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AFFORESTATION OF AGRICULTURAL LAND WITH *PINUS RADIATA* D. DON AND *BETULA ALBA* L. IN NW SPAIN: EFFECTS ON SOIL PH, UNDERSTOREY PRODUCTION AND FLORISTIC DIVERSITY ELEVEN YEARS AFTER ESTABLISHMENT

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ABSTRACT

Afforestation of abandoned agricultural lands has been the main change in land use over the past decade in Europe. However, the impact of tree species and understorey management on production and plant diversity over the medium- and long-term has not been thoroughly studied. This paper aims to evaluate the effects of an afforestation of *Pinus radiata* D. Don and *Betula alba* L. on soil pH, understorey production and plant diversity and life cycle type (annuals vs. perennials) managed with different soil fertilisation treatments over a period of 11 years. The results show an acidification of the soil 11 years after establishment, better vertical growth and diameter of pine compared with birch as usually happens in the region and important variation in the biomass production and composition of the understorey below both tree species. Understorey species remained similar during the first 5 years below both canopies. However, species richness (*S*) was drastically reduced under *Pinus radiata* D. Don plantation compared to *Betula alba* L. ($S_{\text{pine}} = 2$ vs. $S_{\text{birch}} = 17$) after 11 years of tree establishment at a very high density (2500 trees ha^{-1}). Inorganic and organic fertilisation also caused a reduction in floristic diversity. Soil pH, pasture production and floristic understorey plant diversity are better preserved under autochthonous broadleaves, which increased the multiple uses of recently afforested lands in the short- and medium-term. In the European context of high need for sawn wood, the use of autochthonous broadleaved tree species like *Betula* should be promoted due to their better sustainability. Copyright © 2011 John Wiley & Sons, Ltd.

KEY WORDS: broadleaved; conifer; dairy cattle manure sludge; biosolid; Spain

INTRODUCTION

The EU has declared the ambitious objective of stopping the loss of biodiversity in Europe by 2010 (Official Journal of the European Communities, 2002), being change in land use one of the main management causes of biodiversity reduction (Aguiar, 2005). Intensification of agriculture as well as monospecific silviculture and the propagation of non-autochthonous and generalist species are some of the key factors responsible for the soil nutrient status changes, understorey productivity and loss of plant diversity (Augusto *et al*., 2002) in EU forests and agricultural lands all over the world. Since the middle of the 20th century, policies of afforestation and increasing timber production have been imposed in the EU, resulting in the planting of large areas of productive coniferous tree species and a reduction in native deciduous species area (EC, 2004) as happened with *Pinus radiata* in other continents like South America (Paritsis and Aizen, 2008) or Oceania (Brockerhoff *et al*., 2001). Exotic Conifer tree plantations usually cause important changes in soil fertility, understorey production and plant diversity (Michelsen *et al*., 1996; Frank and Finckh, 1997; Raffaele and Schlichter, 2000) compared with native broadleaved species due to their different canopy type, light interception and structure (Brockerhoff *et al*., 2001). Generally, these new forest areas aim to produce timber, which is initially and partly delivered in the first thinning several decades after planting. The use of silvopastoral systems is considered a tool to enhance plant diversity through sympathetic management to indigenous diversity (Brockerhoff *et al*., 2001), while increasing forestry farm profitability (Sibbald, 1996; Fernández-Núñez *et al*., 2007), as intermediate products (wool, meat) are delivered at the same time that tree growth is occurring. Moreover, in Galicia, one of the regions of Europe most prone to forest fires, herbaceous layer promotion through the establishment of silvopastoral systems help to reduce fire risk (Rigueiro-Rodríguez *et al*., 2009), and to protect forest biodiversity.

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Reforestation of Agricultural Land (Regulation EEC n° 2080/1992 n° 1257/1999) caused an increase of 400 000 ha of afforested areas in the Galicia region in the 1990s (MAPA, 2006), mostly with exotic and fast growing species like *Eucalyptus* spp. and *Pinus radiata* D. Don. The latter is the species used most often in afforestation in the Province of Lugo (where the study was carried out) due to its high growth rate (IFN, 1998). However, it could have a negative impact on the production and biodiversity of the understorey component, as *Pinus radiata* creates less suitable conditions (shading) for the development of understorey vegetation than *Pinus pinaster* or those of autochthonous broadleaves like *Betula alba* (Mosquera-Losada *et al.*, 2006). Canopy cover and light interception is a key structural factor when designing and managing plantations to enhance biodiversity (Paritsis and Aizen, 2008). In managed forests, the choice of tree species is one of the fundamental acts of the forester and can have a strong impact on soil (Mosquera-Losada *et al.*, 2006) and understorey development. Recently, Barbier *et al.* (2008) emphasised that studies are needed to test whether hardwoods are more favourable to understorey plant diversity than conifers at a medium- and long-term. Fertilisation as well as ploughing have also been found to have a great impact on soil pH, understorey production and plant diversity in very acidic forest soils of the area (Mosquera-Losada *et al.*, 2009). Fertilisation is the most useful tool to enhance pasture production when no other factors like light, temperature or humidity limits understorey growth. However, this extended practice can cause soil pH depletion due to the increment of cation extractions by pasture and tree and the organic matter mineralisation (Whitehead, 1995). Soil pH depletion could partially be overcome by nutrient recycling of fallen tree leaves (Moreno *et al.*, 2007) of broadleaves compared with conifers. Fertilisation also affects diversity due to the modification of the relationship of species (presence of monocots vs. dicots), different functional ecological traits (presence of annual *vs.* perennial species) or richness. Few studies have been carried out on abandoned agricultural land that has been afforested (Schipper, 1996; Mosquera-Losada *et al.*, 2006 and Mosquera-Losada *et al.*, 2009) with native broadleaved or exotic conifer species taking into account soil, production and richness for a long-term. This study aims to evaluate the effect of *Pinus radiata* D. Don and *Betula alba* L. established in an abandoned agricultural land on soil pH, understorey plant diversity, life cycle type (annuals vs. perennials) and production managed with different soil fertilisation treatments over 11 years. Ultimately, our goal was to determine the effects of extensive exotic tree species plantations as compared with autochthonous species within the context of land use change from agronomic to forest land to provide more sympathetic management to biodiversity preservation at a high tree densities.

METHODS

Location

The experiment was established in Castro Riberas de Lea (Province of Lugo, NW Spain, 43°01′N; 7°40′W). The study area is situated 439 m above sea level. The zone in which the study was carried out is located in the Atlantic Bioclimatic Region (EEA, 2003). The total annual rainfall and average temperature for the last 30 years were 1300 mm and 12·2°C, respectively. Generally, water deficits were registered during the months of July and August, which limited vegetal development in these months due to drought (Pearson and Ison, 1987; Mosquera and González, 1998). The experiment developed from 1995 to 2005 over a soil classified as Umbrisol (FAO, 1998), with a sandy-silty texture (61 per cent sand, 34 per cent silt and 5 per cent clay) that was previously used for agricultural purposes (cultivation of potatoes). The initial pH of the water (1:2·5) was close to neutrality (6·8), indicating optimum availability of nutrients for the plants (Porta Casanellas *et al.*, 2003). The edaphic contents of organic matter and nitrogen were 8·03 per cent and 0·33 per cent, respectively, which could be considered high, but is characteristic of soils dedicated to crops in Galicia (Calvo de Anta *et al.*, 1992). Moreover, the relation of C/N was 14·11, indicating a slow mineralisation rate favouring soil organic matter accumulation.

Experimental Design and Management

The experiment was initiated in 1995 and consisted of 24 treatments and 72 experimental units arranged following a randomised block design with three replicates. Plantation has a size of around 1 ha. Only the results of six treatments out of 24 and obtained in 1995, 2000 and 2005 are described in this paper. The treatments tested two forest species *Pinus radiata* D. Don (from container plants) and *Betula alba* L. (from bare root) established at 2500 trees ha^{-1} (with a distance of 2×2 m between trees) and three different fertilisation treatments. Each replicate or experimental unit of a total of 18 experimental unit (three replicates × six treatments) had an area of 64 m^2 (8×8 m^2), and 25 trees were planted with an arrangement of 5×5 stems, forming a perfect quadrat. In March 1995, land ploughing was carried out. Each experimental unit was sown with *Dactylis glomerata* L. var. Saborto (25 kg ha^{-1}) + *Trifolium repens* L. var. Ladino (4 kg ha^{-1}) + *Trifolium pratense* L. var. Marino (1 kg ha^{-1}). The fertiliser treatments applied in each experimental unit are as follows: *no fertilisation* (NF) for the duration of the experiment; *mineral fertilisation* (M) every year throughout the experiment following a standard procedure for the region: 500 kg ha^{-1} of 8:24:16 (N:P_2O_5:K_2O) fertiliser complex in March and 40 kg of N (calcium ammonium nitrate 26 per cent N) ha^{-1} in May; and *fertilisation with dairy cattle manure sludge* (D) in the first year (1995) at 154 m^3 ha^{-1}, i.e. 160 kg of total N, 85·9 kg of

total P_2O_5 and 23·4 kg of total $K_2O\,ha^{-1}$, with values determined based on total N of sludge. Also, 265·6 kg ha^{-1} of CaO and 32·8 kg ha^{-1} of MgO were added together with the input of the dairy cattle manure sludge (D) treatment in 1995. Dairy cattle manure sludge was only applied in the first year of the study because this is traditionally done in the area due to difficulties related to dairy cattle manure sludge application in high density stands. Moreover, trees are more benefited from fertilisation in the first year of establishment due to the high tree plantation stress and the high tree growth rate at the start of the plantation, which therefore increases stand response to fertilisation at establishment than later. In the following 2 years (1996 and 1997), the plots to which the sludge was applied were not fertilised, but they were fertilised again from 1998 until the conclusion of the study, using the same fertilisers and doses as for M. Dairy cattle manure sludge applied in the plots had 2 per cent DM, 1·3 kg m^{-3}, 0·55 kg $P_2O_5\,m^{-3}$, 0·15 kg $K_2O\,m^{-3}$, 1·7 kg CaO m^{-3} and 0·21 MgO kg m^{-3}. These values were determined in the laboratory. Sludge DM was determined after drying 100 g of sludge. N, P, Ca, K and Mg concentrations were determined after microkjeldahl digestion. N and P concentration was evaluated by colorimetry with an auto analyser (TRAACS-800$^+$), as per Castro *et al*. (1990). K, Ca and Mg concentrations were measured with a VARIAN 220FS spectrophotometer using atomic emission for K and absorption for Ca and Mg (VARIAN, 1989). At the end of 2001, the pine trees were low-pruned (height of 2 m); at the end of 2002, formation pruning was carried out on the birch trees, with the purpose of producing good quality timber.

In this paper, we show the corresponding results at three stages in the development of the system, corresponding to the year of establishment (1995), intermediate development of the system (2000) and the final situation (2005).

Soil Sampling

During the period of study, a composite soil sample (three samples per experimental unit) was collected in each of the established experimental units, using a core to a sample depth of 25 cm (BOE, 1990). The samples were collected in December 1995, March 2001 and January 2006. Soil samples were carried out during the winter period, as is traditionally done in the area in order to quantify fertiliser inputs before the growing season. Once the samples were obtained, they were taken to the laboratory, air-dried and sifted using a 2 mm sieve, after which the pH of the water (1:2·5) was determined using a pH meter (Model GLP 21 Crison).

Tree Measurement

Each year, diameter and height of *Pinus radiata* and *Betula alba* were taken from the nine central trees of each experimental unit, in order to avoid the border effect produced in the trees on the edges of the plot. Distances between replicas were 2 m, so measured trees were at least 6 m away. During the first few years, measurements of diameter were made by calliper at the height of the root-collar; later, the diameter at breast height (1·30 m) was measured by calliper. In order to obtain height measurements, a pole was used in 1995 and 2000, while in the last year (2005) it was necessary to use a hypsometer.

Pasture Sampling

The pasture was harvested four times each year (May, June, July and December) with the exception of the year in which the system was established (1995) and the last year (2005) in the plots established under *Pinus radiata*, when only two harvests (July and December) were carried out. At each harvesting in each experimental unit, the entire surface area delimited by the nine inner six trees of the plot was cleared, the fresh forage was weighed *in situ* and a representative subsample was taken to the laboratory. In the laboratory, the species of 100 g subsamples from each plot were hand-separated to determine botanical composition. The different species were weighted separately to determine dry weight (48 h at 60°C) to perform understorey plant diversity analyses. The species were split into annual and perennial functional groups.

Diversity and Abundance

The diversity index calculations were carried out using plant species data collected in 1995, 2000 and 2005. Once all of the species that were present in the different samples from each harvest of the established plots were identified they were summed up in a year basis and their relative percentage proportion (taking into account the four harvests of the year) was calculated.

A series of indexes were determined relative to the α (species richness (S) (Magurran, 2004), Shannon–Wiener index (H) (Shannon and Weaver, 1949) and β *diversity* (Jaccard Index of Similarity and the Index of Complementarities (Colwell and Coddington, 1994; Lande 1996) to compare the different stages of the experiment) from the relative species percentage proportion (Moreno, 2001) as shown in Table I.

Once the samples of pasture collected from each treatment had been separated by hand for all the species, senescent material (dead plant material) and needles and their dry-weight per cutting obtained, the annual production of each category was determined. From these data, the relative abundance of each species (expressed as part/unit) was obtained and abundance diagrams were produced, ordering the species from most to least abundance (Magurran, 2004). The aim of these diagrams was to reflect the relative evolution of the species under the two established tree covers (*Pinus radiata* and *Betula alba*),

Table I. Beta diversity index

Jaccard (I_j) Equations	
$I_j = c/(a + b - c)$	(i) Comparison between the initial (1995) and final situation (2005) a = initial species number (year 1995) b = final species number (year 2005) c = initial and final shared species (ii) Comparison of the similarity between plots established under the tree cover of pine and birch trees over the three years of the study, where: a = species present in systems established with pine trees b = species present in systems established with birch trees c = number of species present in both systems
Colwell and Coddington index of complementarities (1994) (C_{AB}) Equations	
$C_{AB} = U_{AB}/S_{AB}$	S_{AB} = total richness of species for both plots
$U_{AB} = a + b - 2c$	U_{AB} = number of unique species in each type of plot
$S_{AB} = a + b - c$	a = species present in plots of pine trees
	b = species present in plots of birch trees
	c = common species between plots established under the two canopies.

in each of the treatments (D, M, NF) and at the three different time periods after tree planting: the beginning stage (1995), intermediate stage (2000) and final stage (2005). The evolution of the ecologic groups (annuals vs. perennials) and seeded/native species per treatment and year was also evaluated.

Statistical Analysis

The variables studied were pH, diameter and height of the trees, biomass, and α and β plant diversity. For the first five variables, the results obtained were analysed with Repeated Measures ANOVA, using Mauchly's Criterion to test sphericity. If sphericity assumption was met then univariate approach output was used, otherwise multivaritae output (Wilks' Lambda test was taken into account). The LSD (Least Significant Difference) test was used for subsequent pairwise comparisons after a normalisation test ($\alpha = 0{\cdot}05$) only when factors were significant in the ANOVA analyses. Correspondence analyses (CA) with all the treatment and replicas were carried out to determine how the number of native/spontaneous species and how annual/perennial in each year interacted with the different tree systems (exclusive pine species, exclusive birch species and species shared by both tree systems). The statistical software package SAS (2001) was used for all analyses with the exception of CA which was performed with SPSS (v 15·0).

RESULTS

pH

In the samplings made in the three stages of the study, progressive acidification was produced irrespective of the type of tree established [$F(2, 28) = 36{\cdot}02$; $p = 0{\cdot}001$] (Figure 1). A significant interaction year* fertilisation [$F(4, 28) = 2{\cdot}81$; $p = 0{\cdot}04$] was found in soil pH. Inorganic fertilisers (M) caused a smaller reduction in water pH than D and NF treatments (Figure 1), being this effect more important at the start than at the end of the experiment. Moreover, the effect was also significant at the end of the study and did not depend on tree cover [$F(2, 28) = 2{\cdot}45$; $p = 0{\cdot}10$]. The initial soil water pH was 6·8 (Figure 1), reaching values of 5·40, 6·10 and 5·67 at the end of the experiment (2005) under pine trees, and values of 5·67, 6·10 and 5·90 under birch trees for organic fertilisers (D), mineral fertilisers (M) and no-fertilisers (NF), respectively.

Tree Growth

Mauchly's sphericity tests were significant when the height and diameter of trees were examined; therefore, multivariate approach was selected for testing within subject factors. Tree diameter [$F(2, 94) = 233{\cdot}49$; $p = 0{\cdot}001$] and height [$F(2, 90) = 510{\cdot}77$; $p = 0{\cdot}001$] growth changes with the year of sampling. Significant interactions of year* fertilisation were detected in the multivariate test for tree diameter [$F(4, 188) = 2{\cdot}52$; $p = 0{\cdot}04$] and height [$F(4, 180) = 4{\cdot}47$; $p = 0{\cdot}001$]. Change in mean tree height [$F(2, 90) = 51{\cdot}74$; $p = 0{\cdot}001$] and diameter [$F(2, 94) = 64{\cdot}40$; $p = 0{\cdot}001$] across tree levels depended upon year. The height and diameter growth of *Pinus radiata* 11 years after establishment was greater than that of *Betula alba* (Figure 2). The results show that from the beginning of the experiment, the application of mineral fertiliser (M) reduces the height growth of pine trees compared with no fertilisation treatment by 22 per cent at the end of the study; the same effect was seen in birch trees in 2005 (33 and 42 per cent for height and diameter, respectively). However, birch response

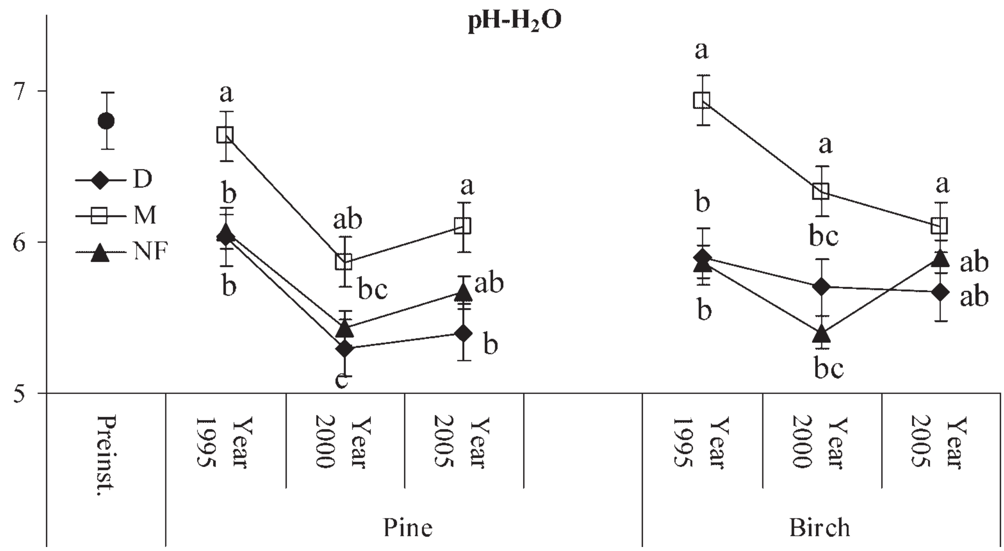

Figure 1. pH at the time of system establishment and in the years 1995, 2000 and 2005 under *Pinus radiata* D. Don (pine) and *Betula alba* L. (birch), where: Preinst. = pre-installation, D = fertilised with sludge, M = mineral fertiliser and NF = not fertilised. Different letters indicate significant differences between the six treatments for each year.

to fertiliser treatments was enhanced by mineral fertilisation in 2000.

Annual Aerial Biomass

Year factor had a significant negative effect on annual pasture production [$F(2, 28) = 39{\cdot}18$; $p = 0{\cdot}001$]. However, senescent [$F(2, 13) = 15{\cdot}29$; $p = 0{\cdot}001$] and needle [$F(2, 13) = 38{\cdot}79$; $p = 0{\cdot}001$] percentages were positively influenced by year. The interaction year* tree was also significant for annual pasture production [$F(2, 18) = 14{\cdot}69$; $p = 0{\cdot}001$] and needle percentage [$F(2, 13) = 38{\cdot}79$; $p = 0{\cdot}001$]. Apart from this, annual pasture production was positively and significantly affected by fertiliser effect [$F(2, 14) = 21{\cdot}91$; $p = 0{\cdot}001$].

The annual pasture production of the different treatments varied between 3·45–5·48, 1·71–3·33 and 0·11–0·33 t DM ha^{-1} in the plots established under pine trees in the years 1995, 2000 and 2005, respectively (Figure 3). In the case of the plots established under birch trees, the ranges were 3·88–5·32, 1·89–5·20 and 3·22–3·86 t DM ha^{-1}, respectively. Tree effect on pasture production was more important at the end of the experiment than at the start, as pine canopy significantly reduced pasture production in 2005. However, pasture production was positively affected by mineral and dairy cattle manure sludge fertilisation in 1995 in pine and birch system, and below birch in 2000.

Senescent material biomass increased from 1995 (below 0·12 t DM ha^{-1} under both tree species) to 2000 (below 0·72 and 0·33 t DM ha^{-1} under pine and birch, respectively) and 2005 (above 0·84 and 0·62 t DM ha^{-1} under pine and birch respectively); this increment was significantly higher under pine in NF and D treatments than under birch due to faster development of the pine canopy (Figure 3). The contribution of the needles to the annual aerial biomass in the pine tree plots was significantly greater during 2005 than in previous years.

Diversity Analysis

In the 3 years studied, a total of 57 species (sp) were recorded, 48 dicotyledonae and nine monocotyledonae. The families with the greatest species richness were *Compositae* (12 sp), *Gramineae* (nine sp), *Leguminoseae* (eight sp), *Caryophillaceae* (six sp) and *Polygonaceae* (five sp) (Table II). In regard to their ecologic groups, 24 species were annuals, and 26 were perennials; the rest (seven) were biennials. In 1995, the percentage of annual species was approximately 60 per cent in the plots established under the canopy of pine trees and 55 per cent under the canopy of birch trees. In contrast, by 2005, the presence of annual species was zero under the canopy of pine trees and varied between 27 and 53 per cent under the canopy of the birch trees (Figure 4).

Year [$F(2, 28) = 276$; $p = 0{\cdot}001$] in a negative way and year* tree interaction [$F(2, 28) = 18{\cdot}11$; $p = 0{\cdot}001$] significantly affected Species Richness (S) and Shanon index [$F(2, 28) = 72{\cdot}72$; $p = 0{\cdot}001$ and $F(2, 28) = 30{\cdot}95$; $p = 0{\cdot}001$, respectively], so tree canopy effect on plant diversity depended on the age of the stand. Fertiliser type effect was also significant for S [$F(2, 14) = 15{\cdot}60$; $p = 0{\cdot}001$] and H [$F(2, 14) = 3{\cdot}55$; $p = 0{\cdot}05$], being usually both higher in no fertiliser than in fertilised treatments. S in the plots under conifers varied between 20–28, 15–18 and 2 species in 1995,

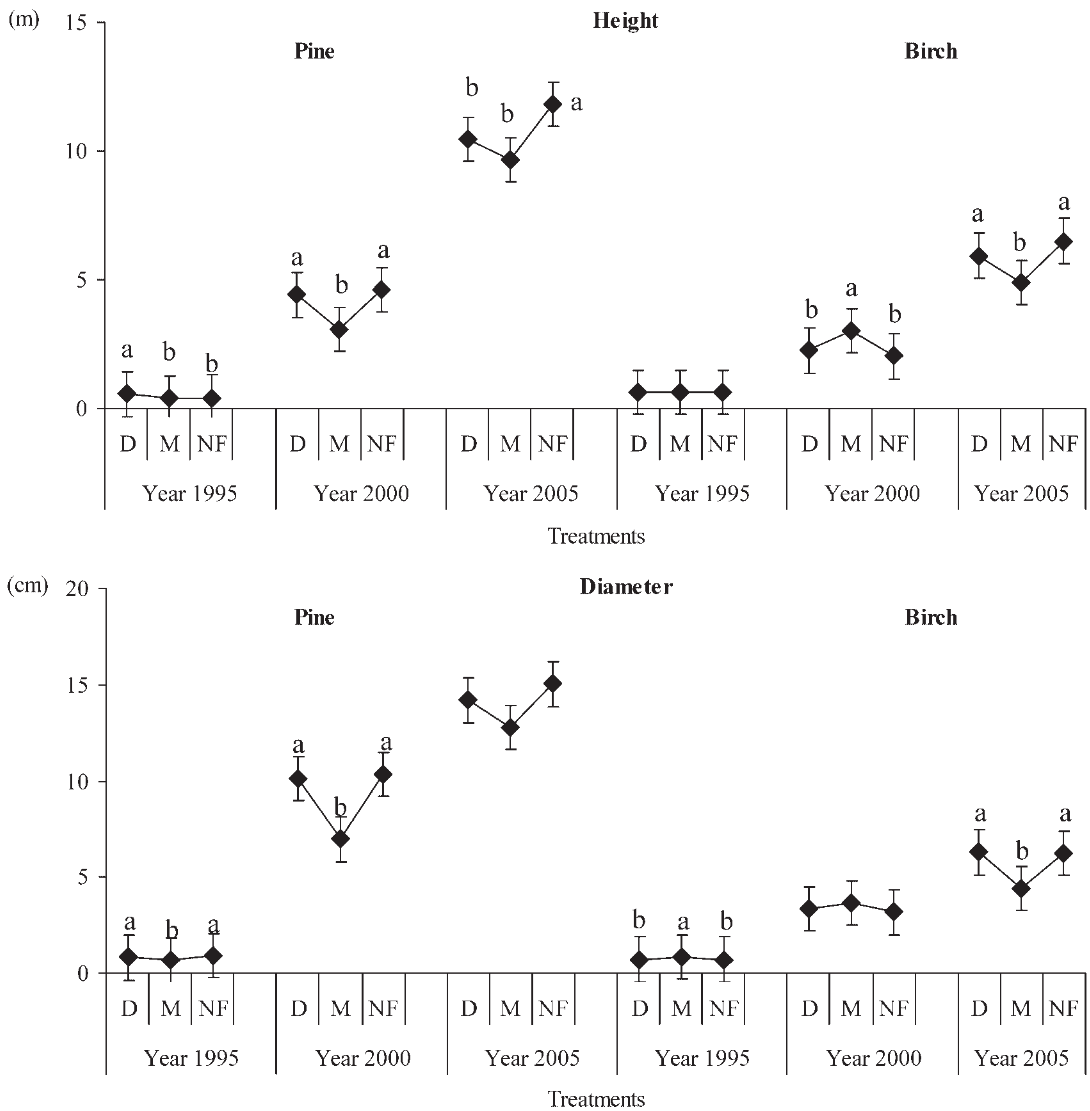

Figure 2. Height and diameter of *Pinus radiata* D. Don (pine) and *Betula alba* L. (birch) for the years 1995, 2000 and 2005. Note: D = fertilised with sludge, M = mineral fertiliser and NF = not fertilised. Different letters indicate significant differences between treatments within each tree species.

2000 and 2005, respectively. In the systems established with birch trees, the S was steadier over time, varying between 18–23, 15–20 and 15–19 species for the same years, respectively (Figure 5). *Dactylis glomerata* was the most abundant species in almost all of the treatments throughout the 11 years of study, with the exception of *Pinus radiata* when *Prunella vulgaris* L. codominated with *Dactylis glomerata*. *Dactylis glomerata* presence was close to or above 50 per cent on many occasions with the exception of the no-fertiliser plots (NF) developed under the canopy of birch trees in 1995 and 2000 (Figure 5).

The Shannon diversity index (H) varied between 1·16–1·94, 1·34–1·79 and 0·04—1·63 for the years 1995, 2000 and 2005, respectively. The effect of tree on H depended on the age of the stand. During the first year, a significant decrease in plant diversity was observed in plots established under the canopy of birch trees and those fertilised with M compared with pine tree treatment (Figure 6). This floristic diversity reduction was also noticed during the last year, when H was significantly reduced in the plots planted with conifers.

The floristic composition of the two established systems showed important differences over the 11 years of the study (Table III). The variation in floristic composition was more noticeable in the case of pasture established under pine trees than under birch trees. Under the canopy of pine trees, only 3 per cent of the species were shared between the initial situation (agricultural land) and the final situation (forest land), while in the case of systems established with birch

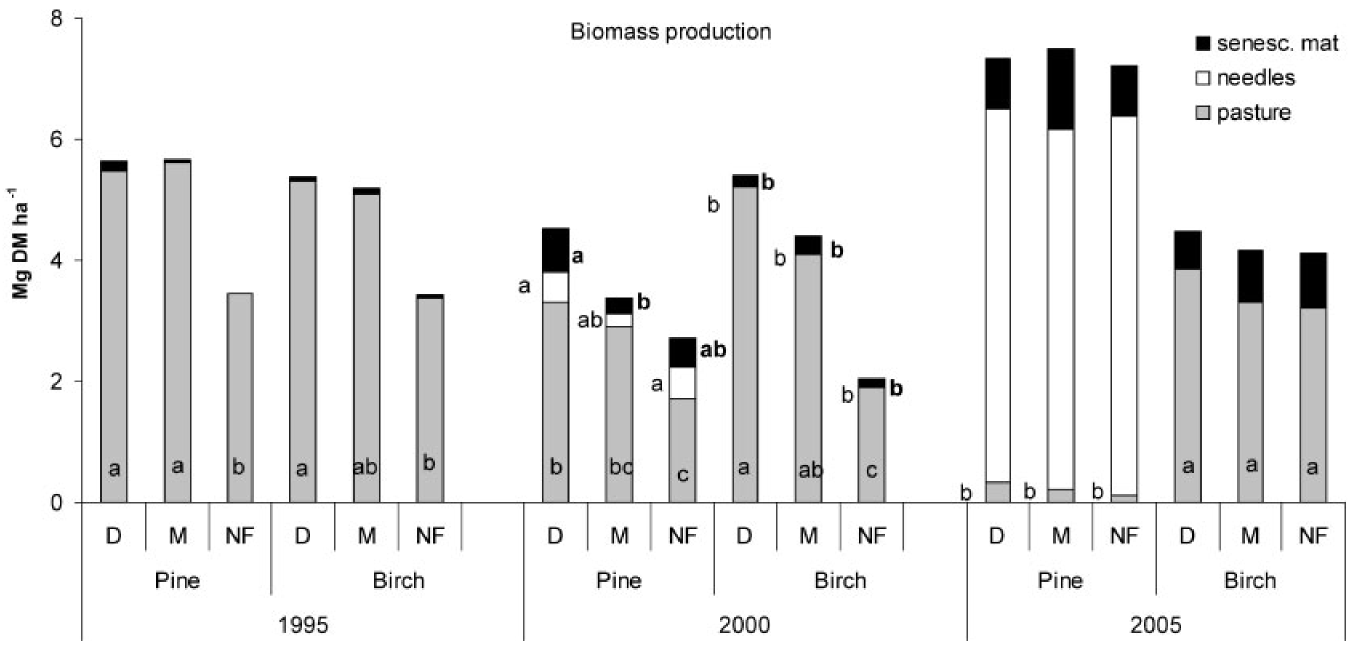

Figure 3. Variation in the biomass components: senescent material (senesc. mat.), needles and pasture production (pasture) under pine and birch, and fertiliser with dairy cattle manure sludge (D), mineral fertiliser (M) and not fertilised (NF). Different letters indicate significant differences between the six treatments per each biomass component in the same year.

trees, this percentage rose to 30 per cent (Table II). In the first two periods of study (1995 and 2000), the floristic composition of the two systems was very similar, sharing 65 and 71 per cent of their species (Table III), respectively; the greatest differentiation in the species was observed in the final period ($I_j = 0{\cdot}11$). In the first 2 years of study (1995 and 2000), approximately 30 per cent of the species were different between the two types of tree canopies (Table III). In the final year, the degree of dissimilarity between the two systems increased substantially (89 per cent).

The correspondence analysis graphical output is shown in Figure 7. The percentage of variance explained of dimension 1 in the graphs native/spontaneous or annual/perennials vs. tree system was very high (89 and 83 per cent, respectively). It can be seen that the number of native and seeded species as well as annual and perennials were mostly related with birch in the year 2005 compared with the other years and pine or shared species of pine and birch.

DISCUSSION

Most of the variables evaluated in this study were differently affected by treatments depending on the evaluated year. Those treatments with higher tree growth rate and pasture production (D and NF) significantly reduced soil pH due to higher cation soil extraction caused by the better development of these components. Cation extraction increment is, among other mechanisms like leaching and mineralisation, one of the main causes of soil acidification (Whitehead, 1995). This effect was especially relevant (and appeared earlier) under the faster-growing species (pine) compared to the native slower-growing species (birch). Under birch, the effect of fertilisation on soil pH decreased over time; this could be explained by fallen birch leaves, which were easily incorporated into the soil. Birch leaves have a high proportion of calcium (Palviainen *et al*., 2004), which reduces the negative impact of soil calcium leaching and extraction caused by pasture and trees (Rigueiro-Rodríguez *et al*., 2009), thus allowing for better nutrient recycling than pine trees, whose needles do not incorporate into the soil easily.

As the tree cover of both established systems developed, a negative relationship was found between pasture production and tree canopy development. This negative relationship was greater in the case of the systems established under the *Pinus radiata* canopy as found by Mosquera-Losada *et al*. (2006), resulting in reduced plant diversity under pine, probably due to its faster growth, which caused earlier canopy closure (Ferris *et al*., 2000). *Pinus radiata* negative effect on pasture production and diversity is probably more important than that created by the native coniferous *Pinus pinaster* species, due to the faster growth rate and the denser aerial structure type of the *Pinus radiata* exotic species, which limits the amount of light reaching the understorey (Rozados *et al*., 2007). Conifer tree plantations also cause important understorey changes compared with native

Table II. List of species found during the period of study in plots established with pine and birch trees

Class.	Family	Specie	Cod.	Cycle	Pine 1995	Pine 2000	Pine 2005	Birch 1995	Birch 2000	Birch 2005
Di	Boraginaceae	*Lithodora prostata* Loisel	Li	P						x^{NF}
	Cruciferae	*Lepidium heterophyllum* Bentham	Lep	P					x^{M}	
		Raphanus raphanistrum L.	Rp	A	x^{3}			X^{DM}		x
	Caryophyllaceae	*Cerastium glomeratum* Thuill	Cer	A	x	x		x^{3}	x^{D}	x^{3}
		Illecebrum verticillatum L.	Il	A	x					
		Scleranthus annuus L.	Sc	A				x^{NF}		
		Silene gallica L.	Si	A	x^{3}			x^{3}		
		Spergula arvensis L.	Sp	A	x^{3}			x^{3}		
		Stellaria media L. (Vill)	St	A	x^{M}					
	Chenopodiaceae	*Chenopodium album* L.	Ch	A	X^{DM}	x		x^{3}	x	x
	Compositae	*Anthemis arvensis* L.	Aa	A	x^{DM}			x		
		Achillea millefolium L.	Ach	P		X^{DM}				
		Chamaemelum mixtum L.	Cham	A						x^{M}
		Coleostephus myconis (L.) Rchb.f	Col	A	x^{NF}					
		Crepis capillaris (L.) Wallr	Cr	A	x			x^{3}		
		Hieracium pilosella L.	H	P						x
		Leontodon saxatilis Lam	Le	P						x^{NF}
		Chamomilla recutita L.	Mat	A-B		x^{M}			x^{NF}	x^{3}
		Sonchus asper L. (Hill)	Sas	A	x^{NF}					
		Senecio jacobaea L.	Se	B-P		x^{3}	x^{M}		x^{3}	x^{3}
		Sonchus oleraceus L.	So	A		x^{3}			x^{3}	x^{DM}
		Taraxacum officinale Weber	Ta	P		x^{3}			x^{3}	x^{3}
Mon	Poaceae	*Agrostis capillaris* L.	Aca	P	x^{3}	x^{3}		x^{3}	x^{3}	x^{3}
		Dactylis glomerata L.	Dg	P	x^{3}	x^{3}	x^{3}	x^{3}	x^{3}	x^{3}
		Elymus repens L.	El	P			x^{NF}			
		Holcus lanatus L.	Hl	P	x^{3}	x^{3}		x^{3}	x^{3}	x^{3}
		Holcus mollis L.	Hm	P	x^{3}			x^{3}		x^{D}
		Lolium multiflorum Lam	Lm	A		x^{D}			x	
		Lolium perenne L.	Lp	P	x^{3}	x^{3}		x^{3}	x^{3}	x^{DM}
		Poa pratensis L.	Poa	P					x	
		Poa trivialis L.	Pt	P	x^{NF}			x^{NF}		
Di	Geraniaceae	*Erodium moschatum* (L.) L'Hér.	Er	A-B						x^{M}
		Geranium dissectum L.	Gd	A-B	x^{NF}			x^{M}		
		Geranium rotundifolium L.	Gr	A	x^{3}				x^{NF}	
	Labiatae	*Mentha suaveolens* Ehrh	Me	P						x^{D}
		Prunella vulgaris L.	Pr	P			x^{D}			x^{NF}
	Leguminosae	*Lupinus luteus* L.	Lpl	A	x			x^{NF}		
		Lotus corniculatus L.	Lt	P						x^{M}
		Medicago sativa L.	Ms	P		x^{NF}		x		
		Ornithopus compressus L.	Or	A	x			x		
		Trifolium campestre Schreber	Tc	A	x^{3}			x^{3}	x^{NF}	x^{M}
		Trifolium pratense L.	Tp	P		x^{NF}		x^{NF}	x	
		Trifolium repens L.	Tr	P	x^{3}	x^{3}		x^{3}	x	x^{NF}
		Vicia sativa L.	Vi	A-B	x^{NF}					
	Linaceae	*Linum bienne* Miller	Ln	A-B	x^{D}					
	Plantaginaceae	*Plantago lanceolata* L.	Pl	P	x	x^{3}		x	x^{3}	x^{D}
	Polygonaceae	*Polygonum aviculare* L.	Pya	A	x^{NF}					
		Polygonum hydropiper L.	Pyh	A	x^{3}			x^{3}		
		Rumex acetosa L.	Ra	P					x^{3}	
		Rumex acetosella L.	Rll	P	x^{3}	x		X^{DM}	x^{D}	x^{3}
		Rumex obtusifolius L.	Ro	P	x	x^{DM}		x^{M}	x	
	Portulacaceae	*Montia fontana* L.	Mon	P				x^{NF}		
	Ranunculaceae	*Ranunculus repens* L.	Rn	P						x^{M}
	Solanaceae	*Solanum nigrum* L.	Sn	A	x^{3}			x		
	Scrophulariaceae	*Rhinanthus minor* L.	Ri	A				x^{NF}		
		Veronica agrestis L.	Va	A						x^{M}
	Umbelliferae	*Daucus carota* L.	Dau	B	x^{3}	x^{3}		x^{M}	x^{3}	x^{3}
		Total species			32	19	4	29	22	27

Where: Class: Di = dicotyledonae, Mon = monocotyledonae; Cod: species code; Cycle: P = perennial species, A = annual species, B = biannual species; X^{DM}: species associated only with fertiliser treatments; X^{NF}: species associated only with plots that were not fertilised (NF), X^{3}: species that appear in both the fertilised and non-fertilised plots, X^{D}: species that are only associated with organic fertiliser, and X^{M}: species that are only associated with inorganic fertiliser.

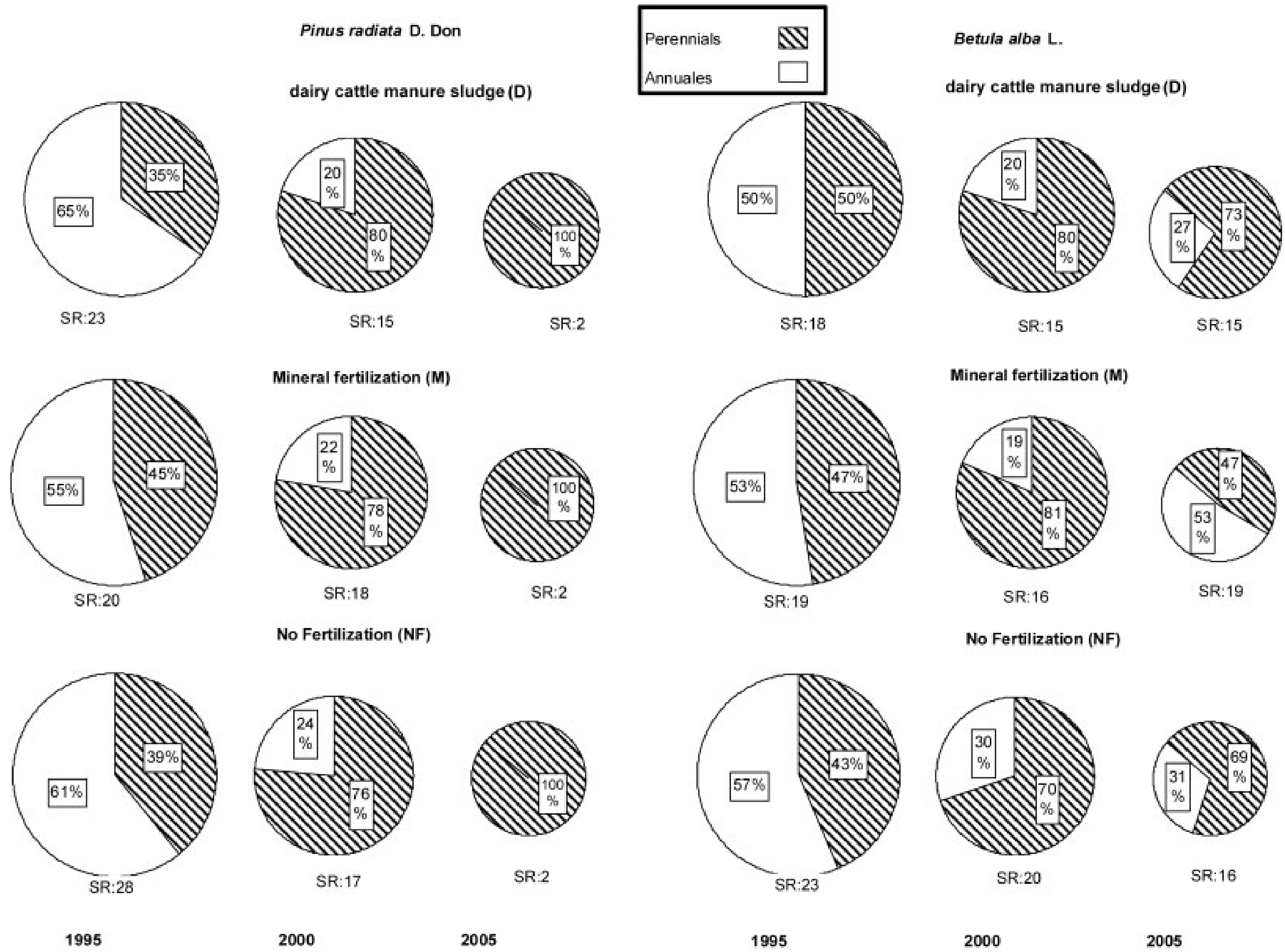

Figure 4. Variation in the percentage of annual and perennial species in the years 1995, 2000 and 2005 of the study in the systems established under the cover of pine and birch trees and for each application of fertiliser where SR = species richness.

broadleaved species due to their different canopy type, light interception and structure (Brockerhoff *et al*., 2001).

There was important variation in the understorey vegetation of the two established systems (conifers and leafy trees) during the 11-year study period, as can be seen with the types (annuals/perennials) and richness (number of species) of species. The factors that contributed to these differences in plant diversity are complex, but can be placed into two main groups: (1) anthropogenic factors (disturbances such as ground preparation, pasture mowing, thinning regime, clearing and fertiliser application) and (2) factors related to the overstorey (growth, canopy development, stand structure, etc.). Anthropogenic factors play an important role in structuring plant communities (Grime, 1982; Terradas, 2001; Augusto *et al*., 2002). In this study, the first disturbance was related to soil preparation in 1995 (ploughing and grading). This intervention favours the germination of annual species, mainly characterised by heliophytes and invaders such as *Anthemis arvensis* L., *Crepis capillaris* (L.) Wallr, *Lupinus luteus* L., *Ornithopus compressus* L., *Polygonum hydropiper* L., *Solanum nigrum* L., *Spergula arvensis* L. and *Silene gallica* L. (all of which were present in the two established systems), *Coleostephus myconis* (L.) Rchb. F., *Sonchus asper* L. (Hill), *Illecebrum verticillatum* L., *Polygonum aviculare* L., *Stellaria media* L. (Vill) (exclusively present in the plots replanted with pine trees), *Rhinanthus minor* L. and *Scleranthus annuus* L. (exclusively present in the plots afforested with birch trees) (Table I). Similar findings in areas with soil disturbance have been obtained by Dyrness (1973), who found that resurgence of residual herbaceous species can follow an initial period of dominance by invading species, or by Wallace *et al*. (1992) or Wallace and Good (1995) in conifer plantations, where ground flora communities were dominated by light-demanding species in the first few years. In the following years, the presence of annual species began to decrease in the understorey vegetation, especially in the systems established under the pine tree canopies (100 per cent of

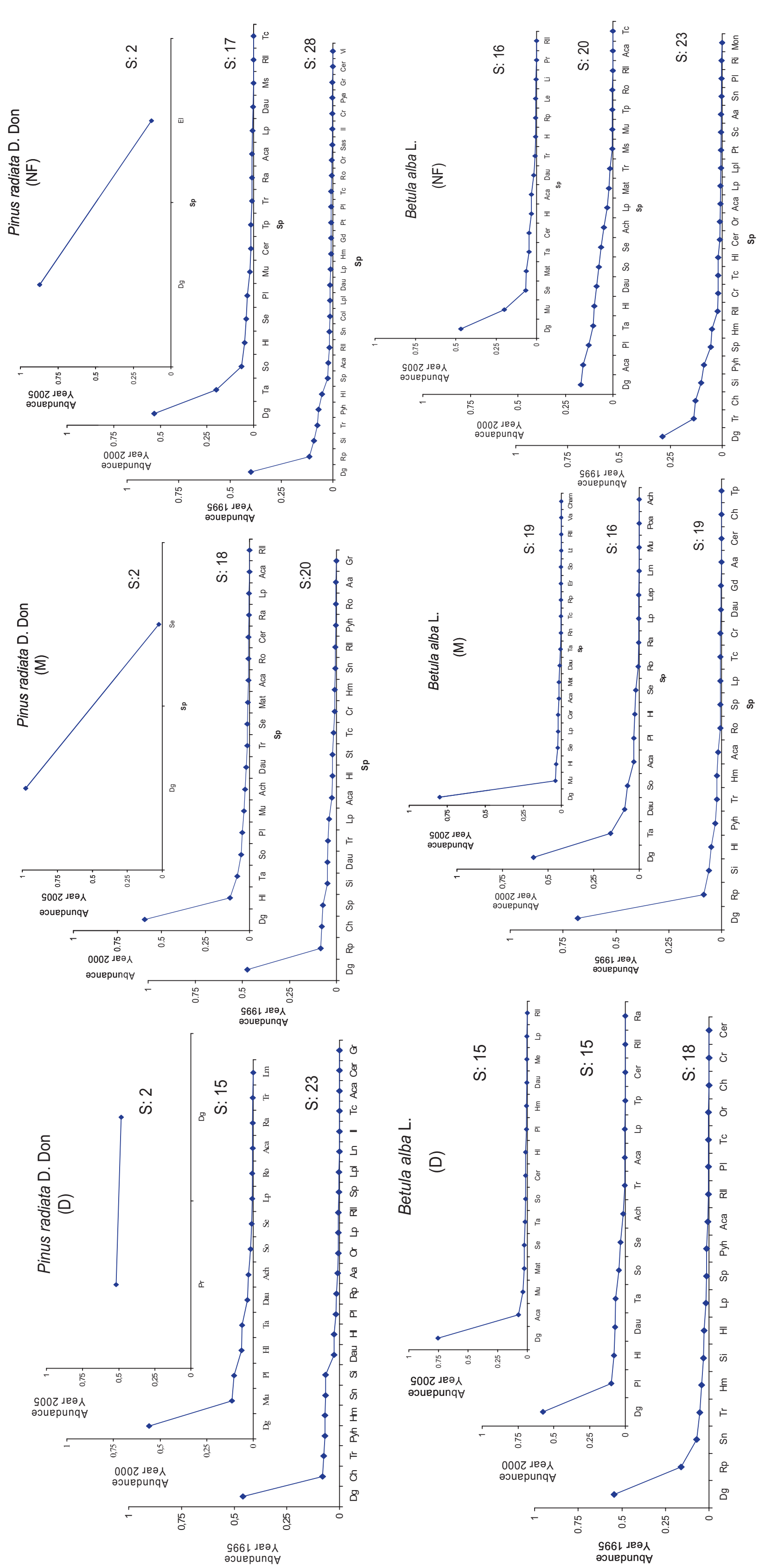

Figure 5. Abundance diagrams (relative proportion of each species based on dry weight) corresponding to the three years of study (1995, 2000, 2005) where: D = fertilised with sludge, M = mineral fertiliser, NF = not fertilised and S = species richness. Species codes are shown in Table II.

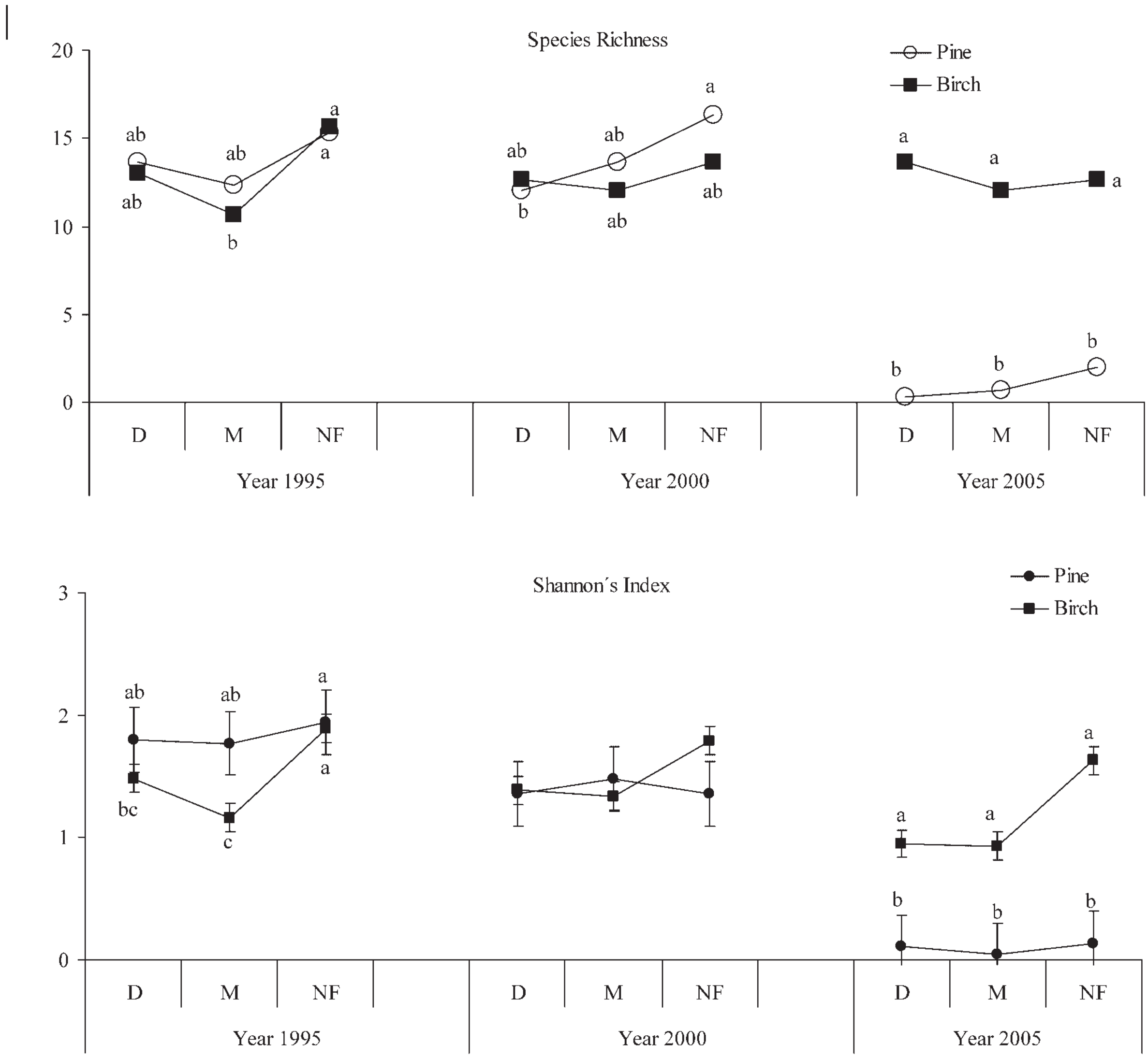

Figure 6. Species richness and Shannon–Wiener indexes determined for each of the fertiliser treatments applied under the two types of trees established and over the 3 years of study where: D: fertilised with dairy cattle manure sludge, M: mineral fertiliser and NF: not fertilised. Different letters indicate significant differences between treatments in the same year.

Table III. Jaccard (I_j) index determination where: (i) comparison between initial situation (1995) and final situation (2005) of the established plots, and (ii) determination for each year of the study. Index of Complementarity (C_{AB}) between the plots established under pine trees and birch trees in the three years of study

Beta diversity index								
Jaccard index					Index of complementarity (C_{AB})			
(i) Comparison between initial and final situation								
Species	a_{1995}	b_{2005}	C	I_j		Year 1995	Year 2000	Year 2005
Pine	32	4	1	0·03	a	32	19	4
Birch	29	27	13	0·30	b	29	22	27
(ii) Determined for each year of the study					c	24	17	3
Year	a_{pine}	b_{birch}	$c_{pine–birch}$	Ij	S_{AB}	37	24	28
1995	32	29	24	0·65	U_{AB}	13	7	25
2000	19	22	17	0·71	C_{AB}	0·35	0·29	0·89
2005	4	27	3	0·11				

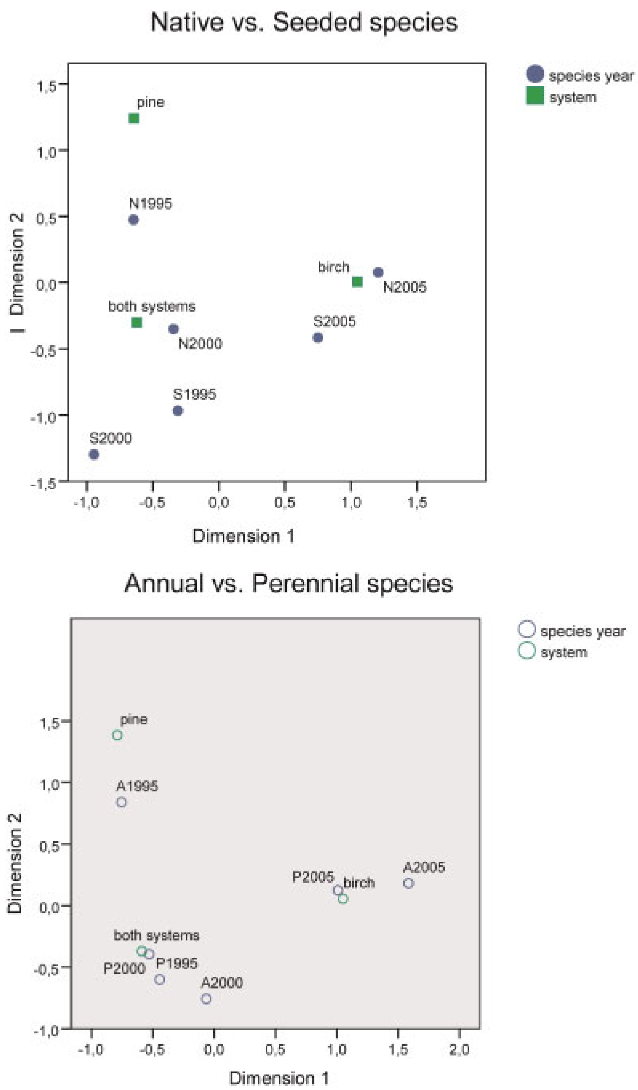

Figure 7. Correspondence analysis graph of categories related to the number of native (N) and seeded (S) species (above) and annual (A) and perennial (P) species (below) in years 1995, 2000 and 2005 present in each tree system (exclusively in pine, exclusively in birch and shared by both systems). This figure is available in colour online at wileyonlinelibrary.com

perennial species in 2005) compared to native birch canopies (between 47 and 73 per cent of perennial species). In the first case, the absence of annual species can be explained by the higher light interception of the conifer canopy and the presence of an important litter layer that prevented the root systems of these species from reaching the soil, as was described when conifer and broadleaved species were compared in the same area (Rozados *et al*., 2007). In the case of the birch tree, the presence of summer drought in the area favoured mortality for perennial species that were incorporated into the soil, leaving gaps that permitted the germination and rapid establishment of annual species, although their proportion was not as high as in the Mediterranean region (Mosquera-Losada *et al*., 2009).

Another anthropogenic factor that influenced variation in plant diversity was the management of the plantation with regard to the type of fertilisation (D or M) used in the system. With both types of fertilisers, there was a decrease in the plant α diversity expressed in the form of S, and the Shannon Index (H), with respect to the non-fertilised (NF) systems. The addition of fertiliser (NPK) and the use of pasture production through harvesting favoured the presence of sown species (Rodríguez *et al*., 1996; Mosquera and González, 2000; Rajaniemi, 2002), especially *Dactylis glomerata*. This Gramineae is characterised by strong growth in height, out-competing shorter species like Leguminae (clovers); these may even disappear from the system completely, thus resulting in an important decrease in the diversity and richness of species under these treatments (Mosquera-Losada *et al*., 2000; Rodríguez *et al*., 2001).

With regard to the factors related to the overstorey, numerous studies indicate that understorey vegetation is influenced by overstorey composition and structure through modifications of resource availability: light, water and soil nutrients and other effects such as the physical characteristics of the litter layer (Barbier *et al*., 2008). Light is commonly considered to be the major limiting factor of forest vegetation cover and/or richness (Barbier *et al*., 2008), and light penetration is affected by leaf size, specific leaf area, canopy form and deciduous leaf mass (Coomes and Grubb, 2000). In our region, the growth of the pine tree in terms of both height and diameter was greater than that of the native birch tree. This faster growth rate resulted in canopy closure 5 years after the beginning of the experiment (2000) in the systems established with pine trees. In our case, canopy closure caused a marked decrease in light in the pine tree plots and favoured the presence of litter, especially during the final year of the study (2005). In this situation, the seeds under the litter were deprived of light, and the seeds on top could not easily take root (Hamrick and Lee, 1987; Facelli and Pickett, 1991; Ellsworth *et al*., 2004). This caused a considerable decrease in the species richness and floristic diversity of the system. Species such as *Cerastium glomeratum* Thuill, *Chenopodium album* L., *Agrostis capillaris* L., *Holcus lanatus* L., *Lolium perenne* L., *Trifolium repens* L., *Plantago lanceolata* L., *Rumex acetosella* L. and *Daucus carota* L. (Table I) virtually disappeared from the understorey vegetation because these plant species were unable to tolerate the deep shade beneath *Pinus radiata*. With canopy closure, the microclimate beneath the tree cover changes, reducing the interior temperature of the system and increasing the interception of rainfall by the trees (Papanastasis, 2004; Moreno *et al*., 2005; Mosquera-Losada *et al*., 2005). In our case, these two situations were emphasised when the canopy consisted of perennial species

(*Pinus radiata*) and involved a decrease in the decomposition of organic material and its accumulation within the system (Marcos *et al*., 2007). As a consequence, greater acidification of the plots occurred, especially in those plots replanted with conifers (Barbier *et al*., 2008) in which the pH was already lower (Mosquera-Losada *et al*., 2006). Moreover, in our case, the results showed a lower acidification rate for those plots that received mineral fertiliser (M) exclusively as found by Mosquera-Losada *et al*. (2006). This could be explained by the lower growth rate of the tree cover and the decreased production of biomass, resulting in a reduced extraction of nutrients and an inferior acidification. This change in the pH of the systems could also have contributed to the reduction in α diversity (Schlenker, 1968). The area went from a high pH (6·8) maintained over time due to potato cropping to a drop of at least one pH point in only a few years. This modifies the availability of nutrients (Mosquera-Losada *et al*., 2006) and can affect the competitive relationships between the different pasture species, favouring the presence of gramineae over the dicotyledonae, which have a greater cation content (Pinto *et al*., 2001) and, therefore, a greater need for cation extraction. On the other hand, and due to the importance of growth rate for the forest species used in our area (Galicia has one of highest potential forest growth rates in Europe), rapid change was produced in an area with a long tradition of managing liming materials and in which the established seed banks were more in agreement with these more acid conditions. For this reason, the graminoide grassland species habitually found in the scrublands typical of the region (Mosquera-Losada *et al*., 2009) and in soils with a pH lower than 5·0 did not appear. Buscardo *et al*. (2008) also suggests that afforestation represents a threat to semi-natural habitats biodiversity like grasslands.

In numerous articles, the variation in diversity along temperature and topographical gradients has been studied, comparing arboreal forestlands established in different areas of the same region and even across a whole country (Brockway, 1998, Gachet *et al*., 2007). In our case, β diversity has been used with the objective of comparing effects on species composition that may be caused by the decision to establish one forest species or another. The results obtained in this study show important variation in the composition of species. As explained previously, the effects of the establishment of a dense plantation of a forest species such as *Pinus radiata* species on the ecosystem are important (decrease in light, variation in pH, accumulation of needles in the soil, etc.); these effects resulted in variation in the floristic composition and, therefore, distancing from the initial characteristics of the system when compared with birch trees. As indicated, in the case of systems established with conifers, only four species were able to adapt to the new changes in the ecosystem (*Dactylis glomerata*, *Senecio jacobea* L., *Elymus repens* L. and *Prunella vulgaris*), while the establishment of birch trees, even after 11 years, led to less important disturbances and allowed species, such as *Holcus lanatus, Holcus mollis* L., *Trifolium campestre* Schreber, *Trifolium repens, Rumex acetosella,* and *Daucus carota* (up to a total of 13 species), to continue to form part of the understorey vegetation. But, the modifications introduced by the tree cover also favoured the appearance of some herbaceous species exclusively under one or another type of tree. Thus, species such as *Illecebrum verticillatum*, *Stellaria media*, *Achillea millefolium* L., *Coleostephus myconis*, *Sonchus asper*, *Elymus repens* L., *Vicia sativa* L., *Linum bienne* Miller and *Polygonum aviculare* appear to be associated with pine trees. Conversely, *Lithodora prostata* Loisel, *Lepidium heterophyllum* Bentham, *Scleranthus annuus*, *Chamaemelum mixtum* L., *Hieracium pilosella* L., *Leontodon saxatilis* Lam, *Erodium moschatum* (L.) ĹHér., *Mentha suaveolens* Ehrh, *Lotus corniculatus* L., *Montia fontana* L., *Ranunculus repens* L., *Rhinanthus minor* L. and *Veronica agrestis* L. appeared only under birch trees.

In conclusion, the establishment of a silvopastoral system with two forest species that are very different in terms of growth and canopy development introduced important modifications over the mid- and short-term. In the case of the soil, both systems caused acidification, but this effect was more important in the systems established with *Pinus radiata*, specially when the fertilisation type (sludge or NF) enhanced tree growth. Pasture production was drastically reduced in both established systems, negatively affecting its use for livestock production and, therefore, the output that the owner could achieve over a mid-term time frame. Pasture production was positively affected by fertilisation when tree canopy development did not reduce pasture response to fertiliser inputs at the start of the experiment. The diversity of both the species (α) and the habitat (β) was notably reduced by the introduction of an conifer (*Pinus radiata*) compared to a native broadleaf species (*Betula alba*), being this effect more iportant in those fertilised treatments, which enhanced pine development (D and NF). This negative effect was obtained in a relatively short period of time in this study due to the high density of the established stand. Initial choice of forest species is highly relevant because a reduction in pasture production and important plant diversity losses can result in the short term. However, more long-term studies should be carried out in order to understand production and plant diversity responses to different canopies, with an emphasis on differences in tree types and density and fertilisation regimes in older stands.

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