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Growth, soil properties and foliage chemical analysis comparison between pure and mixed stands of *Castanea sativa* Mill. and *Pseudotsuga menziesii* (Mirb.) Franco, in Northern Portugal

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Abstract

Ecosystems with mixed species compared to the ones with pure compositions provide a broader range of options in the fields of biodiversity, conservation, protection and restoration.

Industrial plantations in Portugal are mostly monocultures with the predominance of coniferous forests, so the introduction of broadleaves could be important for the biodiversity and sustainability of Portuguese forests.

This study aims to present and discuss field data from pure and mixed-species plantations of European chestnut (*Castanea sativa* Mill.) and douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] with different intercropping densities in a total of 5 treatments with two replicate blocks. In respect to growth, the results showed significant difference ($P < 0.05$, Scheffe) between treatments for survival and basal area. Diameter breast height (DBH), total and top height of the individual *Castanea* tree was positively affected by the presence of *Pseudotsuga*. In general, soil properties results showed a higher fertility for mixed treatments compared with pure ones. Statistically differences ($P < 0.05$, Scheffe) were observed in pH, P, Mn and in the exchangeable acidity. Results for foliar analysis in N, P, Ca and K concentrations are higher for mixed treatments. The obtained results from this study indicate that mixed plantations have a positive effect in the productivity of the *Castanea*.

Additional key words: mixed-species plantation; tree growth, soil fertility; foliar analysis.

Resumen

Comparación del crecimiento, propiedades del suelo y análisis químico del follaje entre masas puras y mixtas de *Castanea sativa* Mill. y *Pseudotsuga menziesii* (Mirb.) Franco en el norte de Portugal

Los ecosistemas con especies mixtas, en comparación con las masas puras, proporcionan una gama más amplia de opciones en el campo de la biodiversidad, la conservación, la protección y la restauración. Las plantaciones industriales de Portugal son en su mayoría monocultivos con predominio de bosques de coníferas, por lo que la introducción de frondosas podría ser importante para la biodiversidad y la sostenibilidad de los bosques portugueses.

Este estudio tiene como objetivo presentar y discutir los datos de campo de plantaciones puras y mixtas de especies de castaño y de abeto de Douglas con diferentes densidades de cultivo intercalado en un total de 5 tratamientos con dos bloques. En lo que respecta al crecimiento, los resultados mostraron diferencias significativas ($P < 0,05$) entre los tratamientos para la supervivencia y el área basal. El diámetro normal (DAP), la altura total y la altura dominante del Castaño se vio afectado positivamente por la presencia de Pseudotsuga. En general, las propiedades del suelo mostraron una mayor fecundidad de los tratamientos mixtos en comparación con los puros. Se observaron diferencias estadísticamente significativas ($P < 0,05$) en el pH, P, Mn y en la acidez intercambiable. Los resultados de los análisis foliares de N, P, Ca y la concentración de K son mayores para los tratamientos mixtos. Los resultados obtenidos de este estudio indican que las plantaciones mixtas tienen un efecto positivo en la productividad del Castaño.

Palabras clave: plantaciones mixtas; crecimiento; fertilidad del suelo; análisis foliar.

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Introduction

Society looks now for forest stands in a different way. Public awareness and the global context of climate change are forcing the increase of mixed forest stands. There is also an increasing ecological view on the production and management of the forest ecosystems (Kanna, 1997).

Natural forests ecosystems are usually a mixture of species (Luis and Monteiro, 1998). There are critics about forest practices that result in wide single-species plantations that differ radically from natural forest ecosystems (Bartelink, 2000; Chen and Klinka, 2003). Even keeping in mind the production perspective, Chen and Klinka (2003) report that mixed-species stands are generally thought to be more productive than single-species stands. These same authors complete this productive perspective, reporting that mixed stands are not only more diverse in respect to tree species, but also provide a more diverse habitat and may also have greater resilience to natural disturbances. For example, mixtures of trembling aspen with conifers are more fire resistant and less susceptible to spruce budworm outbreaks than pure coniferous stands. Mixtures of species also provides a diverse range of products, like fruit and wood, as well as services (Kelty, 2006), and are more favourable to natural regeneration, allows a better use of the soil (Luis and Monteiro, 1998) and proves to be an essential tool for producers to diverse production.

Forest nutrition management is an important factor in the management of wood forests since the rates of production are limited by the supply of one or more nutrients in almost all forests, a mixture of species, each with different nutrient requirements and nutrient cycling properties and efficiency of nutrient use (Khanna, 1997; Binkley and Ryan, 1998; Rouhi-Moghaddam *et al*., 2008; Álvarez-Álvarez *et al*., 2010). This way, high rates of production depend strongly on nutrient supply, efficiency of nutrient use, and, thus, on species selection. Welke and Hope (2005) noted a wide range of effects for mixed woods with respect to nutrient cycling. Studies examining relationships between site index and soil chemical properties report significant relationships which include the following soil chemical measures: total N, available P, mineral soil pH, effective exchange capacity and C:N ratio.

In Portugal, almost all the commercial plantations are single-species plantations and there is a predominance of coniferous forests. Being the single-species plantations problematic, in terms of risk of fires and pathologic problems, the introduction of broadleaves will be important for the sustainability of Portuguese forests. Furthermore, questions about single-species plantations are being raised about the sustainability of their growth and their effects on the site. These questions are related with the removal of nutrients in the harvest wood and other tree components and further losses may occur due to site management practices (Khanna, 1997).

The present study aims to compare growth, soil and foliar properties in mixed-species plantations from pure composition and evaluate which stand composition is more advantageous and have more productivity. This information could be an important tool to forest manager's options. The first goal of this study is to analyse if different species composition will have impact on soil nutrients content, and if in mixed stands these nutritional status will be different from monocultures. The second goal of the study is to understand if these similarities or differences between soil nutritional *status* will have impact on the foliar composition. Finally it is also important to understand if growth will differ from mixed to pure plantations and in which level it will occur.

Material and methods

Study area and site characteristics

Growth, soil properties and foliar analysis were quantified within pure and mixed-species plantations of European chestnut (*Castanea sativa* Mill.) and douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] for different intercropping densities. Effects of species interactions on tree growth, concentrations of soil nutrients, and foliar nutrients on trees were assessed. According to Luis and Monteiro (1998), *Castanea sativa* (Cs) is a native deciduous broadleaf species in Portugal, known since the Miocenic period and cultivated since the Roman times. According with the National Forest Inventory (NFI, 2005/06) *Castanea* covers an area of 32.029 ha (AFN, 2010) and in Portugal the cultivation of chestnut trees is for wood and mainly for nut production, either for internal consumption or export (Vasconcelos *et al*., 2010). *Pseudotsuga menziesii* (Pm) is an exotic evergreen species, introduced in Portugal in 1846, in Sintra. Since then it has been used in reforestation projects, covering nowadays about 4.200 ha (Fontes *et al*., 2003); it proved to be well adapted to mountain sites and shows a large potential for timber production. In mountain silviculture, *Castanea* and *Pseudotsuga* are two very interesting species, not only for timber production but also with a very high ecological potential (mainly the deciduous one) in Portugal. On mountain areas, in the seventies, both species were extensively planted in pure stands, with promising results.

The experimental site is a private land located at Bemlhevai (41°24' N and 7°6' W), 55 km away from Bragança (Fig. 1), Trás-os-Montes region, in northeastern Portugal. It is located at an altitude of 710 m above sea level and is an almost flat (2°), NE turned (60°) site.

According to Luis and Monteiro (1998), the soil at the site is litholic non-humic from sericitic schist. The annual rainfall at Bragança is 685 mm, and the average annual temperature is 13.0°C.

Ruas (1997) compiled climate data obtained by meteorological stations spread across the Bragança district for wild studies, in a period between 1980 and 1995. The mean and standard error (±S.E.) was made for this period and the average annual temperature was 12.5°C (±1.8°C) and the annual rainfall was 690.1 mm (±8.8 mm) in the Bragança district (Fig. 2).

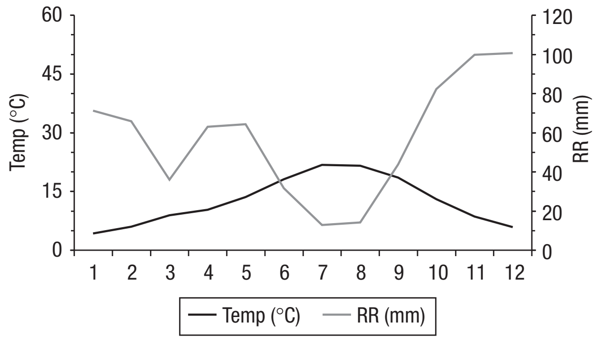

Figure 2. Climate diagram for the study area from the meteorological stations of Bragança since 1980 to 1995 (Ruas, 1997).

Figure 1. Study area location.

Experimental design

The site was composed by twenty one sampling plots with 64 plants each and the trees spacing within the plantations was 4 × 2 m, established by Jaime Sales Luis and Maria Monteiro in the winter of 1981 (Luis and Monteiro, 1998).

The experimental plantations were established using a randomized complete-block design including tree replicate blocks with no buffer between treatments. In each block and treatment, designed by M as the initial word of «Mixture», 4 subsamples were installed which covered two lines. *Castanea sativa* (as target species - *Cs*) and *Pseudotsuga menziesii* (*Pm*) were planted in four proportions (100*Cs*; 25*Cs*:75*Pm*; 50*Cs*:50*Pm*; 100*Pm*) according the next scheme: a) row mixtures with species changing in the planting line; M1 - 1 *Cs* and 3 *Pm* in the line (25*Cs*:75*Pm*); M2 - 1 *Cs* and 2 *Pm* in the line (25*Cs*:75*Pm*); M3 - 1 *Cs* and 1 *Pm* in the line (50*Cs*:50*Pm*); b) line mixtures with species changing between planting lines; M4 - 1 line of *Cs* and 1 line of *Pm* (50*Cs*:50*Pm*); M5 - 1 line of *Cs* and 2 lines of *Pm* (25*Cs*:75*Pm*); and c) monocultures; M6 - pure *Cs* (100*Cs*); M7 - pure *Pm* (100*Pm*) (Fig. 3). These proportions make 7 treatments which were randomly located in each block. The sampling plots size was 512 m^2.

In 2005, all the 7 treatments present in block I and the treatments with the mixtures 50*Cs*:50*Pm* (M3 and M4), present in block II burned. As a result, block II just presents three mixtures proportions (100*Cs* – M6; 25*Cs*:75*Pm* – M1, M2, M5; 100*Pm* – M7). All the plots

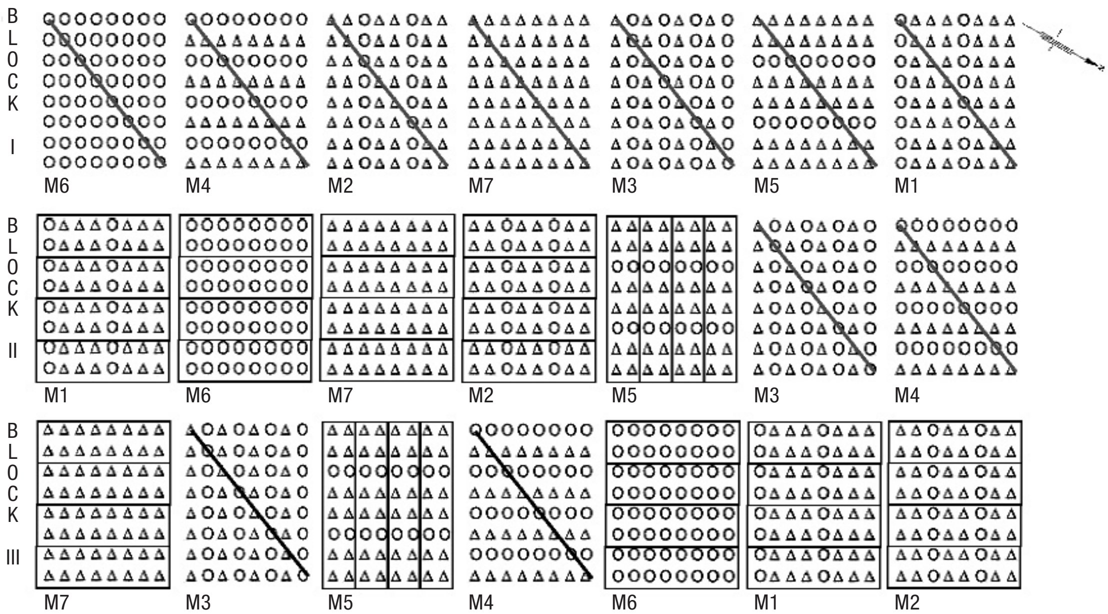

Figure 3. Layout of permanent research plots composed by *Castanea Sativa* (O) × *Pseudotsuga menziesii* (Δ), in Bemlhevai (adapted from Luís and Monteiro, 1998). The grey lines indicate the sampling plots burned. The black lines indicate the sampling plots not analysed because the repetitions in other blocks were burned. The rectangles represent the subsamples installed in each plot.

installed on block I weren't considered in this study. Also plots M3 and M4 (proportions 50*Cs*:50*Pm*) in block III weren't considered due to the non existence of plots on block II with the same proportions.

Growth measurements

Dendrometric variables, such as diameter at breast height (DBH) and total height, were measured for each tree in each sampling plot from block II and block III. All trees were numbered and repeated measures were made in each tree in April 2008 (*Castanea* and *Pseudotsuga* 27 years old) and April 2009 (*Castanea* and *Pseudotsuga* 28 years old).

DBH was measured at 1.30 m from the ground and total height obtained by using a calibrated pole using always the same measurement direction: center of the three-center of the plot, in order to avoid measurements errors because of the non perfect circularity of the stem. The average of the DBH, total height, basal area, top height and survival percentage were calculated for each plot in blocks II and III. Since the plots were installed height was always measured using an hypsometer from the same distance and the same direction. Once again were avoid measurement errors because of the non perfect circularity of the stem. To find the mean top height at each sampling plot it was considered the maximum height of the dominant trees by averaging the four highest trees for each species. The increment in height and DBH was also estimated based on measures made in 2008 and 2009. The survival was measured based on the mortality rate, according to the percentage of dead plants in the planted total in each treatment in April of 2009.

Soil properties

Soil samples were collected at two layers-horizon A: 0-20 cm and horizon B: 21-60 cm depths during February 2010 using a 7.6 cm diameter core sampler (n = 4 cores/plot). These samples were collected in three proportions (100*Cs*; 25*Cs*:75*Pm*; 100*Pm*) at M1, M2, M5, M6 and M7 treatments in blocks II and III.

Then the samples were air-dried and sieved with a 2 mm screen before analysis.

Physical (texture) and chemical analysis, such as pH(H_2O), organic matter content, phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), nitrogen (N), exchangeable acidity (Exc ac), electrical conductivity (EC), were done in order to estimate the effect of plantation type on soil fertility.

Soil texture was determined by the Bouyoucos hydrometer method (Bouyoucos, 1962). pH was measured using a 1:2.5, soil:water or KCl 1M. EC was determined using an Orion Ionalyzer Model 901 EC meter in a 1:2.5, soil:water solution. Soil organic carbon was determined using an elemental analysis equipped with an IR detection. The total nitrogen was measured using a semi Micro-Kjeldhal technique and quantified by molecular absorption spectrophotometry (Bremner, 1996). The available P was determined with spectrophotometer by using Olsen method (Homer and Pratt, 1961). The exchangeable K, Ca, Mg and Na (by ammonium acetate extraction at pH 7) were determined with atomic absorption spectrophotometry (Bower *et al*., 1952). Exchangeable acidity was determined by titration according to Thomas (1996).

Foliage nutrients

The study was carried out with the collection of 64 foliage samples from the 10 sampling plots with the proportions 100*Cs*, 25*Cs*:75*Pm*, and 100*Pm* present in blocks II and III, making 5 treatments (M1, M2, M5, M6 and M7), in July of 2010 (leaves peak period of maturity).

Each foliage sample was composed by 30 leaves collected from the top one third of the tree by clipping two small distal twigs located on the opposite sides of the crown. Four representative trees (one in each row of the sub-plot) per species were selected inside the sampling plots to collect the fully expanded leaves (mature leaves). The collected samples were oven-dried at 70°C to constant weight and grounded in a mill to pass through a 0.25 mm sieve before the chemical analysis. The analysed nutrients were nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg) and carbon (C).

N, P and K were analyzed after digesting the sample in concentrated $H_2SO_4 + H_2O_2$ using Se as catalyst (Milles and Jones Jr., 1996). N and P were determined by molecular absorption spectrophotometry and K by flame emission spectrophotometry. C was determined by an elemental analysis equipped with a NIR detection. Ca and Mg were determined using an atomic absorption spectrophotometry after wet digestion with HNO_3 and $HClO_4$ (Milles and Jones Jr., 1996).

Statistical analysis

All statistical analyses were made using the SPSS v.18 software. Normality of the variables was checked by Shapiro-Wilk test and the homogeneity of the variances was done by the Levene test. One-way analysis of variance (ANOVA) was used to compare tree growth, soil properties data and foliar nutrients data among experimental treatments. The Scheffe test was used to separate the averages of the dependent variables which were significantly affected by treatment. Data from soil parameters were analyzed using a two-way ANOVA with treatments and soil depth as the factors. Significant differences among the treatments averages were tested at $P < 0.05$ using Scheffe test.

Results

Growth measurements

From the analysis of Figure 4, and in terms of survival, there are significant differences between pure and mixed plantations for both species ($P < 0.05$, Scheffe). The treatment M6 (100*Cs*) present the highest survival value (85.16 ± 0.99%) for *Castanea*, only in treatment M2 (25*Cs*:75*Pm*), the survival was slightly lower (71.88 ± 0.88%). The treatment M5 (25*Cs*:75*Pm*) present the highest survival value (89.58 ± 1.20%) for *Pseudotsuga* (Fig. 4a).

There are no significant differences on DBH in both species ($P > 0.05$, Scheffe) among the treatments, so DBH of *Castanea* was unaffected by the presence of *Pseudotsuga* in different proportions (Fig. 4b). The *Pseudotsuga* mixed plantations present slightly higher DBH values in treatment M5 (19.01 ± 2.61 cm) than in pure plantations, M7 (100*Pm*, 16.72 ± 1.41 cm). For the *Castanea*, the heterogeneity is the most notable fact, with treatments of mixed plantations presenting higher values than the pure plantations but also the opposite; the average of DBH ranged from 12.92 cm to 14.55 cm. There is not a logical evolution of this parameter which allows the conclusion if mixed or pure plantations are better.

A similar behaviour occurs in the total height (Fig. 4c) but for both species. The average of total tree height for *Castanea* ranged from 6.73 m to 8.56 m and for *Pseudotsuga* ranged from 11.31 m to 12.43 m with no significant difference ($P > 0.05$, Scheffe) among the treatments in both species. The analysis of top height shows the highest values for *Castanea* and for *Pseudotsuga* in M2 treatment (25*Cs*:75*Pm*) (Fig. 4d).

With respect to the basal area the *Pseudotsuga* presents higher values than the *Castanea* species. For

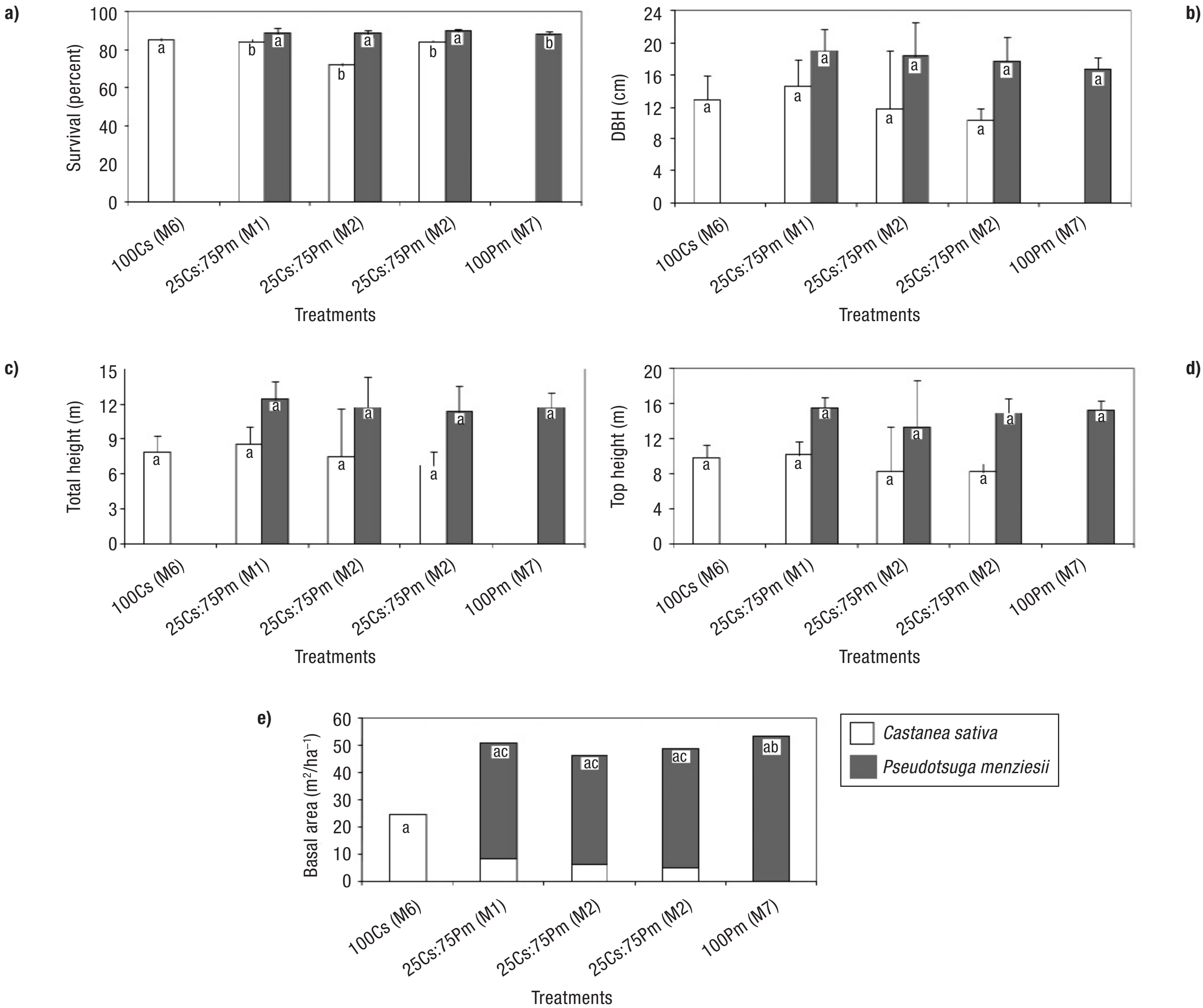

Figure 4. Survival (a), DBH (b), total height (c), top height (d) and basal area (e) of both tree species were separately compared. The letters on different column indicate a different comparison. Mean values with the same letter within a tree species do not differ significantly with each other ($P < 0.05$, Scheffe).

the *Castanea*, basal area is lower in the mixed treatments than in the pure ones. The same occurs for the *Pseudotsuga* (Fig. 4e). A general study of the analysed parameters allows the conclusion that in mixed plantations the *Castanea* presents lower DBH values than in a pure one, but is taller. This means competition between species tends to increase height and decrease DBH. The behaviour of the *Pseudotsuga* is different because while DBH tends to increase in mixed stands, top height tends to be stable.

Figure 5 shows the DBH and total height measured in 2008 and 2009 and the increment that occurred in this period. Analysing the DBH (Fig. 5a) the M1 (25*Cs*:75*Pm*) treatment presents the highest values for each species. The increment for the *Castanea* is lower in the mixed treatments than in the pure ones. For the *Pseudotsuga* the increment is higher in mixed treatments than in pure ones (Fig. 5b).

In respect to the total height for the same period, the M1 (25*Cs*:75*Pm*) also presents the highest values for each species (Fig. 5c). The increment for both species shows higher values in pure treatments (M6 for *Castanea* and M7 for *Pseudotsuga*) (Fig. 5d). When comparing different establishment procedures (M1, M2 and

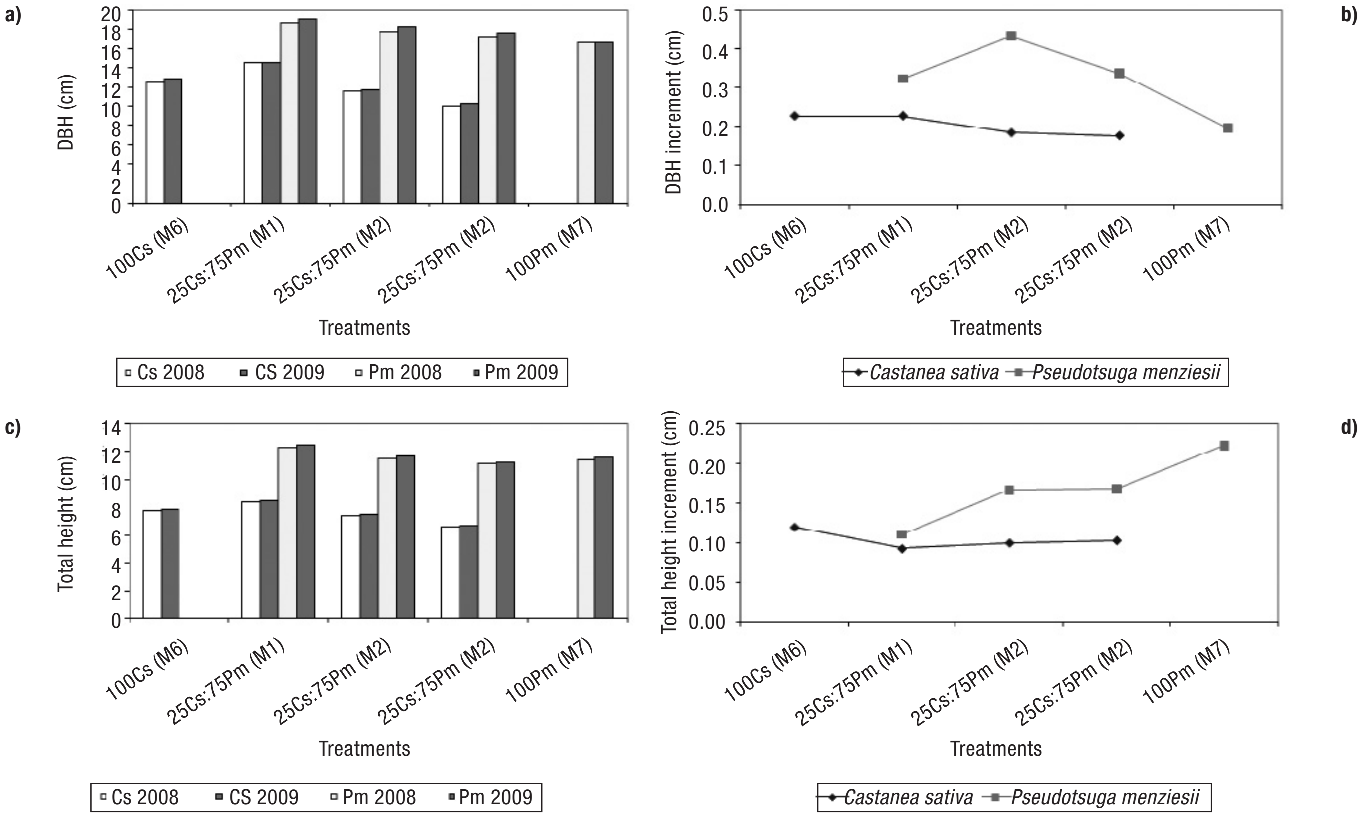

Figure 5. (a) DBH in 2008 and 2009, (b) increment of DBH, (c) total height in 2008 and 2009, (d) increment of total height during this period.

M5), even with the same proportion of trees (25*Cs*:75*Pm*), it can be observed the heterogeneity of these values.

Based on the results the *Pseudotsuga* has a different behaviour and the DBH increment is even more heterogeneous with mixed treatments presenting higher increment values than the pure ones (*e.g.* M2). In terms of the total height increment the tendency is clear, with the pure treatments presenting higher increment values than the mixed ones. In mixed treatments the *Pseudotsuga* DBH increment was positively affected by the presence of *Castanea* (Fig. 5b). Khanna (1997) made a comparison study of growth in pure and mixed stands of *Eucalyptus globulus* and *Acacia mearnsii* and has concluded that the DBH increment of *Eucalyptus* was higher in mixed treatments.

Soil properties

The obtained results showed few differences in soil properties among the treatments. The soils were characterized as medium-textured. Soil pH (H_2O), ranged from 4.69 to 4.85 in horizon A (0-20 cm depth) in M2 and M5 (25*Cs:*75*Pm*) treatments and no significant differences were found. In horizon B (21-60 cm depth) extreme values ranged from 4.59 to 4.89 in M6 (100*Cs*) and M5 (25*Cs:*75*Pm*) treatments with significant differences ($P < 0.05$, Scheffe) among the treatments (Table 1).

There were no significant differences ($P > 0.05$, Scheffe) between pure and mixed treatments in terms of K (pure treatments with 52.50 and 55.00 mg kg^{-1}; mixed treatments with 49.25, 39.25, and 53.00 mg kg^{-1}, for horizon A). Nevertheless, the K content shows a decrease in mixed treatments.

In terms of exchangeable acidity, there are differences between the mixed treatments analysed in both horizons ($P < 0.05$, Scheffe) (for horizon A the pure treatments present 2.53 and 1.71 cmol$_{(+)}$ kg^{-1}; mixed treatments with 1.88, 2.13 and 2.34 cmol$_{(+)}$ kg^{-1}, and for horizon B the pure treatments present 3.69 and 2.69 cmol$_{(+)}$ kg^{-1}; mixed treatments present 2.50, 2.40 and 2.38 cmol$_{(+)}$ kg^{-1}). Once again mixed treatments presented lower values than the pure ones, excluding horizon A for pure *Pseudotsuga*.

Table 1. Soil properties in 5 treatments in two soil layers (A and B horizons) with their standard error

Soil properties[a]	Depth (cm)	100Cs (M6)	25Cs:75Pm (M2)	25Cs:75Pm (M2)	25Cs:75Pm (M5)	100Pm (M7)	ANOVA[b]
pH (H_2O)	0-20	4.73 (0.07)	4.75 (0.06)	4.69 (0.06)	4.85 (0.05)	4.83 (0.07)	ns
	21-60	4.59[a] (0.07)	4.70[b] (0.05)	4.71[b] (0.06)	4.89[c] (0.05)	4.64[a] (0.09)	*
pH (KCl)	0-20	3.77 (0.06)	3.81 (0.09)	3.83 (0.06)	3.84 (0.06)	3.87 (0.07)	ns
	21-60	3.65 (0.08)	3.76 (0.08)	3.85 (0.09)	3.79 (0.09)	3.71 (0.12)	ns
Organic matter (g kg^{-1})	0-20	26.5 (0.03)	23.5 (0.03)	25.0 (0.02)	24.9 (0.03)	23.3 (0.03)	ns
	21-60	8.8 (0.01)	10.5 (0.02)	12.0 (0.02)	11.7 (0.03)	14.1 (0.01)	ns
P_2O_5 (mg kg^{-1})	0-20	20.9[a] (4.62)	12.4[b] (2.15)	11.2[c] (2.71)	9.1[d] (1.92)	15.5[b] (2.21)	*
	21-60	1.7 (0.30)	3.1 (0.89)	3.9 (1.18)	1.9 (0.42)	3.4 (1.20)	ns
K_2O (mg kg^{-1})	0-20	52.50 (5.86)	49.25 (4.73)	39.25 (4.44)	53.00 (7.20)	55.00 (8.98)	ns
	21-60	41.50 (7.33)	47.25 (5.10)	34.00 (2.56)	40.00 (4.34)	45.50 (6.14)	ns
Ca [$cmol_{(+)}$ kg^{-1}]	0-20	0.17 (0.06)	0.14 (0.05)	0.16 (0.09)	0.21 (0.05)	0.16 (0.05)	ns
	21-60	0.14 (0.05)	0.14 (0.08)	0.13 (0.08)	0.31 (0.15)	0.06 (0.01)	ns
Mg [$cmol_{(+)}$ kg^{-1}]	0-20	0.18 (0.05)	0.09 (0.02)	0.11 (0.05)	0.15 (0.04)	0.16 (0.06)	ns
	21-60	0.13 (0.05)	0.10 (0.02)	0.03 (0.01)	0.43 (0.28)	0.06 (0.02)	ns
K [$cmol_{(+)}$ kg^{-1}]	0-20	0.15 (0.01)	0.12 (0.01)	0.09 (0.01)	0.15 (0.02)	0.14 (0.02)	ns
	21-60	0.11 (0.01)	0.13 (0.02)	0.08 (0.01)	0.15 (0.03)	0.11 (0.03)	ns
Na [$cmol_{(+)}$ kg^{-1}]	0-20	0.08 (0.01)	0.11 (0.03)	0.08 (0.01)	0.08 (0.01)	0.09 (0.01)	ns
	21-60	0.08 (0.01)	0.09 (0.01)	0.08 (0.01)	0.11 (0.03)	0.09 (0.01)	ns
Exc ac [$cmol_{(+)}$ kg^{-1}]	0-20	2.53[a] (0.17)	1.88[b] (0.17)	2.13[b] (0.19)	2.34[b] (0.19)	1.71[c] (0.11)	*
	21-60	3.69[a] (0.46)	2.50[b] (0.21)	2.40[b] (0.38)	2.38[b] (0.24)	2.69[c] (0.38)	*
Total N (g kg^{-1})	0-20	1.31 (0.14)	1.11 (0.12)	1.11 (0.13)	1.14 (0.13)	1.14 (0.13)	ns
	21-60	0.52 (0.03)	0.61 (0.08)	0.61 (0.07)	0.61 (0.13)	0.72 (0.07)	ns
EC (dS m^{-1})	0-20	0.04 (0.01)	0.04 (0.01)	0.03 (0.01)	0.03 (0.01)	0.03 (0.01)	ns
	21-60	0.02 (0.01)	0.03 (0.01)	0.02 (0.01)	0.02 (0.01)	0.02 (0.03)	ns

[a] Mean values with the same letter within the soil layer do not differ significantly with each other. [b] ANOVA results: ns = treatment effect not significant. * $P < 0.05$ Scheffe.

For P and for horizon A, M6 (100*Cs*) treatment presents higher values (20.9 mg kg^{-1}), which decreases with the decreasing proportion of the *Castanea* among the mixed treatments (12.4, 11.2 and 9.1 mg kg^{-1}). This means that higher proportions of *Castanea* results in higher values of P in the soil. It is also interesting to report that differences can be observed among the three mixed treatments.

No statistically significant differences were observed for the organic matter content between the treatments in both soil layers (horizon A ranged from 23.3 to 26.5 g kg^{-1} and horizon B ranged from 8.8 to 14.1 g kg^{-1}), although it decreases with a decreasing of *Castanea* proportion in horizon A.

Ca (values ranged from 0.14 to 0.21 $cmol_{(+)}$ kg^{-1} in horizon A and from 0.06 to 0.31 $cmol_{(+)}$ kg^{-1} in horizon B), Mg (values ranged from 0.09 to 0.18 $cmol_{(+)}$ kg^{-1} in horizon A and from 0.03 to 0.13 $cmol_{(+)}$ kg^{-1} in horizon B) and total N (values ranged from 1.11 to 1.31 g kg^{-1} in horizon A and from 0.52 to 0.72 g kg^{-1} in horizon B) exhibited no significant differences among the soil horizons of treatments ($P > 0.05$, Scheffe). Also, no significant differences were observed in the exchangeable Na (values ranged from 0.08 to 0.11 $cmol_{(+)}$ kg^{-1} for both horizons) and electrical conductivity content in both soil layers between the treatments.

A generic analysis of the Table 1 shows that soils are not so different between pure and mixed stands. The only statistically differences were observed for the pH in the horizon B, P in horizon A and the exchangeable acidity for both layers.

Foliage nutrients

Table 2 shows the results of foliage chemical analysis. Once again, and reflecting the previous results

Table 2. Foliage chemical analysis in 5 treatments and in two species (*Castanea* and *Pseudotsuga*) with their standard error

Fully expanded leaves	100Cs (M6)	25Cs + 75Pm (M1)		25Cs + 75Pm (M^1)		25Cs + 75Pm (M5)		100Pm (M7)
	Castanea	*Castanea*	*Pseudotsuga*	*Castanea*	*Pseudotsuga*	*Castanea*	*Pseudotsuga*	*Pseudotsuga*
N (g kg^{-1})	19.5^a (0.56)*	19.9^a (0.42)*	9.2 (0.25)	20.0^a (0.45)*	9.4 (0.47)	23.4^b (0.80)*	10.2 (0.27)	9.5 (0.30)
P (g kg^{-1})	1.94 (0.09)	2.21 (0.10)	1.57 (0.11)	2.06 (0.11)	1.48 (0.09)	2.22 (0.18)	1.57 (0.11)	1.38 (0.10)
K (g kg^{-1})	16.4 (0.92)	16.4 (0.50)	11.2 (0.45)	16.1 (1.05)	10.5 (0.66)	14.4 (0.66)	11.0 (0.41)	9.7 (0.34)
Ca (g kg^{-1})	4.3 (0.40)	5.3 (0.60)	4.8 (0.51)	4.8 (0.52)	4.7 (0.55)	4.3 (0.27)	5.0 (0.28)	4.9 (0.43)
Mg (g kg^{-1})	3.73 (0.46)	3.70 (0.44)	1.55^a (0.06)*	3.03 (0.36)	1.76^b (0.10)*	3.88 (0.51)	1.65^b (0.06)*	1.87^c (0.11)*
C (g kg^{-1})	541.2 (10.07)	539.9 (8.78)	542.8 (10.00)	535.5 (7.87)	563.2 (6.41)	538.4 (5.18)	501.1 (12.33)	564.6 (4.76)

Mean values with the same letter within the two species do not differ significantly with each other. ANOVA results: * = treatment significant ($P < 0.05$ Scheffe).

of soil analysis there is almost no statistically differences between pure and mixed treatments for both species. The results of the analysis of variance (ANOVA) have showed that only for the *Castanea* there was a statistic difference for the N content. In this case, mixed treatments presented higher values for the N content and the highest being in M5 (25*Cs:*75*Pm*) treatment with 23.4 g kg^{-1}. Generally, N and P contents in *Castanea* were higher in the M5 (25Cs:75Pm) treatment. Variations of N in foliage ranged from 19.5 to 23.4 g kg^{-1} for *Castanea* and from 9.2 to 10.2 g kg^{-1} for *Pseudotsuga*. P limit values vary from 1.94 to 2.22 g kg^{-1} for *Castanea* and from 1.38 to 1.57 g kg^{-1} for *Pseudotsuga*.

The others elements (K, Ca, and C) did not present significant differences for *Castanea* leaves between pure and mixed treatments, although K and Ca present a higher concentration (16.4 g kg^{-1} for K and 5.3 g kg^{-1} for Ca) in M1 (25*Cs*:75*Pm*) treatment and C present a higher concentration, with 541.2 g kg^{-1}, in M6 (100*Cs*) treatment. *Pseudotsuga* leaves present significant differences only for Mg among the treatments, with the highest value observed for M7 (100*Pm*) treatment with 1.87 g kg^{-1}.

Discussion

This study area is located in a place where *Castanea* is ecologically well adapted. According to Fontes *et. al*. (2003) the best sites for *Pseudotsuga* growth are located along north coastal to central regions at altitudes between 500 m and 1,000 m with moisture deficit above 1,000 m, and as mentioned above, this study area is installed in a place in accord with these parameters.

Castanea and *Pseudotsuga* survival have significant differences in pure and mixed plantations. Survival of *Castanea* is higher in pure than in mixed plantations with *Pseudotsuga*. Rouhi-Moghaddam *et al.* (2008) reported that survival of *Quercus* in the pure plantations was lower than in the mixed plantations with *Zelkova carpinifolia*. The differences between our results and those obtained by these authors were probably due to differences in nutrient and light requirements of our species and the ones studied by them.

In terms of diameter growth, we found that there were no significant differences between the different treatments, for both species and in result the DBH was not affected by the different mixtures. A similar result was obtained by Rouhi-Moghaddam *et al.* (2008). According to these authors this results could be due to the similarity of the two species in their light needs.

Basal area for the *Castanea* and the *Pseudotsuga* plantations showed significant differences between the pure compared to the mixed plantations. Our results were similar to those obtained by Khanna (1997), Parrotta (1999), Montagnini (2000) and Piotto *et al*. (2004); they found larger basal area for target species in the mixed compared to the pure plantations. Opposite results were obtained by Sayyad *et al*. (2006).

In terms of total height there were no significant differences between pure and mixed plantations for both species. Although it is important to stress that, analyzing only the mixed treatments, it could be observed different results with the same species composition, showing a diversity of responses.

Top height showed a more clear response to species composition. Dominant height is higher for mixed plantations of *Castanea* than in pure ones. This fact is not so clear for the *Pseudotsuga*, where values are more similar. Based on the assumptions of Kayahara *et al*. (1995), *Tsuga heterophylla* does not appear to increase in site index with increasing nutrients, and assuming that top height is a good measure of site index, we can at least assume that top height is depending on species composition, and mixed plantations promote higher values of this structural parameter. Luis and Monteiro (1998) found, for the same experimental design, that *Castanea* and *Pseudotsuga* show significant differences in top height growth patterns, but these patterns were not influenced by the mixtures tested; this contradict what some years late was found for the same study area. Chen and Klinka (2003) reported that productivity was not higher in mixed species stands thus, they have concluded that mixed-species composition in western hemlock and western redcedar stands in southern coastal British Columbia did not increase aboveground productivity. Assuming that the analyzed structural variables are also indicators of the stand productivity, the obtained results were in agreement with these ones, except for the top height.

N is often the most important determinant of plant growth and crop yield. Proteins, which are of great importance in many plant organs, *e.g.* seeds, are compounds of nitrogen whilst chlorophyll, the green coloring matter of the leaves, also contains the element. Our results showed that soil N content does not vary between mixed and pure plantations. Rouhi-Moghaddam *et al.* (2008) reached similar results as this one, and Montagnini (2000) did not observed any significant differences in soil N between pure and mixed plantations. In terms of foliar N concentrations the results was a lower value than the limit for possible deficiency (12 mg g^{-1}, according to Will, 1985) for the pure plantations of *Pseudotsuga*, but not for the pure plantations of *Castanea*.

P plays an important role in the process of photosynthesis, nutrient transport, and energy transfer. The P concentration in soil was significantly different between pure and mixed plantations. It was higher in pure plantations for both species, even if the highest values were found for the pure *Castanea* treatments (M6). It is also important to stress that when testing the same proportion of both species but changing the design of the plantation, P content changes, which could be related with the initial heterogeneity of the soil. In this case P content results are not related with changes of species proportion (because they are the same). The P limit values of foliage concentrations vary from 1.00 to 1.50 g kg^{-1} (Mankovska, 1996). In this study, all the plots showed P foliar concentrations higher than the reported limit values range. The obtained results for P soil content are exactly the inverse in terms of foliar concentrations, which means that while for soil P concentrations values are higher in pure than in mixed plantations, in terms of P foliar concentrations results are the inverse.

K is a major plant nutrient which has to be accumulated in great quantity by roots and distributed throughout the plant and within plant cells. Membrane transport of K can be mediated by K channels and secondary K transporters (Gierth and Mäser, 2007). Álvarez-Álvarez *et al*. (2010) have concluded that for the hybrid chestnut plantations the stands with the highest growth rates were characterized by high foliar concentrations of K, P and Ca. The obtained results show no differences between the K concentration on foliage, for both species, and for pure and mixed plantations. A similar result was obtained for the soil analysis. In all the treatments analyzed, the K levels were higher than the marginal ranges for K (3-5 g kg^{-1}, according to Will, 1985), which means this nutrient is not conditioning plants growth.

According to Hong-Bo *et al.* (2008), in plant cells Ca plays a role as a second messenger, coupling a wide range of extracellular stimuli with intracellular responses. In this study satisfactory foliar Ca concentrations (> 1 g kg^{-1}, according to Will, 1985) were observed in all the treatments. Variations of this element in foliage followed the order: 4.3-5.3 mg g^{-1} for *Castanea* and 4.7-5.0 mg g^{-1} for *Pseudotsuga* leaves. These values are very similar to the ones found by Rouhi-Moghaddam *et. al*. (2008). For the soils there was, once again, no differences between all treatments, which means this study did not found differences between exchangeable Ca from mixed and pure plantations, for both horizons.

In terms of Mg, the obtained values are much higher than the optimal nutritional values for Mg ranging from 0.6 to 1.5 g kg^{-1} (Stefan *et al*., 1997). In terms of soils analysis, there were no statistically differences between the Mg content for pure and mixed plantations, for both horizons. Vejre (1999) found that for norway spruce stands the content of Mg and Ca correlated significantly with stand yield class.

Concerning C, foliar analysis showed no significant differences between pure and mixed plantations for both species. The average C value was near 540 g kg^{-1}.

For horizon B the pH was statistically different between treatments. Pure treatments of *Castanea* (M6) and *Pseudotsuga* (M7) differ from the mixed treatments and M1 do not differ from M2 treatment but differ from M5. Although all the mixed treatments analyzed have the same species proportions the only difference was the way the trees were planted. Generally, when analyzing soil horizons, the differences between pure and mixed plantations were very small. This indicates that it might be a result of a fairly short time frame, *e.g.,* longer time spans are required for an influence of the tree species to develop in the mineral soil. Similar results were obtained by Brandtberg *et al.* (2004), studying *Betula* spp. and *Picea abies* in mixed stands. These authors reported that mixture of broadleaf tree species and conifers are not complementary in nutrient uptake from the forest floor and mineral soil.

The results obtained with this study do not confirm what Luis and Monteiro (1998) stated about the more productive mixtures when compared with the traditional solution of *Castanea sativa* monocultures in an economic evaluation. Nevertheless the experimental design should be followed in the next couple of years not only because improves the biodiversity but also because, according to them, these mixtures perform better over time in productivity and economic terms. So it will be necessary to monitor the treatments in the future and evaluate if mixed plantations really have more ecological and economic advantages than pure plantations.

The present study is a preliminary study of the evolution and behaviour of these two species under different treatments; more definitive conclusions are expected in 10-15 years when the effects at ages closer to the rotation age can be observed, especially the effects induced by *Pseudotsuga menziesii.*

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