 cg g^{-1} d.m. in *Pinus sylvestris,* while Bert and Danjon (2006) and Zhang et al (2009) found values smaller than 54 cg g^{-1} d.m. in *Pinus pinaster* and *Pinus koraiensis,* respectively.

The IPCC suggests using species-specific C-concentration values when they are available. In this respect, our results are relevant because they provide detailed data for three species extensively used in reforestation projects.

Operational forestry needs accurate C estimation to establish sustainable forest management alternatives that increase forest carbon sink. This study expands the knowledge on C in biomass by considering different values at different heights and anatomical parts, instead of taking a single value as is the current practice.

Further research on up-scaling including result aggregation will benefit the application in different stand types (structure, size or distribution) of our findings.

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