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Sewage sludge stabilisation and fertiliser value in a silvopastoral system developed with *Eucalyptus nitens* Maiden in Lugo (Spain)

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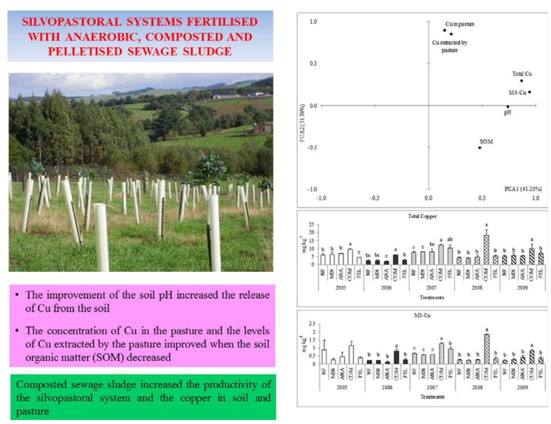
HIGHLIGHTS

- Cu concentration in soil and pasture varies with soil pH and soil organic matter.
- Composted sewage sludge improves the productivity of the silvopastoral system.
- Composted sewage sludge increases Cu concentration in soil and pasture.

GRAPHICAL ABSTRACT



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ABSTRACT

Copper (Cu) is one of the heavy metals with highest proportion in sewage sludge. In Europe, sewage sludge should be stabilised before using it as a fertiliser in agriculture. Depending on the stabilisation process, sewage sludge has different Cu contents, and soil Cu incorporation rates. This study was undertaken to examine the effect of fertilisation with different types of sewage sludge (anaerobic, composted, and pelletised) on the concentration of total and available Cu in the soil, the tree growth, the pasture production, and the concentration of Cu in the pasture when compared with control treatments (i.e. no fertilisation and mineral fertilisation) in a silvopastoral system under *Eucalyptus nitens* Maiden. The results of this experiment show that an improvement of the soil pH increased the incorporation and the mineralisation of the sewage sludge and litter, and therefore, the release of Cu from the soil. Moreover, the concentration of Cu in the pasture and the levels of Cu extracted by the pasture improved when the soil organic matter decreased because the high levels of organic matter in the soil could have formed Cu complex. The composted sewage sludge (COM) increased a) the soil variables studied (pH, total Cu, and available Cu) and b) the Cu extracted by the pasture, both probably due to the higher inputs of cations made with it. In any case, the levels of Cu found in the soil never exceeded the maximums as set by Spanish regulations and did not cause harmful effects on the plants and animals. Therefore, the use of COM as an organic fertiliser should be promoted in silvopastoral systems established in edaphoclimatic conditions similar to this study because COM enhanced the productivity of the system from a viewpoint of the soil and the pasture, without causing any environmental damage.

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1. Introduction

Monocrop production systems are characteristic of many European farms, which are sensitive to soil degradation, climate change impacts, water quality reduction, and biodiversity losses (Lin, 2011). Several studies have shown that agroforestry (AF) practices, in which woody vegetation is integrated into farmland and rangeland, improve nutrient recycling, control soil erosion, promote biodiversity, and increase carbon sequestration (Mosquera-Losada et al., 2009a, 2009b; Howlett et al., 2011). This is while, at same time, food safety and farm resilience are increased, production is diversified, and therefore, the economic and social benefits are increased (Mosquera-Losada et al., 2009b). Recently, AF practices have been promoted by the EU (Council Regulation 1305/2013 (EU, European Union, 2013)) and they have also received favourable evaluations from farmers in Europe (Graves et al., 2009). Silvopastoral systems are the most ancient of AF practices that are used in temperate regions, which are characterised by integrated woody vegetation with forage and livestock production in the same area (Mosquera-Losada et al., 2009b). The establishment of this type of AF in areas such as Galicia (NW Spain), where a high fire risk is recorded, it is essential that the integration of grazing into those high forest fire risk areas reduces the fuel loads in the understory (Rigueiro-Rodríguez et al., 2009).

Eucalyptus plantations cover about 20 million ha around the world (Git, 2009). In Europe, Eucalyptus genera have been used in afforestation since the early 20th century, because of their high productivity and plasticity. In southern Europe, *Eucalyptus globulus* Labill. is the most common species that is used in afforestation, but with an increasing proportion of *Eucalyptus nitens* Maiden., which is grown successfully and as a frost-tolerant species (Prado and Barros, 1989). In Galicia, *E. nitens* Maiden. plantations cover >174.000 ha and produce over 3 million m^3 of round wood annually, which accounts for 23% of the timber harvests in Spain (MMA, Spanish Ministry of Agriculture, Food and Environment, 2005).

In Galician silvopastoral systems, the productivity (of both the understory and the trees) can be limited by low soil fertility as a result of the increased acidity (Zas and Alonso, 2002). Sewage sludge could be used as an organic fertiliser in silvopastoral systems due to the high pH and the macronutrient that it has, particularly N (MMA, Spanish Ministry of Agriculture, Food and Environment, 2006). However, the use of this residue as a fertiliser must take into consideration its copper (Cu) concentration which is higher than that normally found in soils (Smith, 1996). In Europe (European Directive 86/278) (EU, European Union, 1986) and in Spain (R.D.1310/1990) (BOE, 1990), are regulations that limit the total Cu concentration in the soil and in sewage sludge, in order to minimise the harmful effects that sewage sludge fertilisation can cause in the soil, on vegetation, for animals, and human health. Moreover, in Europe, it is compulsory to stabilise sewage sludge before it is used as a fertiliser in agricultural production systems. The stabilisation processes could cause differences in the mineralisation rates (EPA, Environmental Protection Agency, 1994), and therefore, in the fertiliser's potential, but also in the concentration of Cu. Anaerobic digestion and composting are the most important types of sludge stabilisation promoted by the EU (European Directive 86/278) (EU, European Union, 1986). However, both types of waste processing can deliver sewage sludge with high proportions of water which could be reduced by 98% through the pelletisation of anaerobic sludge via a thermic treatment. Consequently, palletisation reduces storage, transport, and spreads costs, when compared with anaerobic or composted sludge (Mosquera-Losada et al., 2010).

Copper is one of the heavy metals with highest proportion in sewage sludge. Cu tends to be more strongly and effectively sorbed in the soil and in plants than other heavy metals as the zinc (Kabata-Pendias and Pendias, 2001), but it can become toxic when high volumes of sewage sludge are applied (Fjällborg and Dave, 2003). Cu is known to have a number of negative effects on both the crops (Baryla et al., 2000) and the microorganisms in the soil, which therefore produces a negative effect on the fertility of the soil (Martensson and Torstensson, 1997). In general, the available Cu in the soil and its transfer to plants depends on different factors such as the soil pH, the parent material, soil organic matter (SOM), and the weather (Smith, 1996; Mosquera-Losada et al., 2011). The Cu availability is normally increased when soil pH is reduced (Smith, 1996) but the high levels of SOM reduce the Cu availability (Kabata-Pendias and Pendias, 2001). Therefore, when sewage sludge is used as a fertiliser, it is important to be aware of its effect on the concentrations of Cu in the soil and in plants, because heavy metals such as Cu cannot degrade, and its negative effect on the soil and the plants can appear several years after the application of the sewage sludge. In general, an initial increase in soil pH, due to sewage sludge application, may occur and modify the mineralization rate and therefore SOM cycling and Cu availability (Rigueiro-Rodríguez et al., 2011).

The aim of this study was to evaluate the effect of fertilisation with different types of sewage sludge (anaerobic, composted, and pelletised) on the concentration of total and available Cu in the soil (total Cu and M3-Cu, respectively), the tree growth, the pasture production, and the concentration of Cu in the pasture when compared with control treatments (i.e. no fertilisation and mineral fertilisation) in a silvopastoral system under *E. nitens* Maiden.

2. Materials and methods

2.1. Characteristics of the study site

The experiment was carried out in A Pastoriza (Lugo, Galicia, NW Spain, European Atlantic Biogeographic Region) at an altitude of 550 m above sea level. Figure 1 shows the mean monthly precipitation and the temperatures from 2005 to 2009 together with the mean data for the last 30 years. The data shows that 2007 was the driest year, with annual precipitation levels (734.4 mm) lower than the annual mean for the study area (1015.99 mm). In 2005, 2006, 2008, and 2009, the total annual rainfall (1024.3 mm, 1157.5 mm, 1222.3 mm, and 1208.6 mm, respectively) was higher than the mean precipitation over the last 30 years due to the especially high precipitation levels at the beginning and at the end of 2006 and in the last months of 2005, 2008, and 2009. However, in these years, the drought periods were also registered and recorded as they would have been unfavourable for tree growth and pasture production. The annual mean temperature was mild (12 °C), in general, with low temperatures at the beginning and at the end of the years under study.

The experiment was established on abandoned agricultural land. The soil texture at the start of the experiment was sandy loam (53.50% sand, 37.35% silt, and 9.15% clay), the water pH (5.52) was moderately acidic, and the organic matter content in the soil was high (17.39%). Moreover, all of the total heavy metal concentrations in the soil (Table 1) were below the maximum threshold for using sewage sludge fertiliser as specified by the European Union Directive 86/278/CEE (EU, European Union, 1986) and the Spanish legislation under R.D.1310/1990 (BOE, 1990).

2.2. Experimental design

At the beginning of the experiment, the soil was double ploughed to a depth of 50 cm, which is a traditional practice in this area, and the pasture was sown with a mixture of *Dactylis glomerata* L. var. Artabro (12.5 kg ha^{-1}), *Lolium perenne* L. var. Brigantia (12.5 kg ha^{-1}), and *Trifolium repens* L. var. Huia (4 kg ha^{-1}), in the autumn of 2004. After pasture sowing in February 2005, plants of *E. nitens* Maiden were planted at a density of 1667 trees ha^{-1}, with a distance between the rows of 3 × 2 m. The experimental design was based upon a randomised block with three replicates and five treatments that were distributed in experimental units of 96 m^2 with twenty five trees arranged in a frame of 5 × 5 trees. The treatments consisted of (1) No Fertilisation (NF),

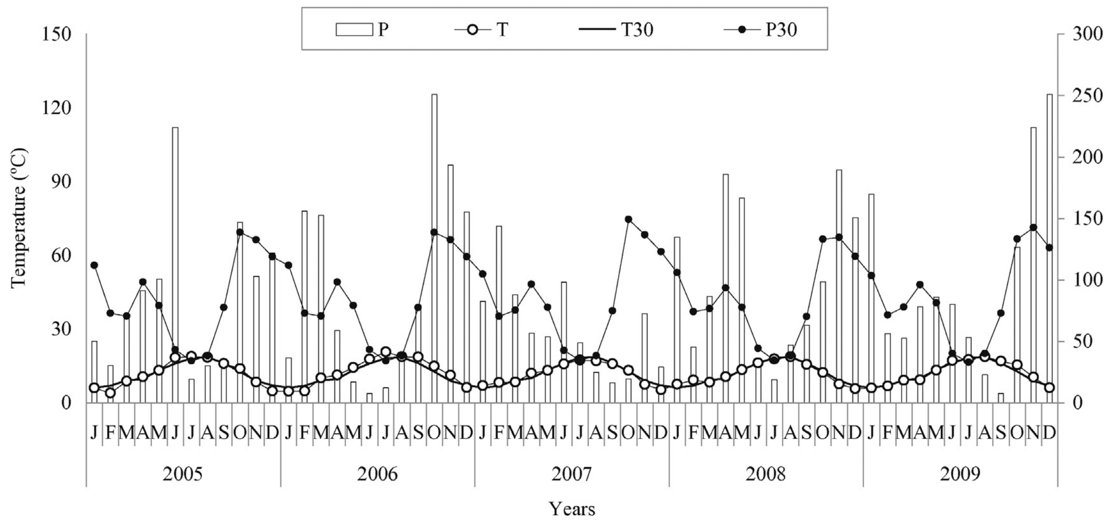

Fig. 1. Monthly precipitation and mean temperatures for the study area from 2005 to 2009 and mean data for the last 30 years. T: mean monthly temperature (°C), T30: mean temperature over the last 30 years (°C), P: monthly precipitation (mm) and P30: mean precipitation over the last 30 years (mm).

(2) Mineral Fertilisation (MIN) with 500 kg ha^{-1} 8:24:16 compound fertiliser (N:P_2O_5:K_2O) at the beginning of the growing season and 40 kg N ha^{-1} before the first harvest; (3) Fertilisation with Anaerobically Digested Sludge (ANA) with an input of 320 kg total N ha^{-1} before the pasture sowing; (4) Fertilisation with Composted Sewage Sludge (COM) with an input of 320 kg total N ha^{-1} before the pasture sowing and (5) an Application of Pelletised Sewage Sludge (PEL), which involved a contribution of 320 kg total N ha^{-1} split as 134 kg total N ha^{-1} just before the pasture sowing in 2004 and 93 kg N ha^{-1} at the end of 2005 and 2006. In all cases, the sewage sludge was superficially applied. In this study the total quantity of ANA, COM and PEL applied to the soil was 41.44 t ha^{-1}, 55.78 t ha^{-1} and 9.45 t ha^{-1}, respectively.

2.3. Sewage sludge

Anaerobically digested sewage sludge, composted and pelletised sludge, were taken from municipal waste treatment plants in Lugo, Valladolid, and Madrid, respectively. Following U.S. Environmental Protection Agency (EPA) recommendations, the doses were based on the percentage of total N and the dry matter content of the sewage sludge (EPA, Environmental Protection Agency, 1994). The EU Directive 86/278/CEE (EU, European Union, 1986) and the Spanish regulation R.D. 1310/1990 (BOE, 1990) regarding heavy metal concentrations in the application of sewage sludge to the soil were also considered. The composition of the sewage sludge that was applied and the total heavy metal inputs to the soil per treatment are summarised in Table 2. The sewage sludge used in the present experiment had a similar composition as the mean composition of the sewage sludge described for plants all over Spain (Mosquera-Losada et al., 2010).

Table 1
Heavy metal concentrations in the soil at the beginning of the experiment and the legal limits established by European Directive 86/278 (EU, European Union, 1986 and Spain R.D. 1310/1990 (BOE, 1990). Limits depend on soil pH (minimum: soil pH < 7; maximum: soil pH > 7).

	Heavy metal concentrations (mg kg^{-1})					
	Zn	Cu	Cr	Cd	Ni	Pb
Initial soil	16.93	5.9	2.35	<0.01	<0.01	<0.01
Spanish legal limits	150–450	50–210	100–150	1–3	30–112	50–300

2.4. Field samplings and laboratory analyses

A composite soil sample per plot was randomly taken at a depth of 25 cm every December from 2005 to 2009 as described in R.D. 1310/1990 (BOE, 1990). In the laboratory, the soil samples were air-dried, passed through a 2 mm sieve, and ground with an agate mortar. The soil pH was determined by KCl (1:2.5) (Faithfull, 2002). The total carbon content in the soil was estimated by oxidation of the total organic matter with potassium dicromate and sulphuric acid. The excess of dicromate was valorated with Mohr salt (Kowalenko, 2001). The percentage of SOM was calculated by multiplying the total C content of the soil by the de Van Bemmelen factor (1.724). Moreover, the total Cu concentration (total Cu) was analysed with a VARIAN 220FS spectrophotometer using atomic absorption (VARIAN, 1989) after a nitric acid digestion that was made in a CEM MDS-2000 microwave (CEM, 1994). The available Cu (M3-Cu) was measured after extraction by the Mehlich 3 method (Mehlich, 1985) using a VARIAN 220FS spectrophotometer that was using atomic absorption (VARIAN, 1989).

The total tree heights and their basal diameters were measured for the inner nine trees of each plot in October during the period 2005–2009. In the first years of the study, the tree heights were measured with a graduated ruler and in the last years with a telescopic pole. The basal diameters were measured with a calliper in all years of the experiment.

The pasture production and the concentration of Cu in the pasture were determined by randomly collecting four samples of pasture in each plot. The four samples were cut with an electric hand clipper at a height of 2.5 cm (0.3 m × 0.3 m) in August and December 2005, in July and December 2006, in April, June and December 2007, and in May and December 2008 and 2009. The pasture was grazed by Galician-bred sheep at a stocking rate of 50 sheep ha^{-1} in a rotational grazing system for one week after each sampling. In the laboratory, the pasture samples were dried for 72 h at 60 °C and weighed to estimate the dry matter production. The cumulative pasture production was calculated by summing the consecutive harvests. Moreover, two of the pasture samples of each plot were ground to determine the concentration of Cu in the pasture with a VARIAN 220FS spectrophotometer using atomic absorption (VARIAN, 1989) after a nitric acid digestion which was made in a CEM MDS-2000 microwave (CEM, 1994).

2.5. Statistical analysis

Data was analysed by a principal component analysis (PCA) based on a correlation matrix for the dependent variables, followed by a

Table 2
Chemical properties of the sewage sludge applied, total loadings supplied with the inputs of different types of sludge in this experiment and legal limits established by European Directive 86/278 (EU, European Union, 1986) and Spanish R.D. 1310/1990 (BOE, 1990). Limits depend on soil pH (minimum: soil pH < 7; maximum: soil pH > 7). Limit values for amounts of heavy metals which may be added annually to soil are based on a 10-years average (kg ha^{-1} year^{-1}).

Parameters	Anaerobic sludge	Composted sludge	Pelletised sludge	Spanish legal limits
Dry matter (%)	29.47	65.19	95.4	
pH	7.25	7.28	7.25	
N (%)	2.62	0.88	3.55	
P (%)	2.14	0.39	1.07	
K (%)	0.19	0.27	0.18	
Ca (%)	0.6	4.98	6.06	
Mg (%)	0.45	1.47	1.24	
Na (%)	0.1	0.04	0.07	
Fe (%)	1.96	1.28	14.15	
Mn (mg kg^{-1})	248.3	90.5	108.8	
Cr (mg kg^{-1})	92.3	3.9	16.6	1000–1500
Cu (mg kg^{-1})	238.5	121.2	136.1	1000–1750
Ni (mg kg^{-1})	69.5	95.3	91.9	300–400
Zn (mg kg^{-1})	1752.3	753.1	1130.4	2500–4000
Cd (mg kg^{-1})	14.4	<0.01	<0.01	20–40
Pb (mg kg^{-1})	281.1	104	58.5	750–1200
Total heavy metal inputs per treatment				
Cr (kg ha^{-1})	1.13	0.14	0.15	3
Cu (kg ha^{-1})	2.91	4.41	1.23	12
Ni (kg ha^{-1})	0.85	3.47	0.83	3
Zn (kg ha^{-1})	21.40	27.39	10.19	30
Cd (kg ha^{-1})	0.18	<0.01	<0.01	0.15
Pb (kg ha^{-1})	3.43	3.78	0.53	15

multivariable analyses of variance. The statistical software package SPSS (V19 Model) was used for the analyses.

Moreover, the soil and the pasture data were analysed using repeated measures ANOVA (proc glm procedure) and Mauchly's criterion was used to test for sphericity. If the sphericity assumption was met, then a univariate approach output was used, otherwise a multivariate output was used (Wilks' Lambda test was taken into account). The statistical model used was $Y_{ij} = \mu + A_i + T_j + TA_{ji} + \varepsilon_{ij}$, where Y_{ij} is the dependent variable, μ is the mean of the variable, A_i is the year i, T_j is the treatment j, TA_{ji} is the year-treatment interaction (year*treatment), and ε_{ij} is the error.

The data obtained for the soil, the trees, and the pasture, in each year, were also analysed by ANOVA (proc glm procedure), but by using this other statistical model $Y_{ik} = \mu + T_i + B_k + TB_{ik} + \varepsilon_{ik}$, where Y_{ik} is the dependent variable, μ is the mean of the variable, T_i is the treatment i, B_k is the block k, TB_{ik} is the block-treatment interaction, and ε_{ik} is the error.

The LSD test was used for subsequent pair wise comparisons ($p < 0.05$; a = 0.05) if the ANOVA analysis was significant. The statistical software package SAS (2001) was used for the analyses.

3. Results

3.1. Soil

3.1.1. Multivariate analysis

In Figure 2, it can be observed that the first component (PCA1) explains 41.23% of the variance, being the M3-Cu (0.94), the total Cu (0.87), and the pH (0.74), positively related. The second axis (PCA2) (31.70% of total variability) illustrates the positive relation between the annual concentration of Cu in the pasture (0.89) and the annual levels of Cu extracted by the pasture (0.85). However, these variables were negatively related to the SOM (−0.51).

3.1.2. Soil pH and SOM

In this study, the soil pH decreased in the last year of the experiment (2009) when compared with the other years that were evaluated (2005: 4.43^{a}, 2006: 4.54^{a}, 2007: 4.46^{a}, 2008: 4.43^{a}, 2009: 4.30^{b}. The different superscript letters indicate significant differences between the years) ($p < 0.05$). The SOM gradually decreased from 2006 (2005: 14.41^{a}, 2006: 10.61^{b}, 2007: 10.83^{b}, 2008: 9.50^{bc}, 2009: 8.93^{c}, expressed as a %) ($p < 0.01$).

Figure 3 shows that the fertilisation with COM increased the soil pH more than the other treatments (NF, MIN, ANA and PEL) in 2005 ($p < 0.05$), 2007 ($p < 0.05$) and 2009 ($p < 0.01$). On the other hand, in 2005, the SOM was higher with the NF treatment and the MIN treatment than with the COM and PEL treatments ($p < 0.05$), but in 2007, the COM increased more the SOM than the NF, the MIN, and the ANA treatments ($p < 0.05$).

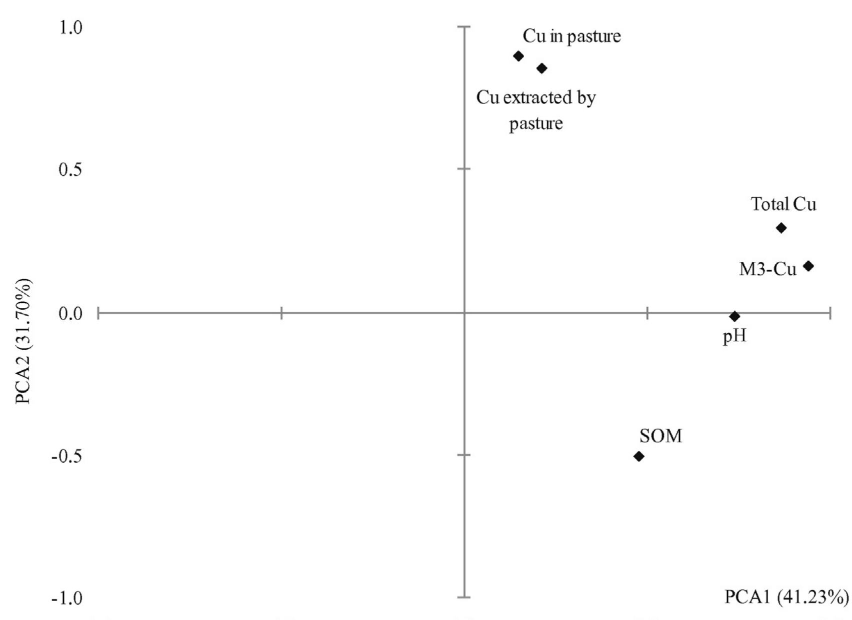

Fig. 2. Multivariate analysis of soil pH, soil organic matter (SOM) (%), total Cu (mg kg^{-1}), Cu extracted by Mehlich 3 (M3-Cu) (mg kg^{-1}), annual concentration of Cu in the pasture (mg kg^{-1}) and annual levels of Cu extracted by the pasture (kg ha^{-1}).

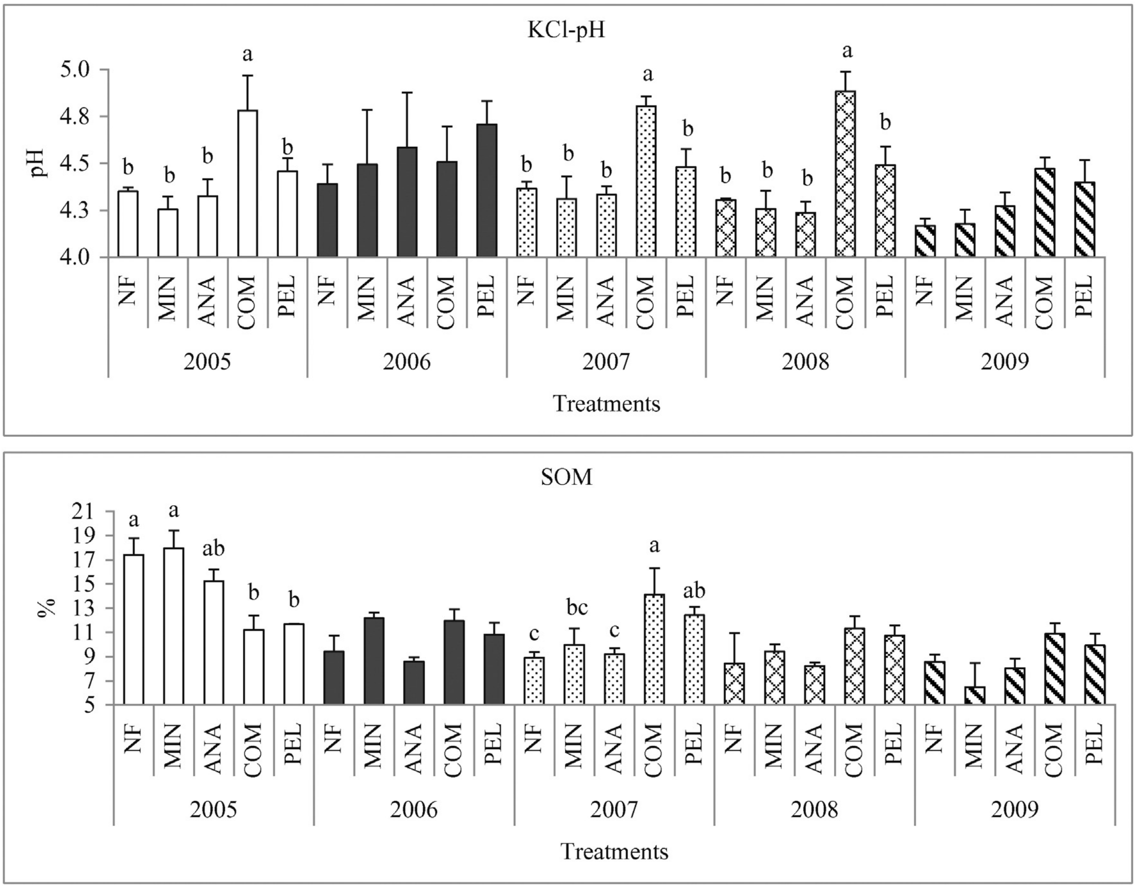

Fig. 3. Soil pH and soil organic matter (SOM) (%) under each treatment from 2005 to 2009. NF: no fertilisation, MIN: mineral; ANA: anaerobic sludge; COM: composted sludge and PEL: pelletised sludge. Different letters indicate significant differences between treatments within the same year and treatments are no significantly different if no letters are shown. Bars in each column indicate the standard error of the mean.

3.1.3. Total Cu and M3-Cu

In this study, it was observed that there was a significant effect of the interaction year* treatment on the total Cu ($p < 0.05$) in the soil. However, the levels of M3-Cu were significantly modified during the study years ($p < 0.05$), being that the levels of M3-Cu were higher in 2007 than in the other years of the experiment (2005: 0.63^{b}, 2006: 0.36^{d}, 2007: 0.82^{a}, 2008: 0.52^{bc}, 2009: 0.46^{cd}, expressed as mg kg^{-1}).

In general, in all of the years of the study, the concentration of total Cu was higher when the plots were fertilised with COM when compared with the other treatments (NF, MIN, ANA and PEL) (Figure 4). COM also increased the levels of M3-Cu more than did the other treatments (NF, MIN, ANA and PEL) from 2006 to 2009 ($p < 0.001$).

3.2. Tree heights and diameters

Figure 5 shows that the tree heights and their diameters were not significantly modified by the fertilisation treatments that were established in any year of the study ($p > 0.05$). However, in general, in 2005 and 2009, the tree heights and their diameters tended to increase with the inorganic (MIN) and the organic fertilisation (ANA, COM and PEL) when compared with the NF treatment. In 2007, the highest tree heights and their diameters seemed to be associated with the NF treatment.

3.3. Pasture

3.3.1. Cumulative pasture production

The cumulative pasture production for the different fertilisation treatments during the experimental period can be seen in Figure 6. In general, in all of the years of the study, the cumulative pasture production was higher in the MIN, COM, and PEL treatments, when compared with the NF treatment and the ANA treatment ($p < 0.001$).

3.3.2. Concentration of Cu in the pasture and the levels of Cu extracted by the pasture

The concentration of Cu in the pasture increased in the last three years of the study when compared with 2005 and 2006 (2005: 3.37^{b}, 2006: 3.57^{b}, 2007: 5.40^{a}, 2008: 5.61^{a}, 2009: 5.59^{a}, expressed as mg kg^{-1}) ($p < 0.001$). Moreover, the levels of Cu extracted by the pasture were higher during the period between 2006 and 2009 than it was in 2005 (2005: 0.015^{c}, 2006: 0.024^{b}, 2007: 0.034^{a}, 2008: 0.025^{b}, 2009: 0.033^{a}, expressed as kg ha^{-1}) ($p < 0.001$).

The concentration of Cu in the pasture was not modified by the fertilisation treatments that were established in any year of the study ($p > 0.05$) (Figure 7). However, in 2006, the levels of Cu extracted by the pasture were higher in the plots fertilised with COM than in the plots which received any of the other treatments (NF, MIN, ANA, and PEL) ($p < 0.05$).

4. Discussion

In this study, the soil acidity significantly increased in 2009 when compared with the other years of the experiment. This result could be explained by the effect of the fertilisation with sewage sludge on the increase of N mineralisation (Rasouli-Sadaghiani and Moradi, 2014), specifically at the step at which NH_4^+ transforms into NO_3^- and H^+ is released into the soil solution media after NO_3^- is leached by rainfall (Whitehead, 1995). This in turn can increase tree and pasture cation extractions from the soil, thus reinforcing the process of soil acidification

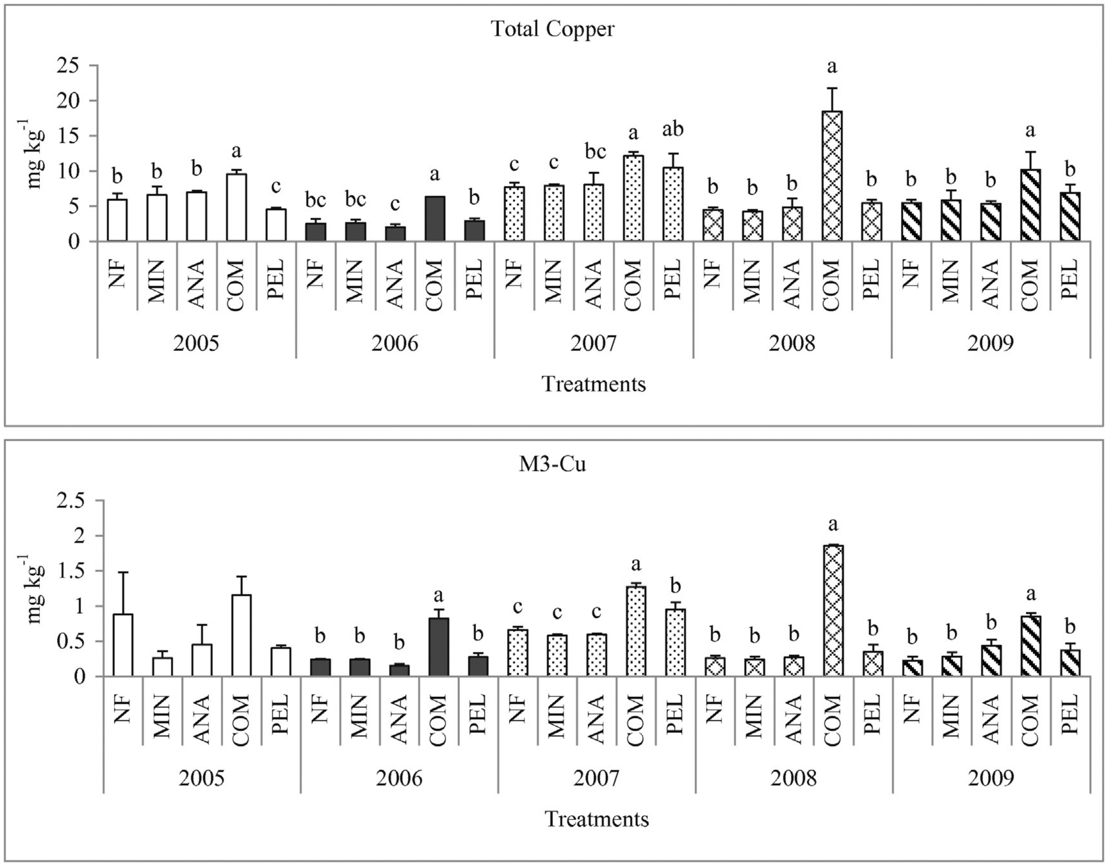

Fig. 4. Concentration of total Cu in the soil (mg kg^{-1}) and levels of Cu (M3-Cu) extracted by Mehlich 3 (mg kg^{-1}) under each treatment from 2005 to 2009. NF: no fertilisation, MIN: mineral; ANA: anaerobic sludge; COM: composted sludge and PEL: pelletised sludge. Different letters indicate significant differences between treatments within the same year and treatments are no significantly different if no letters are shown. Bars in each column indicate the standard error of the mean.

(Mosquera-Losada et al., 2006) in those areas with high rainfall levels. As it was previously observed in numerous other experiments (Ibrahim et al., 2011; Mohd-Aizat et al., 2014), the results obtained in our study showed a positive relation between the soil pH, the total Cu concentration in the soil, and the levels of M3-Cu. In general, an improvement of the soil pH can favour the incorporation and the mineralisation of sewage sludge into the soil which then promotes a Cu release from the sludge. Cu, after Zn, is the most abundant heavy metal in sewage sludge (Mosquera-Losada et al., 2010). However, the levels of M3-Cu decreased in the rainiest years (2005, 2006, 2008, and 2009), when compared with the year with the lowest precipitation (2007). This was probably due to the Cu leaching through the sandy soil profile. This trend was also observed in the case of total Cu by Rigueiro-Rodríguez et al. (2010) in a silvopastoral system established with *Fraxinus excelsior* L. in a sandy soil. This was as it was with the soil in our study, in which it was clearly shown, the important roles that clay minerals have in the immobilisation of heavy metals through their highly specific surface (Zubillaga et al., 2008). In any case, it is important to be aware that the total Cu concentration in the soil was always lower than the limits set by the Spanish regulations for the use of sewage sludge in agriculture for acidic soils (50 mg Cu kg^{-1}) (R.D. 1310/1990) (BOE, 1990). This result may be explained by the fact that this experiment was located in an area without any nearby pollution sources, and the soil had initial low levels of this element when compared to the levels found by others authors, including Egiarte et al. (2008) (13.4 mg Cu kg^{-1}), Antoniadis et al. (2010) (58.5 mg Cu kg^{-1}), and Guan et al. (2011) (37.4 mg Cu kg^{-1}). Moreover, it was also found that the concentration of Cu in the pasture and the levels of Cu extracted by the pasture were negatively related with the SOM, such that the SOM gradually decreased from 2006. In general, the concentration of Cu in the pasture and the levels of Cu extracted by the pasture increased in all treatments, including the NF treatment, from this particular year. The negative relation found between these variables could be due to the high levels of SOM in the soil which could have favoured a Cu complex formation, and therefore, reduce the solubility of this cation when the SOM levels were higher (Kabata-Pendías and Pendías, 2001; Rigueiro-Rodríguez et al., 2011; Ferreiro-Domínguez et al., 2012). Moreover, the increase in the concentration of Cu in the pasture and the levels of Cu extracted by the pasture in the NF treatment could be explained by the graft of the tree roots of the plots without fertilisation with the tree roots of the plots which received fertilisation. The graft of the tree roots could imply a Cu source in the plots without fertilisation, thus increasing the concentration of Cu in the pasture and the levels of Cu extracted by the pasture in the NF treatment. The reduction of SOM over time could be due to the fertilisation with sewage sludge, because the N supplied with this type of organic fertiliser could improve the mineralisation rate by the subsequent reduction of the C/N relationship, but also due to the effect of the trees. It is well known that the *Eucalyptus* litter quality leads to a low biological activity in the soil (Bernhard-Reversat et al. 2001) and results in a low organic matter decomposition rate from the litter and the sludge in *Eucalyptus* plantations which can prevent SOM accumulation. Therefore, the afforestation with *Eucalyptus* not served its task to soil carbon sequestration compared with other tree species as *Pinus radiata* D. Don (Mosquera-Losada et al., 2015) or *Prunus avium* L. (Ferreiro-Domínguez et al., 2016) also established in silvopastoral systems in the same area.

On the other hand, COM was the type of sludge that increased the soil pH, the total Cu and the M3-Cu. The differences found between the three types of sludge analysed in this study could be explained by their different mineralisation rates and composition (Mosquera-

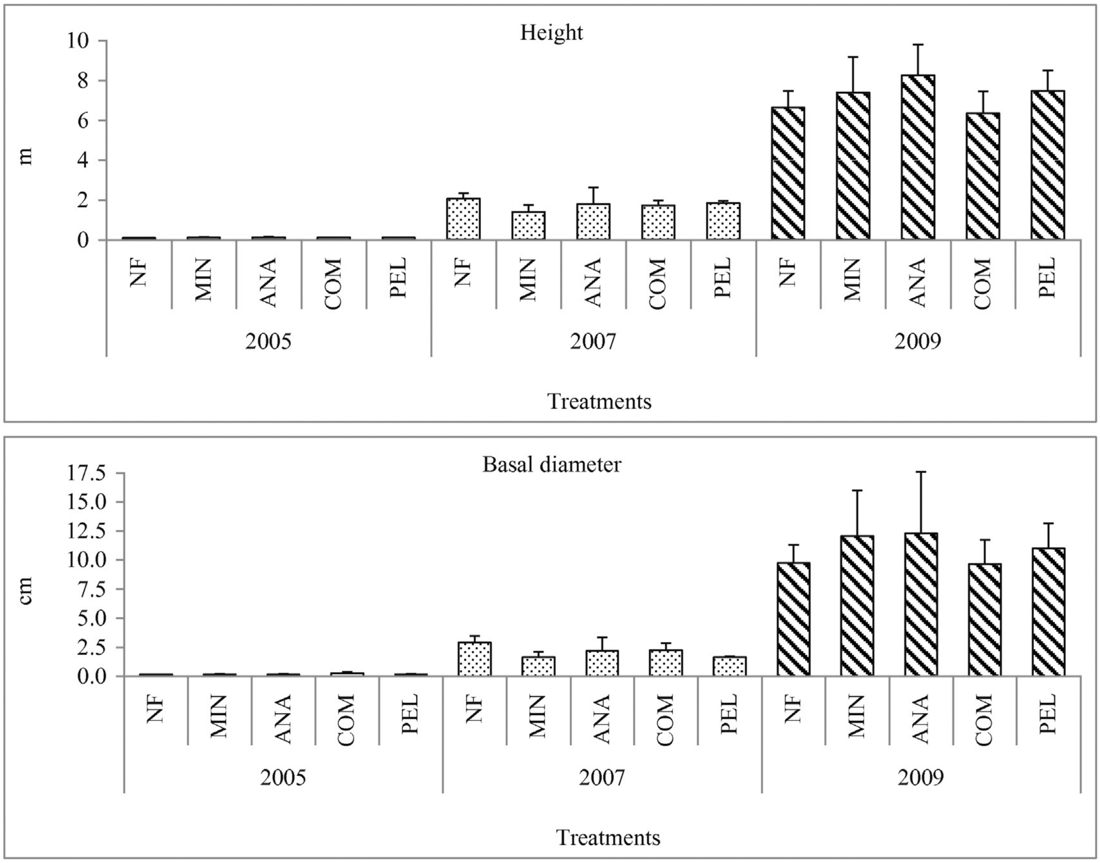

Fig. 5. Tree heights (m) and diameters (cm) under each treatment from 2005 to 2009. NF: no fertilisation, MIN: mineral; ANA: anaerobic sludge; COM: composted sludge and PEL: pelletised sludge. Bars in each column indicate the standard error of the mean.

Losada et al., 2010). As indicated by the EPA (Environmental Protection Agency) (1994), the mineralisation rate is approximately 20% for the ANA treatment when compared with 10% for COM in the first year after the application of the sewage sludge to the soil. Moreover, the composition of COM revealed a lower concentration of N when compared with the ANA treatment and the PEL treatment, and therefore, the dose of COM applied to the soil was higher to meet the N that is required by crops (EPA, Environmental Protection Agency, 1994) than the dose of the ANA treatment or the PEL treatment. This implied that the COM treatment that was applied to the soil around 1810.91 kg Ca ha^{-1} and 4.40 kg Cu ha^{-1}, while meanwhile, only 73.28 kg Ca ha^{-1} and 2.91 kg Cu ha^{-1} were added with the ANA treatment, and 546.25 kg Ca ha^{-1} and 1.23 kg Cu ha^{-1} were the soil inputs with the PEL treatment. The highest inputs of Ca and Cu in the soil were associated to COM and probably explain the increase of the soil pH and the levels of total and available Cu, respectively, with this treatment. Moreover, the higher soil pH in the COM treatment compared with the other treatments could also explain the higher levels of Cu in the soil in the COM treatment because an improvement of soil pH could have increased the incorporation and the mineralisation of the sewage sludge

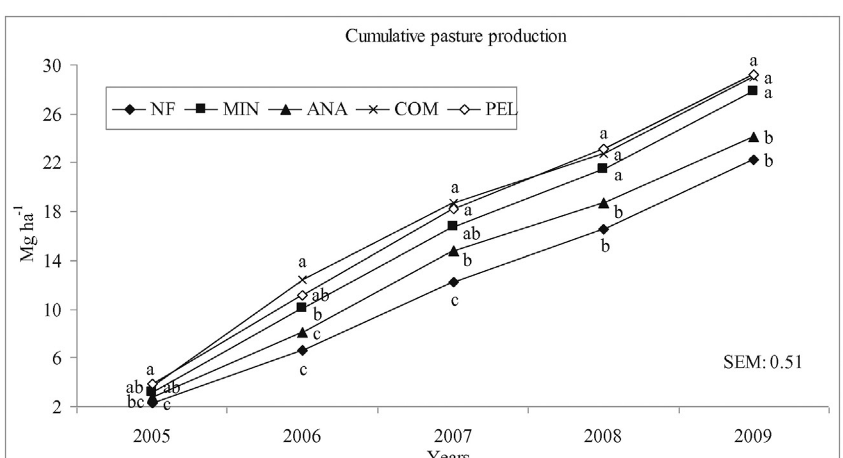

Fig. 6. Cumulative pasture production under each treatment from 2005 to 2009 (Mg ha^{-1}). NF: no fertilisation, MIN: mineral; ANA: anaerobic sludge; COM: composted sludge and PEL: pelletised sludge. Different letters indicate significant differences between treatments within the same year. SEM: standard error of the mean.

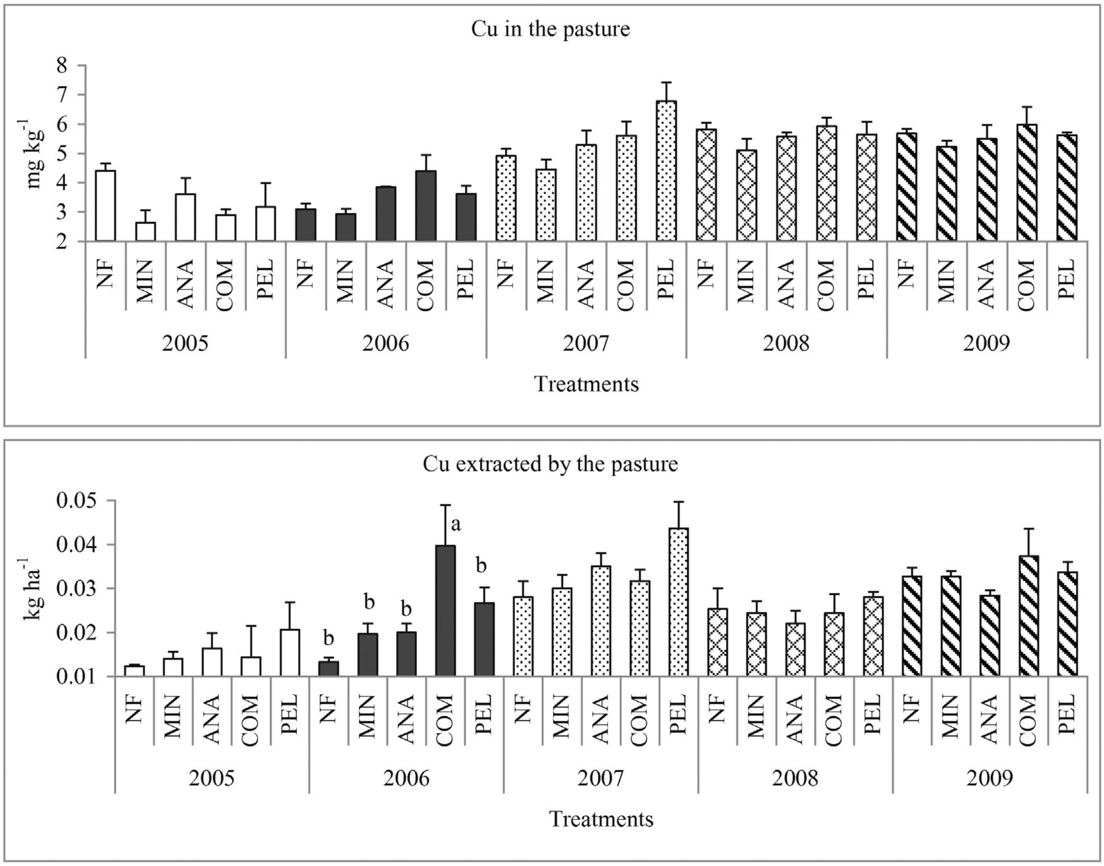

Fig. 7. Annual concentration of Cu in the pasture (mg kg^{-1}) and annual levels of Cu extracted by the pasture (kg ha^{-1}) under each treatment from 2005 to 2009. NF: no fertilisation, MIN: mineral; ANA: anaerobic sludge; COM: composted sludge and PEL: pelletised sludge. Different letters indicate significant differences between treatments within the same year. Bars in each column indicate the standard error of the mean.

and litter, and therefore, the release of Cu from the soil. Similar results were previously observed in several studies in which the effect of different types of sewage sludge on the chemical and physical properties of the soil were also evaluated (Speir et al., 2004; Miao-Miao et al., 2007; Rigueiro-Rodríguez et al., 2010; Vaca et al., 2011). In 2005, the higher Ca supplies of a COM treatment could have activated the mineralisation, and thereby, would have reduced the SOM (Wild 1992). A negative effect of the fertilisation with sewage sludge on the SOM was observed in the year 2005, when the PEL treatment was applied, probably because the dose of the PEL treatment was split several times, thus facilitating the incorporation of the sludge into the soil, and subsequently, the liberation of nutrients such as Ca which favours the increment of the mineralisation rate of the SOM. However, in 2007, a contrary effect of the COM treatment on the SOM was found, probably due to the higher pasture production with this treatment when compared with the NF treatment and the ANA treatment, because it can be assumed that the increase in the SOM depended upon the incorporation of pasture litter into the soil (Mosquera-Losada et al., 2011).

Regarding the effect of different types of fertiliser on the tree growth, in this study, the tree heights and their diameters were not significantly modified by the fertilisation treatments that were established. The absence of any significant differences between the treatments that were applied could be due to the fact that this particular tree species grows very well in the soils within the quality of this experiment (González-Río et al., 2000). When sewage sludge is applied in soils with a lower initial pH than those in this experiment, a positive response of the *Eucalyptus* growth in the sludge or in the mineral soils that were fertilised with these treatments can be compared with those soils where no fertilised trees were found (Mosquera-Losada and Rigueiro-Rodríguez, 2006). In the present experiment, in 2005 and 2009, the tree heights and their diameters tended to increase with the inorganic (MIN) and the organic (ANA, COM and PEL) fertilisation when compared with the NF treatment. In general, the use of fertilisers is not an extended practice that is linked to tree management after afforestation, because it is costly (Dupraz et al., 2005). However, tree growth can be benefited by fertilisation, as shown in our study on *E. nintens* Maiden. and by the study of Ferreiro-Domínguez et al. (2014) on *P. radiata* D. Don, both depending on the initial soil conditions and the type of sewage sludge used in the fertilisation treatments.

Although the tree heights and their diameters tended to improve with the fertilisation, the tree growth found in our study was lower than the growth observed by other authors such as Castillón-Palomeque et al. (2002) in the forest lands of Galicia, where the tree heights reached 3 m in the first two years after the afforestation. The low tree growth observed in our study could be explained by the competition for nutrients and the water generated between the trees and the pasture that was established at the beginning of the experiment. The herbaceous species sown in our study can be considered more extractive than other spontaneous species (Grime et al., 2007), which may have increased the cation soil extractions, and therefore, may have reduced the tree growth. Other authors such as Ferreiro-Domínguez et al. (2011, 2014) in silvopastoral systems under *Quercus rubra* L. and *P. radiata* D. Don, also described that in the first years after the afforestation, the tree development depends on the type of dominant herbaceous species growing in the silvopastoral system. However, over time, the root development of the trees is deeper in the pasture, allowing the trees and the pasture to grow adequately together, as long as the tree canopy allows the passage of light to the understory (Rigueiro-Rodríguez et al., 2012). Therefore, a niche separation of roots in the rhizosphere is important to facilitate tree growth when a high proportion of herbaceous species is present.

Finally, in general, the cumulative pasture production was improved by the MIN, COM, and PEL treatments when compared with the NF treatment and the ANA treatment. The positive effect of COM on the cumulative pasture production could be due to the higher inputs of nutrients into the soil associated with this treatment when compared with the ANA treatment and the NF treatment. Other authors such as Delgado-Arroyo et al. (2002) or Lunas-Zendejas et al. (2011) have also observed a positive effect of COM on the production of crops such as maize (*Zea mays* L.) or bean (*Phaseolus vulgaris* L.), respectively. In the case of the higher cumulative pasture production with the MIN and PEL treatments than with the NF treatment and the ANA treatment could be explained by the fast liberation of nutritional elements by the mineral fertilisers when compared to the slower liberation exhibited by the anaerobic sewage sludge (EPA, Environmental Protection Agency, 1994). This could be together with the annual application of a PEL treatment which could have facilitated the incorporation of the sludge into the soil, and subsequently, the extraction of nutrients by the pasture (Rigueiro-Rodríguez et al., 2010). On the other hand, the annual levels of Cu extracted by the pasture increased with the COM application, probably due to the higher levels of M3-Cu in this treatment than when the ANA treatment and the PEL treatment were applied, due to the faster incorporation of the organic matter (from the litter and the sewage sludge). However, in any case, the levels of 20 and 100 mg Cu kg^{-1}, which are considered excessive and toxic for plants, respectively, were overtaken (Kabata-Pendias and Pendias, 2001). With regard to the animals, the maximum concentration of Cu in the pasture established by NRC (National Research Council) (1980) for bovines (100 mg kg^{-1}), ovine (25 mg kg^{-1}), and equines (800 mg kg^{-1}), was never exceeded, which indicated that the pasture for this experiment was adequate for animal consumption. Moreover, in all of the years, the concentration of Cu in the pasture for this study was below the minimum for the maintenance needs of goats (7 mg kg^{-1}) (Lamand, 1981) and horses (9 mg kg^{-1}) (NRC, 1989), which makes it necessary to supply this element to animals, as was traditionally done in the area. In the case of the bovines (4 mg kg^{-1}) (NRC, National Research Council, 2000) and the ovines (5 mg kg^{-1}) (NRC, National Research Council, 1985), the pasture for this study met with the requirements for the maintenance of these types of animals in 2007, 2008, and 2009, but in the other years (2005 and 2006), supplements of Cu for the animals would be recommended, if their nourishment was derived solely from these pastures.

5. Conclusion

The soil pH was positively related with the total Cu and M3-Cu, probably because the improvement of the soil pH increased the incorporation and the mineralisation of the sewage sludge and litter, and therefore, the release of Cu from the soil. Moreover, the concentration of Cu in the pasture and the levels of Cu extracted by the pasture improved when the SOM decreased due to the high levels of SOM that could have favoured a Cu complex formation, and therefore, reduced the solubility of this cation. The COM was a type of fertiliser that increased the soil variables studied (pH, total Cu, and M3-Cu) and the Cu extracted by the pasture, probably due to the higher inputs of cations made with it, because its N concentration was lower when compared with the other types of sewage sludge applied. However, the levels of Cu found in the soil never exceeded the maximums set by the Spanish regulations and did not cause harmful effects on the plants and the animals, even being below the needed values for the goats and the horses. Therefore, the use of COM as an organic fertiliser should be promoted in silvopastoral systems established in edaphoclimatic conditions similar to this study because this type of sludge enhanced the productivity from a viewpoint of the soil and the pasture, without causing any environmental damage.

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