Ann. For. Res. 57(2): 247-265, 2014
DOI: 10.15287/afr.2014.277

ANNALS OF FOREST RESEARCH
www.afrjournal.org

Long term evolution of tree growth, understorey vegetation and soil properties in a silvopastoral system of northern Greece

S.F. Gakis, M.Z. Orfanoudakis, A.G. Papaioannou, K.T. Mantzanas, V.P. Papanastasis, D.A. Alifragis, D.G. Seilopoulos, S.N. Kostakis

Gakis S.F., Orfanoudakis M.Z., Papaioannou A.G., Mantzanas K.T., Papanastasis V.P., Alifragis D.A., Seilopoulos D.G., Kostakis S.N. 2014. Long term evolution of tree growth, understorey vegetation and soil properties in a silvopastoral system of northern Greece. Ann. For. Res. 57(2): 247-265, 2014.

Abstract. Tree growth, understorey characteristics and soil parameters were evaluated in a silvopastoral system in northern Greece at seven and twelve years after its establishment. The experiment was laid out in 1992 and included three understorey treatments (grass, legume and control - unsown), two tree species - sycamore (*Acer pseudoplatanus* L.) and Scots pine (*Pinus sylvestris* L.) and two tree spacings (2.5 x 2.5 m and 3.5 x 3.5 m). Tree height was taller in the control and in the dense stands while Scots pine had better growth but, higher mortality than sycamore in the both periods. In 2004, understorey vegetation was uniform under the three understorey treatments and a significant decrease in sward above ground biomass was recorded under the dense treatment, compared with the open stand. In the same year, organic C and N, Mg and K concentrations as well as arbuscular mycorrhizal fungi spore numbers were found significantly higher under sycamore than Scots pine plots in the 0-20 cm soil depth. The results show that, although understorey vegetation became uniform over time, the initial plant composition of the understorey treatments continued to affect tree growth, indicating the importance of the understorey vegetation at the establishment stage of silvopastoral systems. However, as time passed initial tree spacing started to affect tree growth and tree species, particularly sycamore, to improve soil properties. These results indicate an evolution of the initial silvopastoral system to a forest.

Keywords agroforestry, tree height, herbage biomass, soil macronutrients, arbuscular mycorrhizal fungi.

Authors. Stergios F. Gakis (stergiosgakis@yahoo.gr) - Forest Directorate of Magnesia Prefecture, Regional Administration of Thessaly and Central Greece, 383 33 Volos, Greece; Michail Z. Orfanoudakis - Forest Soils Laboratory, Department of Forestry and Management of the Environment and Natural Resources, Democritus University of Thrace, 682 00 Orestiada, Greece; Athanasios G. Papaioannou, Dimitrios A. Alifragis, Dimosthenis G. Seilopoulos - Laboratory of Forest Soils, Faculty of Forestry and Natural Environment, Aristotle University, 540 06 Thessaloniki, Greece; Konstantinos T. Mantzanas, Vasilios P. Papanastasis - Laboratory of Rangeland Ecology, Faculty of Forestry and Natural Environment, Aristotle University, 540 06 Thessaloniki, Greece; Stamatis N. Kostakis, Geotechnical Chamber of Greece, 546 31 Thessaloniki, Greece.

Manuscript received September 09, 2014; revised November 10, 2014; accepted November 20, 2013; online first November 27, 2014.

Introduction

Agroforestry systems are a traditional land use practice in Greece. They are widely distributed throughout the country and constitute important elements of the rural and forested landscapes. The area covered by these systems is estimated to be more than 3 million hectares, or 23% of the country (Papanastasis et al. 2009). Silvopastoral systems involving trees and pasture/animals grown on forest and arable land are multiple use systems, well adapted to the Mediterranean environment. These systems include: open or dense warm Mediterranean pine forests, e.g. Aleppo pine (*Pinus halepensis* Mill.) and brutia pine (*Pinus brutia* Ten.), most of the mountainous pine forests, e.g. Austrian pine (*Pinus nigra* Arn.) and Scots pine (*Pinus sylvestris* L.), and the deciduous oak [*Quercus pubescens* Willd., *Q. frainetto* Ten., *Q. petraea* (Mattuschka) Liebl., *Q. cerris* L.] forests that traditionally support herbaceous or shrubby understorey vegetation, mostly used for grazing by livestock, timber and fuelwood production (Schultz et al. 1987, Papanastasis 1999, Pantera & Papanastasis 2001, Papanastasis 2001).

Tree growth in agroforestry systems depends upon various factors such as species, spacing, intercrops, site quality, climate and management practices. The variability in growth between tree species over a long period is largely a consequence of the species genetic potential for exploiting the available resources of the site (Feldhake & Schumann 2005), while differences in survival rates may be explained by the general tolerance of the different species to specific site conditions, such as soil moisture and nutrient status in different years (Moffat et al. 2008). In semi-dry environments, understorey vegetation affects the growth and survival of young trees due to the strong competition for water, at least during the first years of tree establishment (Koukoura et al. 1999, Platis et al. 1999, Chang et al. 2002). However, when trees mature and their crowns are fully developed, the tree canopy architecture often affects understorey vegetation characteristics, by producing different microclimatic conditions, which influences, for example, pasture biomass and its seasonal growth pattern (Mathew et al. 1992, Sibbald 1999, Silva-Pando et al. 2002).

Tree species can influence soil properties via several mechanisms. Differences in foliage and litter quality, root nutrient uptake and activity as well as alterations of microclimate and the soil biological community can result in differences in the physical and chemical characteristics of surface soils under various species (Hagen-Thorn et al. 2004). Also, specific agroforestry managements after several years may cause significant changes in soil chemical properties (Chang et al. 2002). Organic matter accumulation and nutrient return to the topsoil of agroforestry systems varies greatly, depending on factors that determine and control the quantity of plant residues and litter decomposition rate, such as plant species, age and density of the tree stand, chemical composition of the plant residues, climatic and soil conditions as well as microorganism activity (Szott et al. 1991, Rao et al. 1998).

To improve the efficiency of nutrient recycling in agroforestry systems, a better understanding of the soil biological processes is needed. Arbuscular mycorrhizal fungi (AMF) are known to enhance processes of nutrient uptake resulting in increased plant growth (Caravaca et al. 2005). AMF have also been found to control root diseases, improve resistance to drought conditions and contribute to the formation of soil structure (Smith & Read 2008). The role of AMF is well described in a wide range of growth controlled conditions. However, there is insufficient information in the literature concerning the ecology of AMF under natural conditions (Haselwandter & Bowen 1996, Smith & Smith 2011). Land use is known to affect mean AMF species richness and spore abundance (Strümer & Siqueira 2010), but the specific impact of temperate agroforestry systems on AMF is not well understood, as only a few studies have investigated AMF in these systems (Bainard et al. 2011). Generally, AMF are sensitive to changes in soil and plant conditions and their occurrence and persistence are affected by agroforestry combinations (Jefwa et al. 2006). Trees and crops often share the same AMF. Trees may act as reservoirs of mycorrhizal fungi for surrounding crops or other annual vegetation (Ingleby et al. 2007).

Tree-based intercropping systems occur throughout Europe and in the rest of the world and play an important role in the global C cycle. An understanding of the response of these systems to climate change does not only provide necessary information on likely changes to nutrient cycles, but also on feedback of perturbations to global C cycles (Pandey 2002, Palma et al. 2007, Mosquera-Losada et al. 2011, Dhillon & von Wuehlisch 2013). In 1992, a network of silvopastoral systems was established at five sites across Europe within the framework of an EU-funded project (Biogeochemical Cycling in Agriforestry Systems Network, BAFNET, STEP-CT90-0075, EV5V-CT93-0288), to investigate the nutrient cycling interactions of climate and soil type with trees, soil and understorey (Green et al. 1999). One of these sites was located in northern Greece. Sycamore (*Acer pseudoplatanus* L.) was planted in all sites at 800 and 1600 stems ha^{-1} into three different understoreys; perennial ryegrass (*Lolium perenne* L.), white clover (*Trifolium repens* L.) and ground maintained vegetation-free. A second tree species, Scots pine, was planted in the Greek site at the two densities with the same three understorey types. The inclusion of this species, whose southern natural distribution limits in Europe are located in Pieria Mountain, northern Greece (Dafis 2010), permitted comparisons of the relative mechanisms and processes important within broad-leaved and coniferous silvopastoral systems.

Early results from the first six years of the experiment of the Greek site have shown that tree growth was strongly influenced by the presence and type of the understorey species, while tree spacing did not produce any significant differences. Tree growth was found to be higher in vegetation free treatments in response to the greater availability of moisture and nutrients, but the influence of trees (species or spacing) on herbage biomass was not significant (Gakis 2000, Gakis et al. 2004).

The present study aimed at re-examining tree performance and the nutritional status of the soil, by carrying out two new inventories (in 1999 and 2004), in order to understand the tree growth mechanisms and the transfer processes between nutrient pools in the silvopastoral system. The hypothesis was that the interactions between trees, understorey vegetation and soil environment had become more complex as the trees matured. Specifically, we addressed the following questions: (1) How treatments at the establishment phase affected tree growth in the subsequent years? (2) Were trees having any effects on understorey vegetation? (3) Have there been any alterations in soil chemical properties between 1999 and 2004? and (4) Did soil chemical properties remain unaffected by silvopastoral manipulations? Furthermore, since little is known about AMF occurrence and diversity, as well as of the impact of silvopastoral systems on AMF communities, we also investigated AMF spore abundance and community composition.

Materials and methods

Much of the methodology adopted in this paper has also been described in an earlier publication (Gakis et al. 2004).

Study site

The experiment was established in 1992 in a parcel of former agricultural land (about 3.6 ha) within a beech (*Fagus sylvatica* L.) forest, located near the village of Archangelos, prefecture of Pella, in central Macedonia, northern Greece (latitude: 41° 13' N, longitude: 22° 26' E). The area has an uniform, fairly deep (up to 100 cm), sandy clay loam soil, derived from mica schist, with a pH of 6.0, a slope of 8% and is located at 800 m a.s.l. Climate is sub-humid Mediterranean, with a mean annual rainfall of 630 mm and a mean annual temperature of 11° C.

Experimental layout

Prior to the establishment of the experiment, the site was deeply ploughed and cultivated, to remove native herbaceous and woody vegetation, especially bracken fern (*Pteridium aquilinum* L.), which was occupying the majority of the area.

The experimental layout was a split-split plot design with three replications. Main plots (50 x 54 m) were three understorey treatments (grass, legume and control), sub-plots (50 x 25 m) were two tree species (sycamore and Scots pine) and sub-subplots (25 x 25 m) were two tree spacings (dense - 2.5 x 2.5 m or 1600 trees ha^{-1} and open - 3.5 x 3.5 m or 800 trees ha^{-1}). In each main plot, a strip (4 x 50 m) which was not planted with trees was left between the two sub-plots. Around the main plots a 5 m wide buffer zone was left to separate them from each other.

Trees were planted in the autumn of 1991 and herbaceous species were sown in early spring 1992. The grass treatment involved sowing of perennial ryegrass and the legume treatment sowing of white clover, both at a rate of 20 kg ha^{-1}, without fertilization. Two year-old seedlings of both tree species were used at planting time. In the autumn of 1992, dead tree seedlings were replaced with new ones of the same age.

Grass plots were sprayed with the selective herbicide Brominal H (bromoxynil) at a rate of 2 dm^3 ha^{-1} to control the broad-leaved weeds; and the legume plots with 2,4 DB and Fusilade (fluazifop) at a rate of 0.8 dm^3 ha^{-1} each to control thistles and the grass weeds respectively. Control plots were kept weed-free by spraying with the general herbicide Roundup (glyphosate) at a rate of 3 dm^3 ha^{-1}. Glyphosate was also applied to kill any herbaceous plants in an area of 0.5 m radius around each planted tree seedling in all plots. Herbicides were applied in the grass and legume treatments only once, in late spring of 1992, when weeds had developed the first 2-3 leaves. In the control treatment, sprayings were repeated once a year until 1995. In the spring of 1995, sowing in the treatments with herbaceous vegetation was repeated because the plants sown in 1992 had declined significantly. Twenty kilos of grass or clover seed per hectare were applied. The only difference from the first sowing was that perennial ryegrass was replaced by a mixture of tall fescue (*Festuca arundinacea* Schreb.) and cocksfoot (*Dactylis glomerata* L.). Re-sown species gradually declined, too. As a consequence, spontaneous plant species invaded and dominated both the grass and legume treatments. Indigenous weeds also invaded the control treatment after the end of sprayings in 1995. The whole experimental area was fenced out to prevent grazing from livestock.

Field measurements and laboratory analyses

Tree growth. In December 1999 and June 2004, 20 trees in the center of each sub-subplot were measured for height and stem diameter. Height was measured using a telescopic height stick. Diameter was measured using a caliper at the base of the trunk. Survival was calculated from the trees alive at the time of measurement on each group of the core 20 trees.

Understorey vegetation. In June 2004, plant cover and species composition of the understorey vegetation were assessed with the point method (Cook & Stubbendieck 1986), by taking two transects along the diagonals of each sub-subplot. In addition above ground biomass of understorey vegetation was estimated by cutting three randomly located quadrates (0.50 x 0.50 m each) within each sub-subplot. This sampling date was selected because the maximum pasture production usually occurs in early summer at the study area, when temperatures and radiation levels are the highest and precipitation is close to the moderate levels (Gakis 2000, Silva-Panto et al. 2002, Gakis et al. 2004). All samples were weighed in the laboratory after oven-drying at 65° C for 48 hours. A representative sample was ground and analyzed for nutrient content. N was determined by the Kjeldahl method, while Ca, Mg and K were determined by atomic absorption spectroscopy (Alifragis & Papamichos 1995).

Soil pH, organic C and macronutrients. Soil samplings were conducted in April 1999 and June 2004. Soil was sampled in each sub-subplot from two depths, 0–20 and 20–40 cm, using a tube auger. Three randomly selected sub-samples were collected from each sub-subplot and depth and were combined to a composite sample. After drying and sieving the samples, the following determinations were made: organic C by the wet digestion method (Nelson & Sommers 1982), N by the Kjeldahl method (Stevenson 1982), extractable P by the Olsen method (Olsen & Sommers 1982), exchangeable Ca, Mg and K by the CH_3COONH_4 – pH 7 method (Grant 1982). pH determination was conducted electrometrically in a 1:1 water-sample suspension (McLean 1982).

AMF analysis. Additionally, in June 2004, the same soil sampling method was used to collect extra samples, in order to isolate AMF spores. Soil samples were treated as described on Błaszkowski et al. (2006), with 50 spores mounted in water, lactic acid, polyvinyl alcohol/lactic acid/glycerol and a mixture of PVLG and Melzer's reagent (1:1, v/v).

Spores at all developmental stages were crushed to varying degrees by applying pressure to the cover slip and then stored at 65° C for 24 h to clear their contents from oil droplets. AMF were identified according to original descriptions (http://www.lrz.de/~schuessler/amphylo/amphylo_home.html) and descriptions and illustrations presented in Morton & Redecker (2001) and Błaszkowski et al. (2003).

Glomeromycota DNA sequences from ITS and LSU regions of ribosomal DNA (rDNA) were aligned using CLUSTALW2 (Larkin et al. 2007) and regions of high variability with interspecific, but not intraspecific variability, were chosen for the design of taxon-specific primers.

DNA was extracted from spores and root samples using the NucleoSpin Plant II extraction kit (Macherey-Nagel). For this, 10 spores of each AMF isolate or 0.1 g of roots were extracted using PL2 buffer (containing SDS) following instructions provided with the kit. DNA samples were kept at 20°C until analysis.

Nested PCR was used to amplify rDNA from AMF in a region covering part of the ITS region and 5' end of the LSU region. The primers ITS3 (White et al. 1990) and FLR2 (Trouvelot et al. 1999) were used for the first amplification of fungal rDNA sequences. The PCR protocol used was as described by Gollotte et al (2004) and White et al (1990). The PCR products were run on a 1.4% agarose gel in TAE buffer and visualized after ethidium bromide stain, under UV light. Presence/absence of bands was recorded for each sample and primer pair combination.

Statistical analysis

Statistical analysis for understorey and soil (separately for each soil layer) data was performed using ANOVA for the appropriate split-split plot design. Tree data were analyzed by ANOVA separately for each tree species. Percentage cover data of understorey plants were transformed to arcsine values prior to their analysis to meet the normality criterion. Comparisons among means were done with the Duncan's multiple range test. Calculations were performed using the programs MSTAT-C ver. 1.2 and JMPIN ver. 9.0.2.

Results

Tree growth

Tree height, stem diameter and survival are shown in Table 1. For tree height, there was a significant effect of the understorey in both years, with an exception concerning Scots pine in 1999. Specifically, the control treatment produced taller trees than the other two (grass and legume). Tree spacing produced significant results only for Scots pine in 1999, with the dense spacing resulting in taller trees than the open one, although in both species and years there was a similar increasing trend ($p < 0.1$). For stem diameter, the understorey produced significant results in sycamore in both years, again with the control resulting in greater stem diameter than the other two treatments. A significant effect of spacing was observed in 2004 for Scots pine stem diameter when trees in the open treatments were found with greater diameter than those in the dense ones.

Although mean tree height (average of all treatments) of both species was almost the same in 1999, in 2004, Scots pine trees averaged more than one and a half as tall as sycamore. Stem diameter of Scots pine in 2004 was 157% greater than sycamore, while in 1999 that difference was 102%. Scots pine grew

Table 1 Means (and standard errors) of the main effects of the treatments (understorey and tree spacing) on sycamore (*Acer pseudoplatanus* L.) and Scots pine (*Pinus sylvestris* L.) height, stem diameter and survival in the years 1999 and 2004. [Understorey: Control, Grass, Legume; Tree spacing: Dense = 2.5 x 2.5 m, Open = 3.5 x 3.5 m]

	Height (m)				Stem diameter (cm)				Survival (%)			
	Sycamore		Scots pine		Sycamore		Scots pine		Sycamore		Scots pine	
Treatments	1999	2004	1999	2004	1999	2004	1999	2004	1999	2004	1999	2004
Control	4.60^{a} (0.27)	5.57^{a} (0.54)	3.58 (0.17)	6.86^{a} (0.11)	6.4^{a} (0.6)	7.8^{a} (1.1)	9.3 (0.4)	13.7 (0.6)	98.3^{a} (1.1)	98.3 (1.1)	87.5^{a} (5.1)	87.5^{a} (5.1)
Grass	1.91^{b} (0.15)	2.48^{b} (0.32)	3.40 (0.11)	6.43^{ab} (0.12)	2.4^{b} (0.2)	3.1^{b} (0.4)	8.3 (0.2)	13.1 (0.3)	86.7^{b} (3.1)	85.8 (4.0)	73.3^{ab} (4.0)	70.8^{b} (5.1)
Legume	2.83^{b} (0.23)	3.75^{ab} (0.56)	2.86 (0.05)	6.02^{b} (0.09)	3.3 (0.3^{b})	4.5^{ab} (0.7)	7.3 (0.1)	12.3 (0.2)	82.5^{b} (3.1)	77.5 (4.0)	57.5^{b} (10.2)	54.2^{c} (9.4)
	**	*	ns	*	*	*	ns	ns	**	ns	*	**
Dense	3.33 (0.39)	4.32 (0.50)	3.36 (0.14)	6.55 (0.16)	4.2 (0.6)	5.3 (0.7)	8.2 (0.3)	12.7 (0.3)	91.7 (2.5)	90.0 (2.9)	74.4 (5.9)	70.6 (6.5)
Open	2.90 (0.46)	3.54 (0.65)	3.20 (0.14)	6.32 (0.12)	3.9 (0.8)	4.9 (1.1)	8.4 (0.4)	13.4 (0.4)	86.7 (3.4)	84.4 (4.7)	71.1 (7.9)	71.1 (7.9)
Interactions	ns	ns	*	ns	ns	ns	ns	*	ns	ns	ns	ns
	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note. ^{a} Means of the same parameter and in the same year within each understorey treatment marked with different letters are significantly different (according to Duncan's test). ns indicates no significant difference, while * and ** significance at $P < 0.05$ and $P < 0.01$, respectively. The data in paranthesis are the standard errors.

faster than sycamore from 1999 to 2004. Scots pine trees in 2004 were 96% taller and they had 58% greater stem diameter than in 1999 while sycamore was 26% taller and had 24% greater stem diameter respectively.

For survival, understorey treatments produced significant results for Scots pine in both years and for sycamore in 1999 with the control having the highest survival compared to the other two. For sycamore, the difference was apparent ($p = 0.052$) in 2004. Tree spacing did not have any significant effects. Sycamore tree survival was greater than in Scots pine trees. Sycamore had the highest mean survival rate, at 89.2% and 87.2% in 1999 and 2004, respectively and Scots pine the lowest, at 72.8% and 70.9%. Survival was highest for both species in the control treatment, where no tree losses were observed between 1999 and 2004 and lowest in the legume treatment. Particularly in the legume treatment, almost half of the Scots pine trees planted at the beginning of the experiment survived 12 years later. Greater tree losses between 1999 and 2004 were counted for sycamore in the legume treatments (6%) and for Scots pine in the dense treatments (5%). Finally, interactions were not significant.

Understorey vegetation

Cover, above ground biomass and nutrient (N, Ca, Mg and K) concentrations of the understorey vegetation in 2004 are presented in Table 2. Mean cover was about 27%, 47%, 18% and 8% respectively for bracken fern, herbaceous species, woody species and bare soil. Grasses were the dominant group among the herbaceous species. There were no significant effects of the understorey treatments on the various cover components including bare soil. Grass cover was significantly higher under sycamore than Scots pine, while woody species cover and bare soil were significantly higher under Scots pine. Finally, tree spacing produced almost significant differences ($p = 0.054$) on bare soil, suggesting that the dense plots did not favour the establishment of understorey vegetation compared with the open plots. As far as the interactions are concerned, they were statistically significant only between tree species and spacing for the bare soil cover.

For the pasture biomass, understorey and tree species did not produce significant effects, but open spacing had significantly higher biomass than the dense one. Also, no significant interactions were found between any of the treatments employed. For the nutrient concentrations of the herbage, finally, tree species affected both N and K concentrations, with the amounts under Scots pine being significantly higher than under sycamore. The understorey vegetation and tree spacing did not significantly affect nutrient concentrations.

Soil properties

Soil pH, organic C and macronutrient (N, P-Olsen, Ca, Mg and K) concentrations are presented in Table 3 for the upper soil layer (0-20 cm) and in Table 4 for the lower soil layer (20-40 cm). Silvopastoral treatments had no significant effects on soil pH at either depth. Soil pH decreased in all treatments at both depths; from an initial mean value of 6.11 in the upper soil layer in 1999 it became 5.78 in 2004 and in the lower soil layer from 6.02 to 5.62 respectively.

Understorey and tree density had no significant effects on organic C and soil N. Tree species however produced significant differences, but only in the upper soil layer and in 2004, where organic C concentration was 14% and soil N 15% higher in sycamore than in Scots pine plots. Also, no significant interactions were found between treatments in both nutrients. Soil C was decreased between 1999 and 2004, especially in the upper soil layer (from a mean value of 3.42 to 2.96%). On the contrary, soil N tended to increase between the years, especially in the lower soil level (from 0.19 to 0.29%). Concerning P-Olsen concentration,

Table 2 Means (and standard errors) of the main effects of the treatments (understorey, tree species and tree spacing) on understorey vegetation cover as well as on above ground biomass and on N, Ca, Mg and K concentrations in June 2004. [Understorey (1): Control, Grass, Legume; Tree species (2): Sycamore = *Acer pseudoplatanus* L., Scots pine = *Pinus sylvestris* L.; Tree spacing (3): Dense = 2.5 x 2.5 m, Open = 3.5 x 3.5 m]

	Cover (%)						Biomass	N	Ca	Mg	K
Treatments	Bracken fern	Grasses	Legumes	Forbs	Woody species	Bare soil	(kg ha^{-1} dry matter)	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})
Control	36.17(5.46)	14.92(4.59)	6.17(3.48)	13.00(2.38)	21.08(4.56)	8.67(1.94)	2460.37(306.24)	17.44(0.83)	7.05(0.92)	2.01(0.11)	21.60(1.16)
Grass	26.42(5.60)	21.58(6.31)	11.67(3.76)	21.83(5.09)	13.08(3.48)	5.42(1.94)	2927.54(224.43)	15.32(0.55)	6.16(0.89)	1.94(0.12)	19.34(0.76)
Legume	19.33(5.52)	33.33(4.29)	5.50(1.58)	13.92(2.77)	18.42(2.23)	9.50(1.50)	3042.24(234.71)	16.58(1.29)	5.88(0.66)	1.80(0.04)	15.95(1.13)
	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Sycamore	23.78(4.31)	28.61(4.58)	10.67(3.29)	17.72(2.95)	12.94(2.13)	6.28(0.94)	2911.65(179.62)	15.69(0.79)	6.30(0.77)	1.81(0.06)	17.78(0.71)
Scots pine	30.83(5.01)	17.94(4.08)	4.89(1.23)	14.78(3.14)	22.11(3.26)	9.44(1.84)	2708.46(244.78)	17.21(0.74)	6.43(0.57)	2.02(0.09)	20.15(1.16)
	ns	**	ns	ns	**	ns	ns	*	ns	ns	*
Dense	31.56(4.90)	20.33(4.35)	7.17(1.39)	13.28(2.01)	17.56(2.62)	10.11(1.66)	2346.31(188.73)	17.16(0.91)	6.33(0.74)	2.02(0.09)	19.45(1.19)
Open	23.06(4.36)	26.22(4.60)	8.39(2.27)	19.22(3.70)	17.50(3.28)	5.61(1.10)	3273.79(180.21)	15.73(0.59)	6.40(0.61)	1.81(0.06)	18.21(0.75)
	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns
Interactions											
(1)x(2)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*
(1)x(3)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
(2)x(3)	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
(1)x(2)x(3)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note. Abbreviations: ns - no significant difference, * and ** indicate significance at $P < 0.05$ and $P < 0.01$, respectively. The data in paranthesis represent the standard error.

Table 3 Means (and standard errors) of the main effects of the treatments (understorey, tree species and tree spacing) on soil properties (0-20 cm) in the years 1999 and 2004. [Understorey (1): Control, Grass, Legume; Tree species (2): Sycamore = *Acer pseudoplatanus* L., Scots pine = *Pinus sylvestris* L.; Tree spacing (3): Dense = 2.5 x 2.5 m, Open = 3.5 x 3.5 m]

	pH		C (%)		N (%)		P (mg100 g^{-1})		Ca (cmol$_c$ kg^{-1})		Mg (cmol$_c$ kg^{-1})		K (cmol$_c$ kg^{-1})	
Treatments	1999	2004	1999	2004	1999	2004	1999	2004	1999	2004	1999	2004	1999	2004
Control	6.10	5.70	3.59	3.06	0.26	0.29	1.00	2.00	8.69	9.90	1.44	1.29	1.00	0.65
	(0.07)	(0.10)	(0.12)	(0.19)	(0.01)	(0.02)	(0.06)	(0.07)	(0.53)	(1.08	(0.12)	(0.08)	(0.07)	(0.07)
Grass	6.13	5.84	3.24	3.18	0.26	0.28	1.02	2.01	9.28	11.16	1.44	1.32	0.98	0.68
	(0.06)	(0.07)	(0.23)	(0.27)	(0.02)	(0.02)	(0.05)	(0.13)	(0.76)	(1.11	(0.13)	(0.07)	(0.09)	(0.05)
Legume	6.10	5.79	3.43	2.65	0.26	0.31	0.98	1.88	8.62	11.62	1.24	1.21	0.85	0.60
	(0.11)	(0.13)	(0.22)	(0.16)	(0.02)	(0.02)	(0.07)	(0.08)	(1.05)	(1.05	(0.14)	(0.05)	(0.11)	(0.08)
	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Sycamore	6.15	5.84	3.45	3.15	0.26	0.31	1.02	1.85	9.06	11.61	1.36	1.36	0.96	0.71
	(0.06)	(0.08)	(0.17)	(0.15)	(0.01)	(0.02)	(0.05)	(0.08)	(0.69)	(0.87	(0.07)	(0.05)	(0.06)	(0.05)
Scots pine	6.07	5.71	3.38	2.77	0.26	0.27	0.98	2.07	8.67	10.18	1.39	1.19	0.93	0.57
	(0.06)	(0.09)	(0.16)	(0.20)	(0.01)	(0.01)	(0.04)	(0.07)	(0.61)	(0.86	(0.13)	(0.05)	(0.09)	(0.06)
	ns	ns	ns	*	ns	*	ns	ns	ns	ns	ns	**	ns	*
Dense	6.16	5.71	3.42	2.81	0.26	0.29	1.00	1.88	9.15	10.51	1.38	1.23	0.94	0.62
	(0.06)	(0.08)	(0.15)	(0.18)	(0.01)	(0.01)	(0.04)	(0.08)	(0.59)	(0.91	(0.10)	(0.06)	(0.08)	(0.06)
Open	6.06	5.84	3.42	3.11	0.26	0.30	1.00	2.04	8.58	11.28	1.37	1.31	0.94	0.66
	(0.07)	(0.09)	(0.17)	(0.18)	(0.01)	(0.02)	(0.05)	(0.08)	(0.70)	(0.84	(0.11)	(0.05)	(0.07)	(0.05)
	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Interactions														
(1)x(2)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
(1)x(3)	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
(2)x(3)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
(1)x(2)x(3)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note. Abbreviations: ns - no significant difference, * and ** indicate significance at $P < 0.05$ and $P < 0.01$, respectively. The data in paranthesis represent the standard error.

only tree spacing produced significant results in 2004, when open had higher concentration than dense plots in the lower soil layer. Finally, no significant first order interactions were also found at this level, too (Table 4). A considerable increase of P-Olsen concentration was found in all treatments and both depths between years. In 2004, P-Olsen concentration in the upper soil layer averaged almost two times more than the one in 1999 (1.96 to 1.00 mg 100 g^{-1} respectively) and in the lower soil layer almost two times and a half (1.92 to 0.76 mg 100 g^{-1} respectively). There was also a substantial increase of the Ca concentration by 22% in the upper and by 29% in the lower soil level between 1999 and 2004. The silvopastoral treatments though did not produce any significant effects. Mg concentration, on the other hand, changed slightly between the two time periods and tree species produced significant effects in the upper soil layer in 2004 with sycamore exceeding Scots pine. On the contrary, K concentration was decreased considerably by 32% in the upper and by 25% in the lower soil layer between 1999 and 2004. In addition, tree species produced a significant effect in the

Table 4 Means (and standard errors) of the main effects of the treatments (understorey, tree species and tree spacing) on soil properties (20-40 cm) in the years 1999 and 2004. [Understorey (1): Control, Grass, Legume; Tree species (2): Sycamore = *Acer pseudoplatanus* L., Scots pine = *Pinus sylvestris* L.; Tree spacing (3): Dense = 2.5 x 2.5 m, Open = 3.5 x 3.5 m]

	pH		C (%)		N (%)		P (mg100g^{-1})		Ca (cmol$_c$ kg^{-1})		Mg (cmol$_c$ kg^{-1})		K (cmol$_c$ kg^{-1})	
Treatments	1999	2004	1999	2004	1999	2004	1999	2004	1999	2004	1999	2004	1999	2004
Control	5.98 (0.07)	5.51 (0.10)	2.79 (0.16)	2.44 (0.21)	0.19 (0.02	0.24 (0.01)	0.70 (0.03)	1.95 (0.05)	7.48 (0.54)	9.53 (1.17)	1.11 (0.06)	1.17 (0.07)	0.75 (0.07)	0.51 (0.06)
Grass	6.07 (0.09)	5.62 (0.13)	2.17 (0.18)	2.26 (0.20)	0.18 (0.01	0.30 (0.04)	0.75 (0.04)	1.98 (0.11)	7.39 (0.73)	9.53 (1.28)	0.86 (0.07)	1.00 (0.05)	0.61 (0.08)	0.47 (0.04)
Legume	6.00 (0.12)	5.74 (0.17)	2.53 (0.22)	2.16 (0.25)	0.19 (0.05	0.28 (0.03)	0.82 (0.03)	1.82 (0.07)	8.26 (1.00)	10.68 (1.33)	0.91 (0.08)	0.99 (0.07)	0.54 (0.07)	0.45 (0.07)
	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Sycamore	6.02 (0.07)	5.62 (0.10)	2.55 (0.17)	2.37 (0.16)	0.19 (0.01	0.29 (0.03)	0.72 (0.03)	1.88 (0.08)	7.62 (0.63)	10.69 (1.17)	0.97 (0.06)	1.05 (0.06)	0.62 (0.05)	0.51 (0.04)
Scots pine	6.01 (0.08)	5.63 (0.12)	2.44 (0.16)	2.21 (0.20)	0.18 (0.01	0.26 (0.02)	0.79 (0.03)	1.95 (0.05)	7.80 (0.64)	9.14 (0.81)	0.96 (0.07)	1.06 (0.05)	0.65 (0.07)	0.45 (0.05)
	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Dense	6.05 (0.08)	5.57 (0.12)	2.52 (0.18)	2.09 (0.20)	0.19 (0.01	0.27 (0.02)	0.78 (0.03)	1.82 (0.08)	7.98 (0.70)	9.94 (1.05)	0.98 (0.07)	1.03 (0.06)	0.62 (0.07)	0.49 (0.05)
Open	5.98 (0.07)	5.67 (0.10)	2.48 (0.16)	2.48 (0.15)	0.18 (0.01	0.28 (0.03)	0.73 (0.03)	2.01 (0.05)	7.44 (0.56)	9.89 (1.00)	0.95 (0.05)	1.08 (0.05)	0.65 (0.06)	0.46 (0.04)
	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns
Interactions														
(1)x(2)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
(1)x(3)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
(2)x(3)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
(1)x(2)x(3)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns

Note. Abbreviations: ns - no significant difference, *, indicate significance at $P < 0.05$. The data in paranthesis represent the standard error.

upper soil layer in 2004 with sycamore having greater K concentration than Scots pine plots. However, almost all of the interactions among the various treatments were not statistically significant.

Table 5 shows the treatment effects on mycorrhizas. It is clear that they did not produce any significant results in both soil depths except tree species in the upper depth, where total AMF spore number was significantly greater under sycamore than under the Scots pine plots. However, total AMF spore number was highest in the 20-40 cm soil depth in all treatments. Spores were found to belong to four genera: *Glomus*, *Gigaspora*, *Scutelospora* and *Acaulospora*. *Glomus* dominated in both depths (84.4 and 73.3% for the upper and the lower soil layer respectively), followed by *Gigaspora* (11.7 and 16.3%), *Scutelospora* (8.5 and 8.6%) and *Acaulospora* (0.3 and 1.1%). A small proportion of unidentified spores were also encountered (0.2 and 0.7%). As in total AMF, understorey did not significantly affect any of the four genera groups. However, tree

Table 5 Means (and standard errors) of the main effects of the treatments (understorey, tree species and tree spacing) on AMF abundance (spores/200g) in two soil depths in June 2004. [Understorey (1): Control, Grass, Legume; Tree species (2): Sycamore = *Acer pseudoplatanus* L., Scots pine = *Pinus sylvestris* L.; Tree spacing (3): Dense = 2.5 x 2.5 m, Open = 3.5 x 3.5 m]

Treatments	Total AMF		*Glomus*		*Gigaspora*		*Scutelospora*		*Acaulospora*		Other	
	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm
Control	50.33 (17.18)	139.67 (16.31)	39.08 (13.64)	109.67 (12.48)	6.50 (2.60)	18.67 (6.12)	4.50 (2.11)	9.17 (2.61)	0.17 (0.17)	1.08 (0.50)	0.08 (0.08)	1.08 (0.56)
Grass	48.75 (16.74)	179.67 (28.55)	37.67 (12.91)	112.92 (14.38)	5.75 (2.47)	36.00 (8.04)	4.92 (2.25)	26.33 (13.59)	0.25 (0.13)	2.83 (0.92)	0.17 (0.11)	1.58 (0.81)
Legume	77.08 (17.74)	129.25 (10.65)	62.92 (13.85)	106.17 (8.36)	8.42 (2.97)	18.42 (3.33)	5.50 (2.92)	3.25 (0.92)	0.08 (0.08)	1.00 (0.39)	0.17 (0.17)	0.42 (0.23)
	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Sycamore	104.28 (12.23)	128.89 (9.85)	81.06 (10.06)	94.67 (6.62)	13.06 (2.22)	24.67 (4.03)	9.89 (2.23)	7.33 (1.66)	0.28 (0.14)	1.44 (0.43)	0.00 (0.00)	0.78 (0.28)
Scots pine	13.17 (3.18)	170.17 (20.59)	12.06 (3.06)	124.50 (10.84)	0.72 (0.33)	24.06 (6.37)	0.06 (0.06)	18.50 (9.37)	0.06 (0.06)	1.83 (0.66)	0.28 (0.14)	1.28 (0.62)
	***	ns	**	ns	***	ns	**	ns	ns	ns	ns	ns
Dense	66.83 (14.80)	148.72 (17.53)	54.44 (12.58)	110.33 (8.44)	8.22 (2.34)	26.56 (5.99)	3.89 (1.18)	9.22 (4.21)	0.11 (0.08)	1.94 (0.53)	0.17 (0.12)	0.67 (0.27)
Open	50.61 (13.30)	150.33 (16.23)	38.67 (9.22)	108.83 (10.78)	5.56 (1.96)	22.17 (4.51)	6.06 (2.51)	16.61 (8.66)	0.22 (0.13)	1.33 (0.58)	0.11 (0.08)	1.39 (0.62)
	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns
Interactions												
(1)x(2)	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	b=ns
(1)x(3)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
(2)x(3)	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns
(1)x(2)x(3)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note. Abbreviations: ns - no significant difference, * , ** and *** indicate significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. The data in paranthesis represent the standard error.

species produced significant effects in *Glomus* as well as in *Gigaspora* and *Scutelospora* only in the upper depth with sycamore having more spore numbers than Scots pine plots. Tree spacing affected significantly only *Glomus* in the 0-20 cm soil depth with dense plots exceeding the open ones. Again, spore numbers were higher in the lower than in the upper depth in most treatments of the four genera. Finally, the majority of interactions were not statistically significant.

Discussion

Tree growth

The significant differentiation among understorey treatments, concerning the height and the stem diameter of sycamore trees, had already emerged after the second year of establishment (in 1994 for height and in 1993 for stem diameter) where in the control plots the increased water and possibly the increased nutrient availability resulted in better tree growth

(Gakis 2000, Gakis et al. 2004).

For Scots pine, the presence of competitive understorey vegetation of white clover at the early stages of establishment has resulted in limited tree growth due to the competition for water (Gakis 2000, Gakis et al. 2004). The trees in the control and the grass plots were significantly taller than those in the legume plots in 1994 and 1996 while the grass plots produced statistically the same results as in the control, but until 2004 the differentiation in tree height became stronger and trees grew taller in the control treatments.

In fact, trees in the initially unsown plots have still been deriving benefit from the understorey control twelve years later, despite the fact that understorey vegetation cover became almost the same in all treatments after the decline of the sown herbaceous species and the gradual invasion and final dominance of indigenous species in all treatments after 1996. Similar results were also found by Hoepting et al. (2011) in Ontario, Canada, where early control of herbaceous vegetation and an increased duration of this understorey control were associated with increased tree survival and taller trees in a 15 year-old red pine (*Pinus resinosa* Ait.) plantation.

The taller height and larger stem diameter of Scots pine in the experimental area perhaps indicates its potential for a more efficient exploitation of the available resources at the site than sycamore. Scots pine is a pioneer species with high adaptive capacity (Dafis 2010) while sycamore is a shade-bearing species with relatively slow absolute growth rates (Moffat et al. 2008).

It is worth noting that during the first eight growing seasons (1992-1999) sycamore had an average growth rate of 0.33 m year^{-1} for height and 0.4 cm year^{-1} for stem diameter (Gakis et al. 2004). However, over the last four growing seasons (2000-2004) there was a slowdown and height was increased on the average by 0.20 m year^{-1} and stem diameter by 0.3 cm year^{-1}. On the contrary, in the same period, Scots pine continued to get taller by an average rate of 0.79 m year^{-1}. Hazandy & Mencuccini (2008) also observed that sycamore trees in Scotland were growing very rapidly during the younger stages (< 7 years) and the growth rates began to decrease afterwards. Differences in the growth rate of height in Scots pine trees in Great Britain were associated with miscellaneous environmental variables such as competition with other trees, water holding capacity of the surface soil and seasonal rainfall distribution (White 1982).

At the early stage of the experiment, until spring of 1997, no effect of tree spacing on tree height and stem diameter was found (Gakis et al. 2004). Nearly three years later, the difference in tree height between spacings suggests that competition started among trees, especially in the dense treatment. This suggests that the plantation started to function as a forest rather than as a silvopastoral system; in 2004, crown canopy cover in most plots was more than 40% (visual estimation) while in almost all dense Scots pine plots exceeded 80% (Papanastasis et al. 2009). The significant differentiation between spacings concerning stem diameter of Scots pine trees in 2004 was expected. Marcos et al. (2007) reported that lower density results in less intraspecific competition thus permitting greater trunk perimeter and crown diameter in Scots pine stands in the northwestern Spain. Also, in an experiment involving three spacings of an artificial plantation of brutia pine in northern Greece, it was found that, after the tenth year from establishment, both the tree diameter and the amount of herbaceous understorey were increased as tree spacing increased (Platis et al. 1999, Mantzanas et al. 2001).

The lower survival rate of Scots pine trees compared to sycamore may be attributed to the strong competition that its seedlings encountered in the plots seeded with white clover at the early stages of establishment (Gakis 2000, Gakis et al. 2004). Another possible reason may be the interference of the same seedlings by bracken fern, which tended to re-invade the treated areas during early establishment. The latter reason is supported by Dolling (1996) who attributed the low germination and high mortality of Scots pine seedlings to bracken phytotoxicity. On the other hand, the high survival capacity of sycamore is confirmed by Walle et al. (2007), who found a very high survival rate (almost 98%) of the species four years after its establishment in Belgium.

Understorey vegetation

The fact that plant cover was not significantly different among the understorey treatments suggests that the sowings at the establishment stage had ceased to affect the understorey vegetative cover developed twelve years later. This is another indication that the experiment ceased to function as a silvopastoral system. In fact, the sown herbaceous species declined significantly after 1996. This decline together with the interruption of herbicide sprayings resulted in the gradual invasion and a final dominance of indigenous species in all treatments, especially bracken fern. As a consequence, cover of the understorey vegetation was almost the same in all treatments already before the spring of 1999. Only perennial ryegrass was found surviving from its establishment; all the other species were spontaneous. Among these species, the most common grasses were cocksfoot, *Poa* sp. and *Brachypodium sylvaticum* (Huds.) Beauv.; the most common legumes were *Vicia cracca* L., *Melilotus* sp. and *Trifolium pratense* L.; the most common forbs were *Filipendula* sp., *Fragaria vesca* L. and *Potentilla recta* L.; and the most common woody species were *Rubus* sp. and *Rosa canina* L. All these species are perennial which replaced annuals that were recorded in the early years of establishment (Gakis et al. 2004).

It is worth noting that bracken fern, which was the dominant understorey species, was found to have the highest cover in the control and the lowest in the legume plots ($p = 0.085$). Fredericksen et al. (1999) have also found that fern species dominated many forest stands with moderate to high basal area and tree cover in Pennsylvania, USA, as well as that fern had restricted plant diversity. In our study, control plots must have favored bracken fern invasion since they had greater tree size and apparently greater overstorey cover than grass and legume plots. This is also supported by the fact that the control treatment had greater K concentration ($p = 0.072$) in its understorey vegetation than the other treatments apparently due to the increased presence of bracken fern, which is a relatively K-rich species (Reimann et al. 2007).

The significant impact of tree species on grass and woody species cover as well as on N and K concentrations of the understorey vegetation has apparently to do with the structure and size of the canopy and the kind of plant species allowed growing. This is because changes in microclimatic and nutrient status under the tree canopy have impacts on understorey vegetation characteristics (Silva-Pando et al. 2002). As a deciduous species, sycamore provided favourable conditions for the winter growing grasses but not for woody species which grow in late spring and summer. The growing conditions of the latter group were favoured by Scots pine apparently because of its gaps in the canopy cover as a result of the increased tree mortality which allowed more light to reach the understorey. The same reason may also explain the higher N and K concentration found under Scots pine than under sycamore. A similar favourable impact of deciduous trees is also reported by Benavides et al. (2009) who have found that their leafless period enables pasture recovery and their litter smothers pasture less intensely because of its relatively fast decomposition.

The higher understorey vegetation above ground biomass and the consequent less bare soil found in the open spacing should be attributed to the better light conditions compared with the dense spacing. This agrees with findings of several other studies in temperate silvopastoral systems (e.g. Knowles 1991, Koukoura & Papanastasis 1996, Silva-Pando et al., 2002) which concluded that shading by trees was the main factor of reducing pasture productivity.

Soil properties

The lack of significant effects of the understorey treatments on soil pH or organic C and macronutrient concentrations was expected, because organic matter inputs and nutrient uptake were similar since the understorey plant characteristics (cover, biomass, nutrient concentrations) were almost the same. pH was not influenced significantly by tree species or tree spacing, although the introduction of conifers is often assumed to acidify the soil (Hagen-Thorn et al. 2004, Benavides et al. 2009).

The increased organic C and N, Mg and K concentrations in the topsoil of sycamore plots in 2004 indicates higher input and/or more rapid decomposition of litter than in Scots pine plots. This is because sycamore leaves have higher nutrient concentration than Scots pine needles (Gakis 2000, Gakis et al. 2004). Moreover, the fact that mean N and K concentrations in understorey vegetation were higher in Scots pine plots must have contributed towards a reduction in soil N and K availability (Mosquera-Losada et al. 2006). Significantly greater P-Olsen concentrations at the 20-40 cm soil depth in in the open plots in 2004 may be due to the fact that fewer trees were using up fewer nutrients from the deeper soil layer resulting in greater nutrient availability. Tree component in silvopastoral systems often improves soil fertility by increasing most of the nutrient contents beneath the canopy (Moreno Marcos et al. 2007) while decomposition and nutrient release rates are related to quality of leaf material (Partey et al. 2011). Hagen-Thorn et al. (2004) observed differences in soil chemistry in 30 to 40 years-old plantations of six common European tree species, which were much more distinct in the upper (0-10 cm) than in the lower (20-30 cm) soil layer. They concluded that these differences in mineral topsoil chemistry were more likely to have been caused by differences in foliage properties (amount and quality) and the processes taking place on the surface of the forest floor rather than by differences in root activity and turnover.

The decrease of soil organic C concentration from 1999 to 2004, mainly in the surface soil layer, may be attributed to the reduction of understorey vegetation under the influence of stand age. Shading and tree litter accumulation are increasing with stand age and canopy cover (Hoepting et al. 2011). Decreasing photosynthetically active radiation restricts understorey vegetation biomass (Reynolds et al. 2007) and the gradual accumulation of residues in soil surface further reduce the presence of understorey vegetation; in 2004, visual observations in the study site suggested that, although a pure organic horizon had not yet developed, the formation of a thick forest floor consisting of loose litter had started. These alterations resulted in a reduction of understorey vegetation and consequently in a reduction in the size of its rooting system, which mainly develops in the topsoil. Thus, over time, a reduction of organic material arising from understorey plants must have occurred associated with a concomitant reduction in organic C by understorey vegetation rooting system. Nilsson et al. (2008) noted that denser canopy and shadier environment as well as more extensive ground litter cover reduced understorey cover and affected species composition in Scots pine stands in northern Sweden. Marcos et al. (2007) observed noticeable changes in understorey herbaceous and woody species cover in terms of age.

The decrease of soil pH and the increment in N, P and Ca concentrations between 1999 and 2004 may be attributed to the input of acidic compounds as well as to nutrient mineralization due to organic matter decomposition (Singh & Sharma 2007). The decrease in soil K may be due to depletion by uptake from growing trees and leaching, further contributing to the reduction of soil pH. As a monovalent cation, K is easily leached from soil, whereas P and Ca show a much lower rate of leaching (Staaf & Olsson 1994, Aerts & Chapin 2000). N mineralization and nitrate leaching loss are also possible contributing factors to soil acidification (Ridley et al. 1999). The unexpectedly large increase of P-Olsen between the two sampling periods in all treatments is probably also related to the decrease in soil pH. Lal (1989) has found an overall decrease in soil organic matter and most soil nutrients in agroforestry manipulations in Nigeria over a period of five years but a slight increase in soil P, possibly due to pH decrease. Decreasing trends concerning soil pH are also reported by Mosquera-Losada et al. (2006) for silvopastoral manipulations in Spain over a period of four years as a result of depletion of cations, new organic matter deposition and N mineralization. Marcos et al. (2007) mention that the level of soil P concentration tended to increase significantly with Scots pine plantation age while the levels of K tended to decrease over time.

Many of the valuable hardwood trees (e.g. *Acer*, *Juglans* and *Fraxinus* species) associate with AMF, while most coniferous tress (e.g. *Pinus* and *Picea* species) associate with ectomycorrhizal fungi (Bainard et al. 2011 and references therein). This may explain the significantly higher number of AMF spores in the upper soil layer under sycamore. Orfanoudakis et al. (2004) have found a weak mycorrhiza formation in Scots pine roots in plantations of northern and central Greece. Apart from plant species, the number of AMF spores produced depends on light intensity and soil fertility (Hetrick & Bloom 1986, Lesueur & Sarr 2008). Better light and nutritional conditions under sycamore plots may also have favoured AMF occurrence at the upper soil layer.

The finding that AMF spores had greater numbers in the 20-40 cm soil layer in all treatments does not agree with other studies, where numbers of AMF spores strongly decreased in abundance with increasing soil depth (Oehl et al. 2005, Muleta et al. 2008). On the contrary, the dominance of *Glomus* in many ecosystems regardless of the degree of disturbance or land use system has been frequently reported (Oehl et al. 2005, Muleta et al. 2008, Stümer & Siqueira 2010).

The extracted spores from 20-40 cm depth were active and subsequently colonised successfully *Plantago lanceolata* L. trap plants. The larger numbers of spores in the deeper soil layer may be explained by the greater quantities of tree roots which exist in this layer. At this stage of development of the silvopastoral system, the roots of tree species were mostly found deeper than the root system of the understorey plants, which is mainly distributed in the upper soil layer. Apparently, better tree growth and therefore deeper rooting system and more expanded canopy cover probably resulted in greater numbers of AMF spores in the deeper soil layers and a restriction of AMF presence (spores) in the topsoil. Similar results have been reported by Cardoso et al. (2003), who have found higher numbers of AMF spores in the deepest soil layers (40–60 cm depth) in agroforestry coffee fields of Brazil than in coffee fields grown as monocultures. They concluded that deep-rooting trees in such agroforestry systems may promote distribution of AMF (and their spores) at deeper soil levels. Another possible explanation for the higher number of AMF spores in the deeper soil layer could be the presence of a B soil horizon rich in clay which starts at approximately at 30 cm depth (Gakis et al. 2004) and prevents the percolation of water into deeper layers, possibly resulting in AMF spores accumulation due to downwards movement. Therefore, the remarkable N and P increment in the lower soil depth in all treatments between 1999 and 2004 perhaps is also related with a cumulative effect of the greater tree root presence and AMF activity in that depth. Chang et al. (2002) reports, that higher fine root production helps increasing soil C and N concentrations in deeper soil layers. AMF can take up both nitrate and ammonium from the soil and transfer N to the host roots in nutritionally substantial quantities (Tian et al. 2010). For this reason, they are considered responsible for facilitating the dissolution of non-assimilative P forms in the soil (Alifragis 2008).

Conclusions

There are some age related effects on tree growth and soil properties indicating the importance of the early stages of establishment of trees in silvopastoral systems. Also, the degree of alteration of soil chemical properties is influenced by both tree species and stand age. Between the two tree species tested, Scots pine is more robust, but has a higher risk of failure than sycamore.

The introduction of silvopastoral systems composed of Scots pine, as an initially fast growing species, and sycamore, as a valuable hardwood species which over a long term ameliorates soil fertility, may help to conserve soil nutrients, control soil erosion and improve long term site productivity in marginal mountain areas of the Mediterranean region. However, sowing of competitive pasture species, such as white clover and perennial ryegrass at the tree establishment stage should be avoided unless their growth can be controlled with livestock grazing so that they do not interfere with tree seedling establishment and growth. Such a grazing is also necessary to maintain the silvopastoral system, together with thinning of the tree canopy at least in the dense spacing to avoid reduction of understorey biomass. Otherwise, the system will revert to a forest.

As AMF are important components of silvopastoral systems, more field-based research is necessary in future studies, which should focus on seasonal observations on the entire soil profile, providing thereby a better picture of the AMF community in these systems.

Acknowledgments

The authors sincerely thank Dr J.E. Hooker for improving the English of the manuscript.

References

Aerts R., Chapin F.S., 2000. The mineral nutrition of wild plants revisited: a reevaluation of processes and patterns. Advances in Ecological Research 30: 1–67. DOI: 10.1016/S0065-2504(08)60016-1.

Alifragis D., 2008. The soil, genesis-properties-classification, vol. I. Aivazis Press, Thessaloniki, Greece, 582 p. [In Greek].

Alifragis D., Papamichos N., 1995. Description, sampling, laboratory analyses of forest soils and plant tissues. Dedousis Press, Thessaloniki, Greece, 181 p. [In Greek].

Bainard L.D., Klironomos J.N., Gordon A.M., 2011. Arbuscular mycorrhizal fungi in tree-based intercropping systems: A review of their abundance and diversity. Pedobiologia 54: 57-61. DOI: 10.1016/ j.pedobi.2010.11.001.

Benavides R., Douglas G.B., Osoro K., 2009. Silvopastoralism in New Zealand: review of effects of evergreen and deciduous trees on pasture dynamics. Agroforestry Systems 76: 327-350. DOI: 10.1007/s10457-008-9186-6.

Błaszkowski J., Adamska I., Czerniawska B., 2003. *Glomus trimurales*, an arbuscular mycorrhizal fungus (Glomerales) new for Poland and Europe. Mycotaxon 87: 425-436.

Błaszkowski J., Renker C., Buscot F., 2006. *Glomus drummondii* and *G. walkeri*, two new species of arbuscular mycorrhizal fungi (Glomeromycota). Mycological Research 110: 555-566. DOI: 10.1016/ j.mycres.2006.02.006.

Caravaca F., Alguacil M.M., Barea J.M., Roldán A., 2005. Survival of inocula and native AM fungi species associated with shrubs in a degraded Mediterranean ecosystem. Soil Biology & Biochemistry 37: 227-233. DOI: 10.1016/ j.soilbio.2004.06.019.

Cardoso I.M., Boddington C., Janssen B.H., Oenema O., Kuyper T.W., 2003. Distribution of mycorrhizal fungal spores in soils under agroforestry and monocultural coffee systems in Brazil. Agroforestry Systems 58: 33-43. DOI: 10.1023/A:1025479017393.

Chang S.X., Amatya G., Beare M.H., Mead D.J., 2002. Soil properties under a *Pinus radiata* – ryegrass silvopastoral system in New Zealand. Part I. Soil N and moisture availability, soil C, and tree growth. Agroforestry Systems 54: 137-147. DOI: 10.1023/A:1015040006181

Cook C.W., Stubbendieck J., 1986. Range research: basic problems and techniques. Society for Range Management, Denver, Colorado, 317 p.

Dafis S., 2010. The forests of Greece. The Goulandris Natural History Museum, Thessaloniki, Greece, 192 p. [In Greek].

Dhillon R.S., von Wuehlisch G., 2013. Mitigation of global warming through renewable biomass. Biomass and Bioenergy 48: 75-89. DOI: 10.1016/j.biombioe.2012.11.005.

Dolling A.H.U., 1996. Interference of bracken (*Pteridium aquilinum* L. Kuhn) with Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* L. Karst.) seedling establishment. Forest Ecology and Management 88(3): 227-235. DOI: 10.1023/A:1015040006181.

Feldhake C.M., Schumann C.M., 2005. Tree establishment for a temperate agro-forest in central Appalachia, USA. Agroforestry Systems 65: 187-195. DOI: 10.1007/s10457-005-0505-x.

Fredericksen T.S., Ross B.D., Hoffman W., Morrison M.L., Beyea J., Johnson B.N., Lester M.B., Ross E., 1999. Short-term understorey plant community responses to timber-harvesting intensity on non-industrial private forestlands in Pennsylvania. Forest Ecology and Management 116: 129-139. DOI: 10.1016/S0378-1127(98)00452-6.

Gakis S., 2000. Dynamics of nutrients in silvopastoral ecosystems. Ph.D. Thesis, Aristotle University of Thessaloniki, Department of Forestry and Natural Environment. 159 p. [In Greek].

Gakis S., Mantzanas K., Alifragis D., Papanastasis V.P., Papaioannou A., Seilopoulos D., Platis P., 2004. Effects of understorey vegetation on tree establishment and growth in a silvopastoral system in northern Greece. Agroforestry Systems 60: 149-157. DOI: 10.1023/B:AGFO.0000013275.30617.ad.

Gollotte A., van Tuinen D., Atkinson D., 2004. Diversity of arbuscular mycorrhizal fungi colonizing roots of the grass species *Agrostis capillaris* and *Lolium perenne* in a field experiment. Mycorrhiza 14(2): 111-117. DOI: 10.1007/s00572-003-0244-7.

Grant E.G. 1982. Exchangeable cations. In: Page A.L.(ed.), Methods of soil analysis, part 2. American Society of Agronomy and Soil Science Society of America, Madison, Wisconsin, pp. 159-164.

Green J.J., Alifragis D., Bagnaresi J.A., Bohne H., Bulfin M., Edwards A.C., Hooker J.E., Watson C.A., 1999. Impact of understorey vegetation on tree production in European agroforestry systems. In: Papanastasis V.P., Frame J., Nastis A.S. (eds.), Proceedings of ¨Grasslands and woody plants in Europe¨. Thessaloniki (Greece), 27-29 May 1999. European Grassland Federation. Grassland science in Europe, vol. 4, pp. 171-175.

Hagen-Thorn A., Callesen I., Armolaitis K., Nihlgård B., 2004. The impact of six European tree species on the chemistry of mineral topsoil in forest plantations on former agricultural land. Forest Ecology and Management 195: 373-384. DOI: 10.1016/j.foreco.2004.02.036.

Haselwandter K., Bowen G.D., 1996. Mycorrhizal relations in trees for agroforestry and land rehabilitation. Forest Ecology and Management 81: 1-17. DOI: 10.1016/0378-1127(95)03661-X.

Hazandy A.H., Mencuccini M., 2008. Age and size related changes in growth of *Acer pseudoplatanus* and *Fraxinus excelsior* species. American Journal of Plant Physiology 3(4): 137-153. DOI: 10.3923/ajpp.2008.137.153.

Hetrick B.A.D., Bloom J., 1986. The influence of host plant on production and colonization ability of vesicular-arbuscular mycorrhizal spores. Mycologia 78: 32-36. DOI: 10.2307/3793373.

Hoepting M.K., Wagner R.G., McLaughlin J., Pitt D.G., 2011. Timing and duration of herbaceous vegetation control in northern conifer plantations: 15th-year tree growth and soil nutrient effects. Forestry Chronicle 87(3): 398-413. DOI: 10.5558/tfc2011-030.

Ingleby K., Wilson J., Munro R.C., Cavers S., 2007. Mycorrhizas in agroforestry: spread and sharing of arbuscular mycorrhizal fungi between trees and crops: complementary use of molecular and microscopic approaches. Plant and Soil 294: 125-136. DOI: 10.1007/s11104-007-9239-z.

Jefwa J.M., Sinclair R., Maghembe J.A., 2006. Diversity of glomale mycorrhizal fungi in maize/sesbania intercrops and maize monocrop systems in southern Malawi. Agroforestry Systems 67: 107-114. DOI: 10.1007/s10457-004-2370-4.

Knowles R.L., 1991. New Zealand experience with silvopastoral systems: A review. Forest Ecology and Management 45: 251-267. DOI: 10.1016/0378-1127(91)90221-G.

Koukoura Z., Papanastasis V.P., 1996. Establishment and growth of seeded grasses and legumes in pine silvopastoral systems. In: Etienne M. (ed.), Western European silvopastoral systems, INRA, France, pp. 83-91.

Koukoura Z., Vrahnakis M.S., Karatassiou M., 1999. Effect of *Dactylis glomerata* and *Festuca ovina* on *Pinus brutia* seedling survival. In: Papanastasis V.P., Frame J., Nastis A.S. (eds.), Proceedings of ¨Grasslands and woody plants in Europe¨. Thessaloniki (Greece), 27-29 May 1999. European Grassland Federation. Grassland science in Europe, vol. 4, pp. 181-185.

Lal R., 1989. Agroforestry systems and soil surface management of a tropical alfisoil: III: Changes in soil chemical properties. Agroforestry Systems 8: 113-132. DOI: 10.1007/BF00123116.

Larkin M.A., Blackshields G., Brown N.P., Chenna R., McGettigan P.A., McWilliam H., Valentin F., Wallace I.M., Wilm A., Lopez R., Thompson J.D., Gibson T.J., Higgins D.G., 2007. ClustalW and ClustalX version 2. Bioinformatics 23(21): 2947-2948. DOI: 10.1093/bioinformatics/btm404.

Lesueur D., Sarr A., 2008. Effects of single and dual inoculation with selected microsymbionts (rhizobia and arbuscular mycorrhizal fungi) on field growth and nitrogen fixation of *Calliandra calothyrsus* Meissn. Agroforestry Systems 73: 37–45. DOI: 10.1007/s10457-008-9111-z.

Mantzanas K.T., Platis P.D., Papanastasis V.P., 2001. Yearly changes in the growth of overstorey and understorey vegetation in a young Pinus brutia plantation with different tree spacing. In: Papachristou T.G., Dini-Papanastasi O. (eds.), Proceedings of ¨Range science at the threshold of the 21st century¨. Ioannina (Greece), 4-6 October 2000. Hellenic Range and Pasture Society, 2nd Pan-Hellenic Rangeland congress. pp. 181-186. [in Greek].

Marcos J.A., Marcos E., Taboada A., Tárrega R., 2007. Comparison of community structure and soil characteristics in different aged *Pinus sylvestris* plantations and a natural pine forest. Forest Ecology and Management 247: 35-42. DOI: 10.1016/j.foreco.2007.04.022.

Mathew T., Kumar M., Suresh B.K.V., Umamaheswaran K., 1992. Comparative performance of four multipurpose trees associated with four grass species in the humid regions of southern India. Agroforestry Systems 17: 205-218. DOI: 10.1007/BF00054148.

McLean E.O., 1982. Soil pH and lime requirement. In: Page A.L. (ed.), Methods of soil analysis, part 2, American Society of Agronomy and Soil Science Society of America, Madison, Wisconsin, pp. 199-223.

Moffat A., Foot K., Kennedy F., Dobson M., Morgan G., 2008. Experimental tree planting on U.K. containment landfill sites: results of 10 years' monitoring. Arboriculture & Urban Forestry 34(3): 163-172.

Moreno Marcos G., Obrador J.J., Garcia E., Cubera E., Montero M.J., Pulido F., Dupraz C., 2007. Driving competitive and facilitative interactions in oak dehesas through management practices. Agroforestry Systems 70: 25-40. DOI: 10.1007/s10457-007-9036-y.

Morton J.B., Redecker D., 2001. Two new families of Glomales, Archaeosporaceae and Paraglomaceae, with two new genera *Archaeospora* and *Paraglomus*, based on concordant molecular and morphological characters. Mycologia 93(1): 181-195. DOI: 10.2307/3761615

Mosquera-Losada M.R., Fernádez-Núñez E., Rigueiro-Rodríguez A., 2006. Pasture, tree and soil evolution in silvopastoral systems of Atlantic Europe. Forest Ecology and Management 232: 135-145. DOI: 10.1016/j.foreco.2006.05.057.

Mosquera-Losada M.R., Freese D., Rigueiro-Rodríguez A., 2011. Carbon sequestration in European agroforestry systems. In: Kumar B.M., Nair P.K.R. (eds.), Carbon sequestration potential of agroforestry systems: opportunities and challenges. Advances in Agroforestry 8: 43-59. DOI: 10.1007/978-94-007-1630-8.

Muleta D., Assefa F., Nemomissa S., Granhall U., 2008. Distribution of arbuscular mycorrhizal fungi in soils of smallholder agroforestry and monocultural coffee systems in southwestern Ethiopia. Biology and Fertility of Soils 44: 653-659. DOI: 10.1007/s00374-007-0261-3.

Nelson D.W., Sommers L.E., 1982. Total carbon, organic carbon and organic matter. In: Page A.L. (ed.), Methods of soil analysis, part 2. American Society of Agronomy and Soil Science Society of America, Madison, Wisconsin, pp. 539-577.

Nilsson C., Engelmark O., Cory J., Forsslund A., Carlborg E., 2008. Differences in litter cover and understorey flora between stands of introduced lodgepole pine and native Scots pine in Sweden. Forest Ecology and Management 255: 1900-1905. DOI: 10.1016/j.foreco.2007.12.012.

Oehl F., Sieverding E., Ineichen K., Ris E.A., Boller T., Wiemken A., 2005. Community structure of arbuscular mycorrhizal fungi at different soil depths in extensively and intensively managed agroecosystems. New Phytologist 165: 273-283. DOI: 10.1111/j.1469-8137.2004.01235.x.

Olsen S.R., Sommers L.E., 1982. Phosphorus. In: Page A.L. (ed.), Methods of soil analysis, part 2. American Society of Agronomy and Soil Science Society of America, Madison, Wisconsin, pp. 403-427.

Orfanoudakis M., Alifragis D., Papaioannou A., Panagiotidis S., 2004. Mycorrhizal fungi in exotic conifer plantations grown in acid plantations, preliminary results. In: Proceedings of the 10th Pan-Hellenic Soil Science conference. Volos (Greece), 22-25 September 2004. pp. 489-503. [In Greek].

Palma J.H.N., Graves A.R., Bunce R.G.H., Burgess P.J., de Filippi R., Keesman K.J., van Keulen H., Liagre F., Mayus M., Moreno G., Reisner Y., Herzog F., 2007. Modeling environmental benefits of silvoarable agroforestry in Europe. Agriculture, Ecosystems and Environment 119: 320-334. DOI: 10.1016/j.agee.2006.07.021.

Pandey D.N., 2002. Carbon sequestration in agroforestry systems. Climate Policy 2: 367-377. DOI: 10.3763/cpol.2002.0240.

Pantera A., Papanastasis V.P., 2001. Grazing effects on forage production and botanical composition in a Valonia oak silvopastoral system. In: Radoglou K. (ed.), Proceedings of ¨Forest research: A challenge for an integrated European approach¨. Thessaloniki (Greece), 27 August – 1 September 2001. NAGREF, Forest Research Institute, vol. 2, pp. 681-687.

Papanastasis V.P., 1999. Grasslands and woody plants in Europe with special reference to Greece. In: Papanastasis V.P, Frame J., Nastis A.S. (eds.), Proceedings of ¨Grasslands and Woody Plants in Europe¨. Thessaloniki (Greece), 27-29 May 1999. European Grassland Federation. Grassland science in Europe, vol. 4, pp. 15-24.

Papanastasis V.P., 2001. Pine silvopastoralism in Greece. In: Radoglou K. (ed.), Proceedings of ¨Forest research: A challenge for an integrated European approach¨. Thessaloniki (Greece), 27 August – 1 September 2001. NAGREF, Forest Research Institute, vol. 2, pp. 667-672.

Papanastasis V.P., Mantzanas K., Dini-Papanastasi O., Ispikoudis I., 2009. Traditional agroforestry systems and their evolution in Greece. In: Rigueiro-Rodríguez A., McAdam J., Mosquera M.R. (eds.), Agroforestry in Europe: current status and future prospects. Advances in Agroforestry, Springer Netherlands, vol 6, pp. 89-109. DOI: 10.1007/978-1-4020-8272-6_5.

Partey S.T., Quashie-Sam S.J., Thevathasan N.V., Gordon

A.M., 2011. Decomposition and nutrient release patterns of the leaf biomass of the wild sunflower (*Tithonia diversifolia*): a comparative study with four leguminous agroforestry species. Agroforestry Systems 81: 123–134. DOI: 10.1007/s10457-010-9360-5.

Platis P.D., Mantzanas K.T., Papanastasis V.P., 1999. Effects of tree spacing and annual cutting on herbage production in a young *Pinus brutia* plantation. In: Papanastasis V.P., Frame J., Nastis A.S. (eds.), Proceedings of ¨Grasslands and woody plants in Europe¨. Thessaloniki (Greece), 27-29 May 1999. European Grassland Federation. Grassland science in Europe, vol. 4, pp. 221–225.

Rao M.R., Nair P.K.R., Ong C.K., 1998. Biophysical interactions in tropical agroforestry systems. Agroforestry Systems 38: 3-50. DOI: 10.1023/A:1005971525590.

Reimann C., Arnoldussen A., Boyd R., Finne T.E., Koller F., Nordgulen Ø., Englmaier P., 2007. Element contents in leaves of four plant species (birch, mountain ash, fern and spruce) along anthropogenic and geogenic concentration gradients. Science of the Total Environment 377: 416-433. DOI: 10.1016/j.scitotenv.2007.02.011.

Reynolds P.E., Simpson J.A., Thevathasan N.V., Gordon A.M., 2007. Effect of trees on corn and soybean photosynthesis, growth, and yield in a temperate tree-based agroforestry intercropping system in southern Ontario, Canada. Ecological Engineering 29: 362-371. DOI: 10.1016/j.ecoleng.2006.09.024.

Ridley A.M., Simpson R.J., White R.E., 1999. Nitrate leaching under phalaris, cocksfoot, and annual ryegrass pastures and implications for soil acidification. Australian Journal of Argicultural Research 50: 55-63. DOI: 10.1071/A98038.

Schultz A.M., Papanastasis V.P., Katelman T., Tsiouvaras C., Kandrelis S., Nastis A., 1987. Agroforestry in Greece. Working document. Laboratory of Range Science, Aristotle University of Thessaloniki, Thessaloniki, Greece. 45p.

Sibbald A.R., 1999. Silvopastoral agroforestry: soil-plant-animal interactions in the establishment phase. In: Papanastasis V.P., Frame J., Nastis A.S. (eds.), Proceedings of ¨Grasslands and woody plants in Europe¨. Thessaloniki (Greece), 27-29 May 1999. European Grassland Federation. Grassland science in Europe, vol. 4, pp. 133-144.

Silva-Pando F.J., González-Hernández M.P., Rozados-Lorenzo M.J. 2002. Pasture production in a silvopastoral system in relation with microclimate variables in the atlantic coast of Spain. Agroforestry Systems 56: 203-211. DOI: 10.1023/A:1021359817311.

Singh B., Sharma K.N., 2007. Tree growth and nutrient status of soil in a poplar (*Populus deltoides* Bartr.)-based agroforestry system in Punjab, India. Agroforestry Systems 70: 125-134. DOI: 10.1007/s10457-007-9048-7.

Smith S.E., Read D.J., 2008. Mycorrhizal Symbiosis. Academic Press, London, UK. 800 p.

Smith S.E., Smith F.A., 2011. Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. Annual Review of Plant Biology 62: 227-250. DOI: 10.1146/annurev-arplant-042110-103846.

Staaf H., Olsson B.A., 1994. Effects of slash removal and stump harvesting on soil water chemistry in a clearcutting in SW Sweden. Scandinavian Journal of Forest Research 9: 305-310. DOI: 10.1080/02827589409382844.

Stevenson F.J., 1982. Nitrogen-organic forms. In: Page A.L. (ed.), Methods of soil analysis, part 2. American Society of Agronomy and Soil Science Society of America, Madison, Wisconsin, pp. 625-641.

Stümer S.L., Siqueira J.O., 2010. Species richness and spore abundance of arbuscular mycorrhizal fungi across distinct land uses in Western Brazilian Amazon. Mycorrhiza 21(4): 255-267. DOI: 10.1007/ s00572-010-0330-6.

Szott L.T., Fernandes E.C.M., Sanchez P.A., 1991. Soil – plant interactions in agroforestry systems. Forest Ecology and Management 45: 127-152. DOI: 10.1016/0378-1127(91)90212-E.

Tian C., Kasiborski B., Koul R., Lammers P.J., Bücking H., Shachar-Hill Y., 2010. Regulation of the nitrogen transfer pathway in the arbuscular mycorrhizal symbiosis: gene characterization and the coordination of expression with nitrogen flux. Plant Physiology 153: 1175-1187. DOI: 10.1104/pp.110.156430.

Trouvelot S., van Tuinen D., Hijri M., Gianinazzi-Pearson V., 1999. Visualization of ribosomal DNA loci in spore interphasic nuclei of glomalean fungi by fluorescence in situ hybridization. Mycorrhiza, 8(4): 203-206. DOI: 10.1007/s005720050235.

Walle I.V., van Camp N., van de Casteele L., Verheyen K., Lemeur R., 2007. Short-rotation forestry of birch, maple, poplar and willow in Flandres (Belgium) – Biomass production after 4 years of tree growth. Biomass & Bioenergy 31: 267-275. DOI:10.1016/j.biombioe.2007.01.019.

White E.J., 1982. Relationship between height growth of stand and open growth single trees of Scots pine (*Pinus sylvestris* L.) and site factors in Great Britain. Forest Ecology and Management 4(3): 247-259. DOI: 10.1016/0378-1127(82)90003-2.

White T.J., Bruns T.D., Lee S., Taylor J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M.A., Gelfand D.H., Sninsky J.J., White T.J. (eds.), PCR protocols, a guide to methods and applications. Academic Press, San Diego, California, pp. 315-322. DOI: 10.1016/B978-0-12-372180-8.50042-1.