Effect of grazing on carbon sequestration and tree growth that is developed in a silvopastoral system under wild cherry (Prunus avium L.)

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1. Introduction

The reduction of the atmospheric concentration of greenhouse gases (GHGs), particularly CO₂, has captured the world’s attention during the recent past. The Kyoto Protocol established some mechanisms in order to facilitate the achievement of emission reduction targets by the signatory countries, and since then, silvopastoral systems have been considered a good tool for carbon sequestration (CS). Soils are a major reservoir of C in terrestrial ecosystems (Nair et al., 2010). Spain, as a signatory of the Kyoto Protocol, should be able to determine whether its soils are a sink or a source for atmospheric C through its soil C storage (SCS) accounting.

Silvopastoral systems are a type of agroforestry system that is defined as a mixture of trees, grass, and animal components, all on the same land, and in an interaction and in a combination of these factors. The establishment of silvopastoral systems in Europe is promoted by the European Union (Council Regulation 1698/2005; EU, 2013) as being a sustainable use of land with reported benefits to farmers, such as grazing for livestock production, a greater diversity of production, a fire risk prevention, and a better soil use for increasing SCS (Sharrow and Ismail, 2004; USDA, 2008; Nair et al., 2010). In the region of Galicia (NW Spain), land uses have been shifting from traditional farm crops and pastures (wheat, rye, rape, vetch, and maize) (Lloveras, 1982), to mostly short-rotation exotic tree plantations or to abandoned lands (Marey-Pérez and Rodríguez-Vicente, 2008), which could be considered as a way to increase the CS potential in soils.

Soil organic carbon (SOC) is an important variable for soil fertility and it is used as an indicator of soil health. Moreover, since soils are a major reservoir of C (Nair et al., 2010), an understanding of SOC is particularly important. From the silvopastoral point of view, SCS occurs in two major segments: inputs from aboveground derived from the specific parts of trees, herbaceous layers, and animal excreta; together with belowground inputs from living biomass such as trees, herbaceous roots, and soil organisms (Nair et al., 2010). Soil size fractions and soil aggregates are known to have an important effect on the retention of C in the soil (Six et al., 2004). The size of the soil fractions provides information on the SOC age and its amount in the soil, which varies with each soil fraction. Several studies on SCS in agroforestry systems have found that the highest concentration of C is in the macroaggregates (250–2000 μm), while the lowest concentration is linked to microaggregates (53–250 μm) and to the smaller aggregates (<53 μm) of soil size fractions (Nair et al., 2010; Howlett et al., 2011a,b). SOC associated with macroaggregates is, on average, the youngest, because the C
in these particles is less stable when there are management changes; while the C linked to the smaller soil fractions (53–250 μm and <53 μm) is more stable, and as a result, on average, is the oldest (Puget et al., 2000). The increment of C concentration in recalcitrant microaggregates, within the macroaggregates, increases the amount of long-term sequestration of C in the soil (Six et al., 2004), but a disruption of the process of macroaggregate formation, and consequently, the loss of C, occurs where tillage is implemented on previously untilled soils, increasing the amount of bioavailable sources of C from smaller aggregates (Six et al., 2000).

Wild cherry (Prunus avium L.) is one of the most important European timber species of the Rosaceae family (Russell, 2003) due to its rapid growth which promotes its valuable timber and the delivery of better returns than other broadleaf species (Horgan et al., 2003). Wild cherry is an appropriate tree for growth in silvopastoral systems because it allows an adequate pasture production due to the low shade it generates during the winter and autumn, together with a reduction of the negative effects of droughts on pasture production by maintaining a soil humidity and by reducing light inputs, and thus, extending the grazing period before dry summers (Horgan et al., 2003; Chifot et al., 2006).

Generally, grazing studies have focused on the management practices of pasture production and its effect on animals (Sharow, 1994; Rigueiro-Rodríguez et al., 2011b), although some recent studies have focused on the effect of grazing on SOC and N (Schuman et al., 1999; Reeder and Schuman, 2002; Ingram et al., 2007; Tanentzap and Comps, 2012). Grazing could increase pasture production and control the quantity and the chemical composition of SOC and the distribution of N and C in the soil layers (Schuman et al., 1999). Different rates of stocking with sheep could also increase or depress SCS (He et al., 2011; Ingram et al., 2007).

This study was undertaken in order to examine the effects of live stock grazing at two different stocking rates [Light Stocking Rate (LS; 4 sheep ha−1) and Heavy Stocking Rate (HS; 8 sheep ha−1)], and then compared with No Grazing (NG) pastures on: (i) the soil chemical (pH) and physical (bulk density and the percentage of sand, silt, and clay) properties, (ii) the amount of C stored in the whole soil and in three different soil fractions (250–2000, 53–250, and <53 μm), at each of four soil depths (0–25, 25–50, 50–75, and 75–100 cm) and (iii) the tree growth in a silvopastoral system under Prunus avium L.

2. Materials and methods

2.1. General description of the study site

The study was conducted on the plot A Mota, which belongs to Bosques Naturales S.A., a farm located in Boimorto in the region of Galicia, Spain (42°59′ W, 8°11′ E) at an altitude of 380 m above sea level. The climate is considered Atlantic, with long periods of rainfall during the winter period. The total annual precipitation amounts in 2010 and 2011 were 1537 mm and 1256 mm, respectively. Drought periods were observed from July to September in 2010 and 2011, which probably restricted the tree growth and the pasture production. Most of the precipitation during the years of the study fell between October and March. The mean annual temperature was 12 °C.

The experiment was carried out on an abandoned dairy farm. The soil was classified as Humic Cambisol (FAO, 1998) or Inceptisol (USDA, 2006). The initial soil texture was loam (42% silt, 31% sand, 27% clay) with an increase in the clay percentage below 25 cm, and the pH (KCl) was acidic at 5.16 in the upper soil layer (Table 1). Table 1 shows the initial values of C concentration and C storage per hectare, which decreased with soil depth. In the upper 25 cm, the largest amount of C was in the 250–2000 μm soil fraction. However, at greater depths, the largest amount of C was found in the 53–250 μm soil fractions.

| Soil depth (cm) | 0–25 | 25–50 | 50–75 | 75–100 |
|----------------|------|------|------|-------|
| KCl pH         | 5.16 | 5.02 | 4.79 | 4.55  |
| Bulk density (kg m−1) | 892.14 | 1499.89 | 1654.95 | 1439.75 |
| Percentage sand | 48.78 | 35.17 | 20.83 | 23.12 |
| Percentage silt | 37.27 | 42.19 | 42.28 | 44.24 |
| Percentage clay | 13.95 | 22.64 | 36.89 | 32.64 |
| g C kg−1 (whole soil) | 40.84 | 14.19 | 1.51 | 1.00 |
| g C kg−1 (250–2000 μm) | 19.31 | 4.22 | 0.20 | 0.16 |
| g C kg−1 (53–250 μm) | 15.14 | 5.54 | 0.59 | 0.40 |
| g C kg−1 (<53 μm) | 7.95 | 4.36 | 0.78 | 0.39 |
| Mg C ha−1 (whole soil) | 90.30 | 53.84 | 6.19 | 3.63 |
| Mg C ha−1 (250–2000 μm) | 43.14 | 16.05 | 0.82 | 0.59 |
| Mg C ha−1 (53–250 μm) | 33.33 | 20.84 | 2.43 | 1.44 |
| Mg C ha−1 (<53 μm) | 17.56 | 16.60 | 3.24 | 1.43 |

2.2. Silvopastoral experimental plots

Afforestation with five-year-old wild cherry bare-root trees was carried out in the year 2003 at a density of 400 trees ha−1 on the plot A Mota, which had an area of 120 ha. Tree-understory competition was reduced by the annual application of herbicides, following the tree rows, and with mechanical ploughing between the rows. The tree diameters at breast height and tree height were 10.71 cm and 8.67 m, respectively, at the beginning of the experiment. Silvopastoral research plots were initiated in May 2010. The field experiment was planned following a randomised experimental design block with three treatments and two replicates distributed in experimental units of one hectare. Two of the treatments consisted of grazing by mature sheep (Galician breed Ovela Galega) at (a) a Light Stocking Rate (4 sheep ha−1; LS) and (b) a Heavy Stocking Rate (8 sheep ha−1; HS), while the third treatment was No Grazing (NG). The continuous stocking system was performed during the entire grazing season (May–August 2010; November 2010–January 2011; March–September 2011). The treatment of NG was performed following the previous chemical and mechanical control of the herbaceous understory, which was done before the implantation of the silvopastoral research plots. A total of six plots were studied.

2.3. Field samplings and laboratory determinations

Soil samples were collected from each plot of the study site to a soil depth of 1 m in March 2010 and March 2011 using a stainless steel cylinder of a known volume with a cutting edge that was inserted with a mechanical hammer and removed by using a platform-stabilised pulley (Moreno et al., 2005). The samples taken in March 2010 were used in order to know the initial values before the establishment of the silvopastoral system at the Boimorto farm. Soil cores were divided into four subsamples corresponding to the sampling depth (0–25, 25–50, 50–75, and 75–100 cm). The soil roots were separated by hand from each soil sampling depth and then dried and weighed. Using a cylinder of known volume, the soil root biomass and the soil bulk density were calculated at each depth. Bulk soil samples were taken from the field to the laboratory and were air dried at room temperature (20–25 °C) to a constant weight and were then passed through a 2 mm sieve. Material that did not pass through the 2 mm sieve was separated, weighed, and then discarded. The weight of the discarded fraction was noted down in order to convert the eventual data derived from the 2 mm sieved fraction back to a field condition (Rodríguez-Murillo, 2001). The <2 mm soil particle samples are henceforth referred to as “whole soil”. In the laboratory, the soil pH was measured for the whole soil in KCl 0.1 M (Faithfull, 2002).
Subsamples of the whole soil were physically fractionated according to Elliott (1986) and Six et al. (2002). A sample (25 g) of 2 mm sieved air-dried soil of known moisture content was placed in a 250 mL beaker. To promote slaking, sufficient distilled water to completely cover the soil (~150 mL) was poured into the beaker. The slaking process breaks up aggregates which are water-unstable in the soil, leaving water-stable aggregates for further analysis. After waiting for 5 min, the slaked soil was poured on top of the 250 µm sieve. The soil solution was wet-sieved manually by moving the sieve up and down about 5 cm in each direction 50 times in 2 min. The fraction that did not pass through the 250 µm sieve was backed with distilled water into a pre-weighed and numbered aluminium plate. The remaining soil solution was next manually sieved using a 53 µm sieve for 2 min. The fraction that did not pass through the 53 µm sieve was backed into a pre-weighed and numbered aluminium plate. The remaining soil solution that passed through the 53 µm sieve was poured into a pre-weighed and numbered aluminium plate as were the previous fractions. The resulting three soil fractions, 250–2000 µm, 53–250 µm, and <53 µm, were dried overnight at 60 °C, and then weighed, ground for homogenization, and stored in individual plastic bags ready for a C analysis. The whole soil and the three soil fraction samples of known moisture content were analysed using a LECO CNS Elemental Analyser for the percentage of C within two weeks after the whole soil was air-dried. The Van Bemmelen factor (1.724) was used to convert the total SOC into a soil organic matter (SOM) content.

The percentage of C was used to calculate the soil C concentration in the whole soil and in the soil fractions, and was expressed in grams of carbon per kilogramme of total soil using the following formula (Mosquera-Losada et al., 2015):

\[
\frac{\text{gC}}{\text{g whole soil}} \times \frac{\text{g whole soil}}{\text{g bulk soil}} \times \left(\frac{1}{\text{k}g \text{bulk soil}}\right) \times \left(\frac{\text{g fraction soil}}{\text{g whole soil}}\right)^{-1} = \frac{\text{gC}}{\text{kg soil}}.
\]

(1)

\[\text{gC/kg soil.}\]

This final term is required only to calculate the g per total kg of soil in the fractionated soil.

In addition, to convert the C percentage in the whole soil and the soil fractions to C storage per hectare (Mg C ha\(^{-1}\)) at a specific depth using Formula 2, the mean bulk density of the soil at each sampling depth is required.

\[
\frac{\text{gC}}{\text{g whole soil}} \times \frac{\text{g whole soil}}{\text{g bulk soil}} \times \frac{\text{g bulk soil}}{\text{cm}^3} \times \frac{25 \text{ cm}}{1} \times 100 \times \left(\frac{\text{g fraction soil}}{\text{g whole soil}}\right)^{-1} = \frac{\text{Mg C ha}^{-1}}{}.
\]

(2)

This final term is required only to calculate Mg C ha\(^{-1}\) in the fractionated soil.

In order to calculate the mean SCS at 1 m for each soil fraction and in the whole soil, the SCS for each soil depth (0–25, 25–50, 50–75, and 75–100 cm) and the treatment (LS, HS, and NG) was summed, taking into account the soil bulk density.

The tree height and the tree diameter at breast height were measured with a vertex and a calliper, respectively, in December 2010 and 2011.

2.4. Statistical analyses

The soil variables and the root biomass were analysed using ANOVA (PROC GLM procedure) and following the model \(Y_{ijk} = \mu + T_i + D_j + B_k + TD_{ij} + TB_{ik} + DB_{jk} + e_{ijk}\), where \(Y_{ijk}\) is the dependent variable, \(\mu\) is the variable mean, \(T_i\) indicates the treatment \(i\), \(D_j\) is the soil depth \(j\), \(B_k\) is block \(k\), \(TD_{ij}\) is the treatment–soil depth interaction (treatment \(\times\) soil depth), \(TB_{ik}\) is the treatment–block interaction, \(DB_{jk}\) is the soil depth–block interaction, and \(e_{ijk}\) is the error. Moreover, the effects of the soil fractions (250–2000, 53–250, and <53 µm) on the SCS in the soil were also taken into account.

The data obtained from the trees was also analysed by ANOVA (PROC GLM procedure) using the model \(Y_{ij} = \mu + T_i + B_j + TB_{ij} + e_{ij}\), where \(Y_{ij}\) is the dependent variable, \(\mu\) is the variable mean, \(T_i\) is the treatment effect \(i\), \(B_j\) is block \(j\), \(TB_{ij}\) is the treatment–block interaction, and \(e_{ij}\) is the error.

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

Data was also analysed by a principal component analysis (PCA) based on a correlation matrix for the dependent variables, followed by multivariable analyses of variance. The statistical software package SPSS (V19 Model) was used for these analyses.

3. Results

3.1. Soil

3.1.1. Soil pH

Soil depth was a significant factor affecting the soil pH in KCl (\(p < 0.001\)) (Fig. 1), with lower values being found in the deeper soil layers than in the upper ones in all of the treatments. Values were between 5.26 and 5.40 in the 0–25 cm soil depth and between 4.33 and 4.93 in the 75–100 cm soil depth. Moreover, the soil pH was significantly affected by the treatments (\(p < 0.001\)) and by the interaction of treatment \(\times\) soil depth (\(p < 0.05\)), with higher values being found for the NG treatment than for the LS treatment and the HS treatment in all soil sampling depths, except for the 0–25 cm soil depth, where significant differences were not found (\(p > 0.05\)).

3.1.2. Soil bulk density and soil fractions

Soil bulk density, expressed as kilogrammes per cubic metre, was significantly affected by the soil depths and the treatments (\(p < 0.001\)) (Fig. 2 (a)). Higher values of soil bulk density were found in the 50–75 cm soil layer, whereas lower values were found in the upper soil layer. It was observed that in all soil sampling depths, the NG treatment showed higher values of bulk density than did the LS treatment and the HS treatment, except for the 50–75 cm soil layer, where no significant differences between the treatments were found.

The percentages of each soil fraction (sand: 250–2000 µm; silt: 53–250 µm; clay: <53 µm) were calculated and it was found that the soil fractions were significantly affected by the interaction of the treatment \(\times\) soil depth (\(p < 0.05\)). The percentage of sand decreased with the soil depth in all of the treatments, with higher amounts of sand being found in the upper 25 cm of soil than in the other soil depths (Fig. 2 (b)), and where the soil density was significantly lower (\(p < 0.001\)). In the 50–75 cm soil depth, the LS treatment showed a higher percentage of sand than did the HS treatment, while in the 75–100 cm depth, it was the NG treatment which showed a higher value of sand than did the HS treatment. No significant differences between the treatments were found in the upper 50 cm of soil.

Conversely, the greatest percentage of silt was found in the deepest soil layer and lower values were found in the 0–50 cm depth (Fig. 2 (c)). Significant differences between the treatments were only found in the 50–100 soil layers, where the HS treatment showed a higher percentage of silt than did the LS treatment and the NG treatment.

Moreover, the percentage of clay increased with soil depth, with higher values being found in the 50–100 cm layer when compared with the other soil sampling depths (Fig. 2 (d)). Significant differences between the treatments were only found in the 75–100 cm depth, where the LS treatment showed a higher percentage of clay than did the HS treatment and the NG treatment.
3.1.3. Carbon

Soil depth was a significant factor affecting the SCS and was expressed as grams of carbon per kilogramme of total soil, Mg C ha$^{-1}$, and a relative percentage of C ($p < 0.001$) (Fig. 3) in the whole soil, and in all soil fractions, with higher values being found in the upper soil layers (0–25 cm) than in the deeper ones.

SCS (g C kg$^{-1}$ soil, Mg C ha$^{-1}$, relative percentage of C) was significantly affected by the soil fraction and the interaction of the soil depth * soil fraction ($p < 0.001$). The C concentration was higher in the upper soil layers in all of the soil fractions, more than in the deeper soil layers, with greater amounts of C being found in the upper soil layers (0–50 cm); these are expressed as grams of carbon per kilogramme of total soil being found in the 250–2000 μm soil fraction (Fig. 3 (a)). However the largest amount of C concentration in the deeper soil layers (50–100 cm) was to be found in the 53–250 μm soil fractions. Moreover, the same results were observed with regard to the C storage per hectare (Fig. 3 (b)). The SCS expressed by a relative percentage showed that the percentage of SCS in the 250–2000 μm

![Fig. 1. Soil KCl pH under the three treatments (LS: Light Stocking Rate, HS: Heavy Stocking Rate, and NG: No Grazing) at four soil depths (0–25, 25–50, 50–75, and 75–100 cm). Different uppercase letters indicate significant differences between the soil depths within the same treatment, while different lowercase letters indicate the differences between the treatments within a specified soil depth. Horizontal lines indicate the mean standard error.](image1)

![Fig. 2. Soil bulk density (a), percentage of each soil fraction: sand: 250–2000 μm (b), silt: 53–250 μm (c), clay: <53 μm (d), at four soil depths (0–25, 25–50, 50–75, and 75–100 cm) and under the three treatments (LS: Light Stocking Rate, HS: Heavy Stocking Rate, NG: No Grazing). Different uppercase letters indicate significant differences between the soil depths within the same treatment, while different lowercase letters indicate the differences between the treatments within a specified soil depth. Horizontal lines indicate the mean standard error.](image2)
soil fraction decreased with soil depth; conversely, in the 53–250 μm and <53 μm soil fractions, this value increased in the deeper soil layers. In addition, in the upper soil layers (0–50 cm), the 250–2000 μm soil fraction had a higher relative percentage of C than did the <53 μm soil fraction. However, in the deeper soil layers (50–100 cm), it was the 53–250 μm soil fraction which had a higher relative percentage of C than in the other soil fractions (250–2000 μm, and <53 μm) (Fig. 3(c)).

In the experiment, a significant effect of the treatments and the interaction of treatment * soil depth on the SCS in the whole soil, and in the different soil fractions, expressed as a C concentration (p < 0.001) and as a C storage per hectare (p < 0.01), was observed. Regarding the C concentration in the upper soil layer, it was found that the LS treatment had a higher amount of C than did the NG treatment in the whole soil and in the 53–250 μm soil fraction (Fig. 4(a)). However, no differences between the treatments were found in the 250–2000 μm and <53 μm soil fractions at this depth of soil (0–25 cm). The same response to the treatments was found in the 25–50 cm depth, where the LS treatment showed higher values in

Fig. 3. Soil C concentration (g C kg⁻¹) (a), C storage per hectare (Mg C ha⁻¹) (b), the relative percentage of C (c), in three soil fractions (250–2000 μm, 53–250 μm, and <53 μm), and in the four soil depths (0–25, 25–50, 50–75, and 75–100 cm). Different uppercase letters indicate significant differences between the soil depths within the same soil fraction, while different lowercase letters indicate the differences between the soil fractions within a specified soil depth. Horizontal lines indicate the mean standard error.

Fig. 4. Mean soil C storage expressed as grams of carbon per kilogramme of total soil (g C kg⁻¹) (a), the C storage per hectare (Mg C ha⁻¹) (b), in the whole soil and the three soil fractions (250–2000 μm, 53–250 μm, and <53 μm) and at four soil depths (0–25, 25–50, 50–75, and 75–100 cm) with the three treatments (LS: Light Stocking Rate, HS: Heavy Stocking Rate, NG: No Grazing). Different letters indicate significant differences between the treatments in the whole soil and in the same soil fraction. Horizontal lines indicate the mean standard error.
each soil fraction, than did the NG treatment. In the 50–75 cm soil layer, higher values of C concentration were found in the NG treatment than in the LS treatment in the whole soil and in the 53–250 μm soil fractions. However, in the <53 μm soil fraction, higher values were found in the LS treatment and the NG treatment than in the HS treatment. No differences between the treatments were found in the 250–2000 μm soil fraction in the 50–75 cm soil depth. In the deepest soil layers (75–100 cm), no differences were found between the treatments in the whole soil and in the soil fractions.

Regarding the C storage per hectare in the 0–25 cm soil depth, differences were not found between the treatments, as happened with the soil pH (Fig. 4 (b)). However, in the 25–50 cm soil depth, it was observed that the LS treatment showed higher values of Mg C ha
−1 than did the NG treatment in the whole soil and in the 53–250 μm soil fractions. Moreover, in the <53 μm soil fraction, higher values of C storage per hectare were found with the LS treatment and the NG treatment than those that were apparent in the HS treatment in the 25–50 cm soil depth. In the 50–75 cm soil depth, the amount of C storage per hectare in the whole soil and in the soil fractions of 53–250 μm and <53 μm were higher with the NG treatment than with the HS treatment. Finally, in the deepest soil layers (75–100 cm), the C storage per hectare in the whole soil and in the 53–250 μm soil fractions was lower under the LS treatment when compared with the NG treatment.

In addition, in the range of 1 m soil (0–100 cm), the SCS expressed as grams of carbon per kilogramme of total soil and Mg C ha
−1 was significantly affected by the soil fractions (p < 0.001). A higher C concentration (250–2000 μm: 29.27a; 53–250 μm: 23.92b; <53 μm: 10.40c, expressed as g C kg
−1) and the C storage per hectare (250–2000 μm: 66.02a; 53–250 μm: 53.77b; <53 μm: 24.61c, expressed as Mg C ha
−1) (in all cases, the values followed by superscript letters showed significant differences between the fractions) were found in the 250–2000 μm soil fractions than in the other fractions (53–250 μm and <53 μm). Furthermore, it was found that the C concentration in the range of 1 m of soil (0–100 cm) was significantly affected by the treatments (p < 0.001). Fig. 5 shows that the LS treatment gave a higher C concentration than did the NG treatment in the whole soil and in the 250–2000 μm and 53–250 μm soil fractions. No differences between the treatments were found in the C concentrations in the <53 μm soil fraction. However, the C storage per hectare was not significantly affected by the treatments (p > 0.05) (data not shown).

### 3.1.4. Root biomass

Root biomass was significantly affected by the soil depth (p < 0.001). A higher amount of root biomass was found in the upper 25 cm of the soil than in the deeper soil layers (Fig. 6). In addition, it was observed that in the 50–75 cm soil depth, a significant effect of the treatments occurred (p < 0.05), with the root biomass being higher under the LS treatment than under the other treatments (HS and NG). In the other soil layers (0–25, 25–50, and 75–100 cm), a clear effect on the root biomass of the treatments that were applied was not seen.

### 3.2. Tree height and diameter

In Fig. 7, it can be observed that the tree height was not significantly affected by the treatments at the end of 2010 (p > 0.05), but it was significantly modified in 2011 (p < 0.05), where higher values were found with the NG treatment than with the LS treatment and the HS treatment. Moreover, the tree diameter at breast height was significantly affected by the treatments in 2010 (p < 0.05) and 2011 (p < 0.01). As was observed for the tree height, in both of the years of the study, the NG treatment caused a greater increase in the tree diameter at breast height than did the LS treatment and the HS treatment.

### 3.3. Multivariate analysis

In Fig. 8, it can be observed that in the first centimetres of the soil (0–50 cm) and the deepest soil layers (50–100 cm) of soil, C sequestration is related to the amount of roots. However, in the upper 50 cm of soil, the soil density was inversely related to the root biomass, which means that those treatments with high levels of root biomass (LS) are related to low soil densities and vice versa. Moreover, in all of the soil layers, an increment of the percentage of silt and clay implied a reduction in the percentage of sand.

### 4. Discussion

The soil C sequestration was differently affected by biological and physico-chemical characteristics depending upon the soil depth. A superficial soil C sequestration was more dependent on the biological activity of the trees, while a deep soil C sequestration was more affected by the proportion of the soil-size classes. In this experiment, it has been...
demonstrated that soil C sequestration depended on soil chemical characteristics, like the pH, the tree roots, and the litterfall inputs into the soil, together with the soil physical properties which changed with the soil depth profiles. This study was carried out in an acid soil, which could limit the microbial activity, and therefore, the mineralisation of SOM (Jobbágy and Jackson, 2000), the tree growth, and the pasture production (Whitehead, 2000). The higher soil pH in the upper soil layers when compared to the deeper ones could be due to previous lime and fertilisation inputs in the system, derived from the previous agronomic use of this soil (Bailey, 1995), as was found in the silvopastoral systems under *Populus canadensis* Moench (Mosquera-Losada et al., 2011) and *Quercus rubra* L. (Ferreiro-Domínguez et al., 2011), that were established on former agrarian lands. On the contrary, exclusive forest soil use leads to a higher pH in the deeper soil layers than in the upper ones, due to the lack of lime inputs in the above part of the soil and the meteorisation of the rock parent material (Adams et al., 2001; Rigueiro-Rodríguez et al., 2011a).

The greater presence of roots in the upper soil layers than in the deeper ones that were observed in this experiment, probably explains the lower soil density and the silt and clay proportions, with a higher proportion of sand and SCS (g C kg$^{-1}$ and Mg C ha$^{-1}$) in the upper soil layers when compared to the deeper ones (Jobbágy and Jackson, 2000; Six et al., 2000; Dawson et al., 2001; Cresswell and Hamilton, 2002; Howlett et al., 2011a,b; Mosquera-Losada et al., 2011). In the top layer of the soil, the high proportion of root biomass could have favoured the downward displacement of finer soil particles, by increasing the presence of biopores (Jobbágy and Jackson, 2000) in the soil surface layers (cylindrical macro-pores formed by soil biota and decaying plant roots), which favour the movement of small particles into the deep soil layers. In several studies, it has been observed that the soil bulk density at the surface is increased by the presence of live-stock, which compacts the soil (Greenwood and McKenzie, 2001; Sharrow, 2007). However, this effect was not observed in our study, probably due to the low number of animals used in this experiment and the higher proportion of roots found in the upper soil layers when grazing was applied when compared with the NG treatment (He et al., 2011).

In this experiment, the C storage per hectare in the upper 25 cm of soil ranged from 73 Mg C ha$^{-1}$ to 82 Mg C ha$^{-1}$ in the whole soil. However, in the agroforestry study of Howlett et al. (2011b), the values

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**Fig. 6.** Soil roots expressed in Mg roots ha$^{-1}$ with the three treatments (LS: Light Stocking Rate, HS: Heavy Stocking Rate, and NG: No Grazing) at four soil depths (0–25, 25–50, 50–75, and 75–100 cm). Different uppercase letters indicate significant differences between the soil depths within the same treatment, while different lowercase letters indicate the differences between the treatments within a specified soil depth. Horizontal lines indicate the mean standard error.

**Fig. 7.** Tree height (m) (a) and tree diameter at breast height (cm) (b) with each treatment (LS: Light Stocking Rate, HS: Heavy Stocking Rate, and NS: No Grazing) in 2010 and 2011. Different letters indicate significant differences between the treatments within the same year. Vertical lines indicate the mean standard error.
ranged from 80 Mg C ha$^{-1}$ to 102 Mg C ha$^{-1}$. These differences between these two studies could be due to the higher soil bulk density in the experiment by Howlett et al. (2011b) (1150 kg m$^{-3}$) than in ours. This is probably due to the greater tree biomass of Pinus radiata D. Don and Betula pendula Roth. in the study of Howlett et al. (2011b) when compared to the biomass of Prunus avium L., which favoured SCS due to the greater stabilisation of C over the long term through the formation of stable organo-mineral complexes (Hassink, 1997). On the contrary, the C storage per hectare was lower in the soils of Howlett et al. (2011b) in the 25–75 cm soil depths than in our soils, probably due to the higher soil bulk density of our soils when compared to those of Howlett et al. (2011b) in the deep soil layers. This may also be due to a clear effect of soil management. In the experiment by Howlett et al. (2011b), no ploughing was performed after the radiata pine plantation, which meant that the surface soil roots were not damaged and the trees were not forced to put their roots into the deeper soil profiles, and therefore, the soil density was maintained, which did not happen in our study and was also demonstrated by Dupraz et al. (2005). On the other hand, the highest C concentration and the C storage per hectare as observed in this study were also found in the upper 50 cm of soil that were linked to root biomass. This mainly occurred in the soil macroaggregates (250–2000 μm), due to the higher presence of...
of sand at this depth and the preferential stabilisation of SOM in the macroaggregates, rather than in the microaggregates, as was found in the study by Howlett et al. (2011a) of an agroforestry system under cork oak. It is highly relevant to bear in mind that the C associated with macroaggregates is also important in SCS, due to the process of macroaggregate turnover, which has an indirect effect on the microaggregate formation (Six et al., 2004), allowing the soil C to be allocated over the longer term than in the macroaggregates, and therefore, contributing to a more stable C. However, in the deeper soil layers, the C concentration and the C storage per hectare mainly occurred in the microaggregates (53–250 μm) and were linked to soil density and the development of the presence of root biomass in these layers. This addition of deep organic matter by root biomass plays an important role in SCS. Deep SCS is one of the main advantages of agroforestry systems when compared with traditional agricultural systems (Sharrow and Ismail, 2004; Mosquera-Losada et al., 2011).

Regarding the effect of the treatments that were established, the presence of animals in silvopastoral systems usually affects the soil properties such as the soil pH (Whitehead, 2000), the soil bulk density (Sharrow, 2007), and the SCS (Schuman et al., 1999; Reeder and Schuman, 2002; He et al., 2011), when compared with ungrazed systems. In this study, the higher values of soil pH were found with the NG treatment than with the LS treatment and the HS treatment in all of the soil depths, with the exception of the lack of differences between the treatments in the 0–25 cm soil layer. The addition of ammonium from the urea in animal urine in grazed treatments may decrease the soil pH due to the increment of the nitrification rate, and therefore, the release of H⁺ (Whitehead, 2000). However, this difference was not found in the upper 25 cm of the soil. This could be explained by the fact that soil pH is probably more affected by the former lime and current litter inputs (and therefore calcium) at this depth than in the deeper soil layers. Moreover, the root biomass in the deeper soil layers was significantly more developed in the grazed (LS) treatment than in the ungrazed system (NG), probably due to the higher availability of nitrate. Increased nitrate availability in the deeper soil layers could also cause an increase in the uptake of cations by the tree roots, and therefore, an important recycling of cations from the deeper to the surface soil layers when tree leaves fall in autumn, superficially reducing the difference in the soil pH between grazed and ungrazed treatments.

As previously mentioned, the root distribution in the soil depth profiles could be explained by the fact that the highest proportion of sand was found in the 50–75 cm soil layer with the LS treatment and the highest proportion of small particles was found in the 75–100 cm depth, while on the contrary, the NG treatment showed the highest percentage of sand at this depth (75–100 cm). However, the higher values of bulk density in the NG treatment could also be explained by the higher tree growth with this treatment than with the grazing treatments, which may increase soil compactness due to the tree biomass and the transmission of wind forces through it to the soil (Greacen and Sands, 1980). Furthermore, in the NG treatment, the control of tree-understory competition was performed with heavy equipment, which also generates downward pressures on the soil surface, increasing the soil bulk density with this kind of treatment (Greenwood and McKenzie, 2001). The chemical and mechanical control of tree-understory competition could also explain the higher tree growth with the NG treatment due to the lower competition between the trees and between the trees and understory (Dawson et al., 2001). In the same way, this lower competition could also allow the trees to develop their root systems in the upper soil layers, rather than in the deeper ones, leaving less root biomass in deeper layers with this treatment than in the grazed treatments. Dawson et al. (2001) found a similar result with sweet cherry, which showed a higher amount of tree roots in the upper soil layer after the application of herbicide to control grasses than without any chemical control. The energy used by the tree in developing a large and deep root system in grazing treatments could explain the lower tree growth rate, which could later promote tree growth and should therefore be further evaluated. In any case, wild cherry showed an increase of 1 m during the studied years, which is a really good growth rate for this species (Horgan et al., 2003).

The C concentration and the C storage per hectare were affected by treatments as a result of the previously described effects on the pH, the soil bulk density, the root biomass, and the soil fraction distribution through the soil profiles. A better root biomass development and the addition of organic matter by animal excreta in the LS treatment increased the C concentration (Holland and Detling, 1990; Schuman et al., 1999) in the whole soil and in the 53–250 μm soil fractions in the upper 25 cm of the soil, together with all of the soil fractions in the 25–50 cm soil layer when compared with the NG treatment. In the top layer of the soil, despite the higher inputs of animal excreta and urine to the soil, and therefore N, in the HS treatment more than in the LS treatment, the effect of the HS treatment on the SCS was similar to the effect of the NG treatment. This was probably due to the acceleration of the mineralisation rate of the SOM in the HS treatment. Finally, the C storage per hectare followed the same trend as that previously described for the C concentration, except in the 0–25 cm soil depth, where significant differences between the treatments were not found. The increment of C in the large fractions (250–2000 μm and 53–250 μm) of the LS treatment represents an important accumulation of new C in the soil, and in addition, the presence of C in the small fraction (<53 μm) also indicates that the LS treatment favours the accumulation of stable SOM (Christensen, 2001). The SOM in the NG treatment was lower than in the other treatments, in spite of the higher tree biomass production in the NG treatment. A possible explanation for the lower C concentration and C storage per hectare in the NG treatment in the upper 50 cm of soil could be explained by the low organic matter inputs from the herbaceous vegetation roots in the soil due to the chemical and mechanical control of the herbaceous understory — but also by the higher pH in the NG treatment than in the grazed treatments, which increases organic matter mineralisation (Whitehead, 2000), and decreases SCS (Six et al., 2000).

On the other hand, in the 50–75 cm soil depth, the NG treatment showed higher values of SCS (g C kg⁻¹ soil, and Mg C ha⁻¹) than did the HS treatment in the whole soil and in the 53–250 μm and <53 μm soil fractions. The higher SCS in the NG treatment at this depth could be explained, as this treatment tended to have a higher proportion of finer soil particle fractions (silt and clay) when compared with the HS treatment. This fact explains why the levels of SCS in the whole soil were higher with the NG treatment than in the HS treatment, as the contribution of C linked to silt and clay and to the whole soil represented almost 75% of the whole soil C.

Moreover, no differences were found between the treatments of C concentration in the deepest soil layer (75–100 cm), and the NG treatment showed a better promotion of C storage per hectare in the whole soil and in the 53–250 μm soil fractions. This followed the same trend as in the 50–75 cm soil depth, probably due to the higher soil density.

In the end, in the range of 1 m of soil (0–100 cm), it is important to be aware that the LS treatment enhances more SCS, when expressed as grams of carbon per kilogramme of soil, than did the NG treatment in the whole soil and in the 53–250 μm and <53 μm soil fractions. This is due to the increment of organic matter inputs from the upper to the deeper soil depths. However, in the small fraction (<53 μm), there was no difference between the grazed and ungrazed treatments. However, the C storage per hectare at a depth of 1 m was not modified by the treatments, because in the NG treatment, the higher soil density due to the lower disturbance of the deep soil layers compensates for the increased root biomass that occurs with the LS treatment.

5. Conclusion

The presence of trees plays an important role in SCS which could be modified by the presence of livestock by direct or indirect chemical (pH) and physical (soil bulk density and soil fractions) modification of
the soils. The major SCS was found in the first 50 cm of soil and it was linked with macroaggregates and occurred in the LS treatment due to the greater presence of root biomass and organic matter additions by animal excreta than in the NG treatment. In the deeper soil layers, the major SCS was related to microaggregates and was enhanced by the NG treatment due to a higher soil bulk density and the indirect effect of the greater presence of roots in the LS treatment than in the NG treat-

ment. However, it is relevant to bear in mind that this high presence of tree roots in the LS treatment opens up biopores and adds SOM at larger depths, increasing SOC over time, which would lead to an SCS pool that would be stable over the long term.

Further studies should be carried out to evaluate the global C storage per hectare as a result of increased soil C concentration in the larger soil size fractions in grazed systems.

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