



How do management techniques affect carbon stock in intensive hardwood plantations?



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ARTICLE INFO

Article history:

Received 12 September 2016

Accepted 29 November 2016

Keywords:

Walnut
Silvopastoral systems
Legumes
Soil organic matter
Fine root biomass
Tree biomass increment
Mineral fertilisation

ABSTRACT

Recent studies in temperate regions have shown that agroforestry systems, especially silvopastoral systems, have greater carbon (C) sequestration potential than monocropping systems or pastures, or even forest plantations. In Europe, there is growing interest in establishing high quality wood plantations with intensive management comprising irrigation, fertilisation and chemical weed control to reduce rotation length. However, these operations can have major environmental impacts similar to the effects of intensive agriculture, such as impoverishment of soil C. The aim of this study is to identify optimum management practices for intensive systems of quality wood production to optimise soil C stock and plantation productivity. An experiment was conducted in Extremadura, mid-west Spain, from 2011 to 2014, in a 13-year-old hybrid walnut (*Juglans major* × *regia* mj 209xra) plantation with a density of 333 trees ha⁻¹. Two essays were established: one with three techniques to control competition from herbaceous strata beneath trees – mowing, ploughing and sheep grazing (1 sheep ha⁻¹) – and the other to test implementation of legumes (mixture of *Trifolium michelianum* and *Ornithopus compressus* complemented by the same quantities of phosphorous and potassium as mineral treatment) as an alternative to traditional mineral fertilisation (40 kg N ha⁻¹, 40 kg P₂O₅ ha⁻¹ and 50 kg K₂O ha⁻¹). The C stock estimate was based on soil organic carbon (SOC) and aboveground (tree trunks and branches) and belowground biomass (tree and pasture roots). Most of the C stock was contained in SOC, at 50% in the uppermost soil layer (0–25 cm), followed by aboveground biomass. The response of SOC in each treatment was higher than the other parameters analysed, suggesting that SOC is a more sensitive pool to management techniques. Grazing as control of herbaceous vegetation and legume implementation as nitrogen supply are suitable techniques for optimising soil C stocks and also achieve adequate tree growth in the longer term.

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1. Introduction

The importance of C sequestration in the land use scenario lies in its potential as a climate change mitigation strategy (Nair, 2012; IPCC, 2014). The Climate Change 2007 Synthesis Report (IPCC, 2014) proposed several management strategies in the agricultural sector to mitigate CO₂ concentrations in the atmosphere, including cropland and grazing land management and restoration of organic soils. The most cost-effective mitigation options in forestry are afforestation, sustainable forest management and deforestation reduction, although their relative importance differs greatly across regions.

Europe has a shortage of quality wood, resulting in a growing interest in the establishment of hardwood plantations. In Spain, hardwood species are commonly harvested after long rotations of up to 50 or 60 years, although intensive management including irrigation, fertilisation and chemical weed control can reduce rotation length by half (to 20–25 years) (Rigueiro-Rodríguez et al., 2009). However, these operations can have major environmental impacts similar to intensive agriculture, such as impoverishment of soil C (Babcock et al., 2003). Sustainable forest management must be applied using multicriteria objectives that optimise both increasing biomass and C sequestration (Lal, 2005; Bravo et al., 2008). Management systems that maintain a continuous canopy cover and mimic regular natural forest disturbance are likely to achieve the best combination of high wood yield and C storage (Lal, 2005; Jandl et al., 2007).

Recent studies in temperate regions have shown that agroforestry systems, especially silvopastoral systems (integrated

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land-use systems combining wood production and pasture production) have greater C sequestration potential than open fields (crops or pastures) (Dube et al., 2012), mainly in the uppermost soil layer, due to increased C input with rhizodeposition and aboveground residue return (Ramesh et al., 2015). Agroforestry systems have attracted special attention in this regard because of the perceived advantages of the large volume of aboveground biomass and deep root systems of trees (Nair, 2012). Dube et al. (2012) observed that individual trees in silvopastoral systems sequestered nearly 30% more C in total biomass than in a pine plantation, as tree growth in the silvopastoral system was enhanced by lower tree competition, resulting in larger amounts of C being sequestered.

In intensive hardwood plantation, control of competing herbaceous vegetation is required to avoid tree-herbage competition for soil resources and fire risk. Grazing controls understorey vegetation. Its intensity may affect C stocks by modifying net C flows from the atmosphere to vegetation and soil as a result of changes to the amount, plant type composition and decomposition rates of residual plant material. Under grazing conditions, the residence time of aboveground C is very short (10–50 days). It varies according to the probability of defoliation and digestion of leaf tissues and the associated release of C into the atmosphere. In contrast, the residence time of belowground C is long (c. 1 to >1000 years) in grassland ecosystems (Soussana and Lemaire, 2014).

Implementation of forage legumes as an alternative to mineral fertilisation could lower the economic costs of high quality intensive wood plantations, increase the available nutrients in soil (especially N), improve pasture production and quality, and optimise the environmental functions of plantations, i.e. provide soil cover to control erosion (Gabriel and Quemada, 2011; McCartney and Fraser, 2010; O'Dea et al., 2015). López-Díaz et al. (2014) observed an improvement in N availability in soil by almost 200% compared to the control. No competition for soil water and nutrients by forage legumes was noted with legume presence.

Forest ecosystems store more than 80% of all terrestrial aboveground C and more than 70% of all SOC (Montero et al., 2005). It is well known that soils are the largest reservoir of C in territorial ecosystems. Consequently, changes to soil C sequestration, whether positive or negative, could significantly alter atmospheric CO₂ concentrations and impact the global climate of the future (Lal, 2005; Ciais et al., 2013; Riggs et al., 2015). Forest soil C stock can be increased through forest management, which includes site preparation, fire management, afforestation, species management/selection, use of fertilisers and soil amendments. Changes in SOC due to management practices are difficult to quantify because they occur slowly, as claimed by Ramesh et al. (2015). Damien et al. (2015) observed that seven years of grazing at varied intensities modified vegetation but not soil C stocks. Soil C storage is important not only because of its role in the global C cycle, but also because it affects forest productivity, as soil C is a principal source of energy for nutrient recycling (Nave et al., 2010).

Patterns of aboveground biomass distribution in terrestrial ecosystems are reasonably well understood, whereas knowledge of belowground biomass and its distribution is still limited due to methodological difficulties in determining fine root biomass (Finér et al., 2011). Other researchers found that belowground biomass is a defined portion of aboveground biomass, reporting values of 25–40% depending on factors such as the nature of the plant and its root system and ecological conditions (Montero et al., 2005; Alfás et al., 2015).

One aspect of the organic C pool that remains poorly understood is the vertical distribution of fine roots in the soil and accompanying relationships with climate and vegetation (Jobbágy and Jackson, 2000). Finér et al. (2011) reported that fine roots are very dynamic and play a key role in forest ecosystem C and nutrient cycling and accumulation.

The aim of this study is to evaluate the profitability of alternative techniques of control of competing vegetation and fertilisation in intensive hardwood plantations and the implications of these techniques in the C stock of the systems, raising the following research questions:

1. How does the type of treatment (traditional or alternative) affect SOC? The management system with least disturbance was expected to maintain highest soil C storage.
2. How does the type of treatment (traditional or alternative) modify the productivity of the system (i.e. quality timber production) in the short and long term? The productivity of alternative techniques would be similar to or better than traditional (and more intensive) practices in the medium and long term.
3. What is the importance of fine roots as a C sink under the various treatments? This parameter is not usually evaluated and could significantly increase C stock of forest plantations.
4. Which is the most important component (aboveground or belowground biomass, soil) as a C pool in an intensive forest plantation? Soil could be the most important C sink of agricultural and forestry systems.
5. Which treatments maximise the potential of silvopastoral systems as C sinks? Treatments that improve the most important C pool of these systems should primarily be taken into account to maximise their potential as C sinks.

2. Materials and methods

2.1. Study site characteristics

The experiment was conducted from 2011 to 2014 in northern Extremadura, mid-west Spain (ETRS89 Zone 20: X:298.303 Y:4.442326; 309 m a.s.l.), in a 13-year-old hybrid walnut (*Juglans major* × *regia* mj 209xra) plantation, characterised by fast growth and scarce fruit, with a density of 333 trees ha⁻¹. Trees were planted in 1998. Before planting, the land use was agricultural (maize). At the beginning of the essay, mean height and diameter at breast height (DBH) were 8.33 m and 17.8 cm, respectively.

The area is in the Mediterranean biogeographic region (European Environment Agency, 2006). Mean annual precipitation is 952 mm and mean annual temperature is 15.6 °C. A period of drought usually occurs from June to September. The experiment was performed in a sandy loam soil more than 140 cm in depth with less than 5% slope. Initial soil analyses revealed an acidic pH (pH 5 in water) and medium SOC levels (2.6%). Soil characteristics and history are similar.

2.2. Experimental design

Two essays were established: one experiment with three techniques to control competition from herbaceous strata beneath trees (“Grazed Walnut”), and the other to test alternatives to traditional inorganic fertilisation (“Fertilised Walnut”).

The treatments (Table 1) to control competing vegetation (Grazed Walnut) were applied in early spring for three years (2012–14): a) mowing understorey vegetation (herbage); b) ploughing; and c) grazing (introducing a stock of 1 sheep ha⁻¹). In all plots mineral fertilisation was applied (NPK: 40 kg N ha⁻¹, 40 kg P₂O₅ ha⁻¹ and 50 kg K₂O ha⁻¹) in autumn. Doses were based on tree requirements. Plots that were mowed and ploughed were fenced to prevent grazing.

In the Fertilised Walnut essay, three treatments (Table 1) were applied for four years (2011–14): d) inorganic fertilisation: application of 40 kg N ha⁻¹, 40 kg P₂O₅ ha⁻¹ and 50 kg K₂O ha⁻¹, e) legume sowing (complemented by the same quantities of PK as a

Table 1
Description of the essays and treatments.

Essay	Treatments	Description
Grazed Walnut (In all plots mineral fertilisation (NPK) was applied in autumn: 40 kg N ha ⁻¹ , 40 kg P ₂ O ₅ ha ⁻¹ and 50 kg K ₂ O ha ⁻¹)	Mowing Ploughing	
Fertilised Walnut (Sheep were introduced in late spring after the grass had dried)	Grazing Inorganic fertilisation Legume sowing No fertilisation	- Introducing a stock of 1 sheep ha ⁻¹ - Application of 40 kg N ha ⁻¹ , 40 kg P ₂ O ₅ ha ⁻¹ and 50 kg K ₂ O ha ⁻¹ - Application of the same quantities of PK - Sowing of 25 kg ha ⁻¹ <i>Trifolium michelianum</i> and 10 kg ha ⁻¹ <i>Ornithopus compressus</i> after ploughing No fertilisation or sowing

mineral treatment), and f) no fertilisation treatment (no fertilisation or sowing). In the treatment legume sowing, in October 2011 and 2013, a mixture of 25 kg ha⁻¹ *Trifolium michelianum* and 10 kg ha⁻¹ *Ornithopus compressus* was sown under the trees after ploughing. The whole area was fenced to prevent grazing. Sheep were introduced in late spring after the grass had dried.

Nine replicate blocks were used for each of the six treatments (54 plots in total) in a completely randomised design. Each plot (95 × 15 m) had three alleys and two rows of 20 trees.

2.3. Field sampling

Variations in C stock were calculated from SOC and aboveground biomass of trees (stem and branches), and belowground biomass (fine roots of herbaceous and trees and thick roots of trees), all based on data collected in 2014. Fifty four soil cylinders (six per treatment; 6 cm diameter) were extracted every 10 cm to 1 m depth to assess SOC content and fine roots. Roots were separated into tree (black) and pasture (white), dried and weighed to obtain the belowground biomass. To determine SOC content, soil samples were taken every 10 cm depth from the same cylinders used to measure roots. Soil samples were air dried, sifted through a 2 mm sieve, and gravels (>2 mm) and fine earth weighed separately after oven dried (105°, 24 h) to obtain soil bulk density. Carbon content was measured by the Walkley-Black method (Walkley and Black, 1934).

Tree diameter at breast height was measured from 2011 to 2015 every January. To determine the relation between DBH and tree biomass, 12 trees with DBH of 11 to 22 cm were felled in December 2014. Stems and thick and fine branches were weighed and the humidity percentage was determined to calculate dry weight. Allometric equations were fitted to these data (linearised least squares regression) using models according to the formulas of Montero et al. (2005) as explained below. Carbon sequestered by vegetation was calculated by multiplying the aboveground and belowground (fine roots) biomass by 0.5 (Nair, 2012).

2.4. Determination of soil organic carbon

Carbon content vs. depth was measured in percentage of total mass. Thus, if $S(z)$ is the C content (in %) at depth z , the total amount of C (C) in Mg ha⁻¹ between depths z_1 and z_2 is given by

$$C = \int_{z_1}^{z_2} \rho(z)S(z)dz,$$

where ρ is the soil bulk density (the range was 1.10–1.49 Mg m⁻³). The volumetric ratio of gravels was taken into account. As measures of SOC were available only at specific depths from 0 to 100 cm, to estimate the integral, the values were interpolated before numerical integration using trapezoidal rule.

2.5. Determination of aboveground and thick root biomass

To parameterise the relation between dry weight (W) and DBH (d) for the aboveground of walnut trees, the procedure used by Montero et al. (2005) was followed, fitting an allometric curve in the form

$$W = Ad^b,$$

where A and b are the fitting parameters.

The linearised fit yielded a very high determination coefficient ($R^2 = 0.93$) and very significant values for the parameters ($p < 0.001$ for the slope and $p < 0.001$ for the intercept). The allometric parameters were obtained from the values of this fit. Table 2 compares these parameters with the values obtained by Montero et al. (2005) for hardwood trees.

The values of the exponent, the parameter that defines the rate of dry weight increase, were very similar.

As the coarse roots of walnut trees were not sampled, for the estimate of the values of the parameters for this data it was assumed that the ratios between the two fits (aboveground biomass and thick roots) obtained by Montero et al. (2005) for hardwood trees were the same for the walnut trees in this study.

Because this ratio changes with tree size, the quotient between the two allometric fits obtained by Montero et al. (2005) was applied.

$$R = \frac{A_r d^{b_r}}{A_T d^{b_T}} = C d^{b'},$$

where $C = A_r/A_T$ and $b' = b_r - b_T$.

From Montero et al. (2005), the values $C = 1.637417$ and $b' = -0.33079$ were obtained, giving the following estimate for the walnut tree allometric parameters:

$$A_r = C \times A_T = 1.637417 \times 0.08792737 = 0.1439738$$

$$b_r = b' + b_T = -0.33079 + 2.33298190 = 2.002192.$$

2.6. Statistical analysis

The effects of fertilisation and control of herbaceous understorey on SOC content, aboveground biomass and fine roots and

Table 2
Comparison between the allometric fit to the data and the values obtained by Montero et al. (2005) for hardwood trees.

Parameter	Walnut fit	Montero et al. (2005)
A	0.0879274	0.153338
b	2.33298	2.29843
SEE	0.134491	0.014718

were determined using Analysis of Variance (ANOVA). Where ANOVA yielded statistically significant differences ($p < 0.05$), a least squares difference (LSD) test was used for subsequent pairwise comparisons if ANOVA was significant. All statistical analyses were performed with the R software (R Development Core Team, 2011).

3. Results

3.1. Soil organic carbon

A sharp decrease in SOC with depth (z) (Fig. 1) was found in all cases. The decrease followed an exponential decay law

$$SOC(z) = b_0 + b_1 \exp(-z/d_1),$$

where b_0 is an offset describing SOC at greater depths, b_1 depends on the SOC content at the surface, and d_1 is a characteristic depth regulating the decay rate. For any given fraction p of the SOC content increment at the surface with respect to the baseline level, (i.e. $S_0 = SOC(0) - b_0$), the depth (d) at which this amount is pS_0 is obtained from the characteristic depth d_1 via the formula $d = d_1 \ln(1/p)$. Thus for a characteristic depth of 25 cm, the depth at which SOC is just 20% of the surface amount would be approximately 40 cm ($d = 25 \times \ln(1/0.2) \approx 40.23$).

Comparing each depth (Fig. 1), herbaceous vegetation control treatments (Grazed Walnut) significantly modified this parameter

in the first 50 cm depth. In this layer (0–50 cm depth), mowing recorded the highest SOC content ($2.46 \pm 0.14\%$ at 0–10 cm depth, $1.61 \pm 0.05\%$ at 10–20 cm depth and $0.95 \pm 0.09\%$ at 40–50 cm depth), but was similar to grazing ($2.06 \pm 0.08\%$ at 0–10 cm depth, $1.48 \pm 0.10\%$ at 10–20 cm depth and $0.75 \pm 0.11\%$ at 40–50 cm depth) and greater than ploughing ($1.86 \pm 0.10\%$ at 0–10 cm depth; $1.46 \pm 0.13\%$ at 10–20 cm depth; and $0.57 \pm 0.13\%$ at 40–50 cm depth). Below 50 cm, there was a depletion of SOC (0.93% at 50–75 cm depth) and values were similar in all treatments. No change in SOC content was noted with fertilisation treatments.

From these comparisons, an exponential decay curve was fitted for each treatment in Grazed Walnut essay and as there were no statistically significant differences between treatments, a single exponential decay curve was fitted for the Fertilised Walnut experiment. All fits were performed using the nonlinear least squares Levenberg-Marquardt algorithm implemented in the minlapack.lm package of the R statistical software (Elzhov et al., 2013) Table 3.

The values of the offset b_0 are very small, but positive, which could seem unrealistic, because the SOC content should be zero for greater depths. However, these values are an artefact of the nonlinear fit, caused by the lack of data at greater depths. The values of b_1 are determined, as stated above, by SOC at the surface ($b_0 + b_1 = S(0)$). The most important parameter, because it defines the shape of the SOC vs. depth profile, is characteristic depth, d_1 . The greater the characteristic depth is, the deeper relevant amounts of

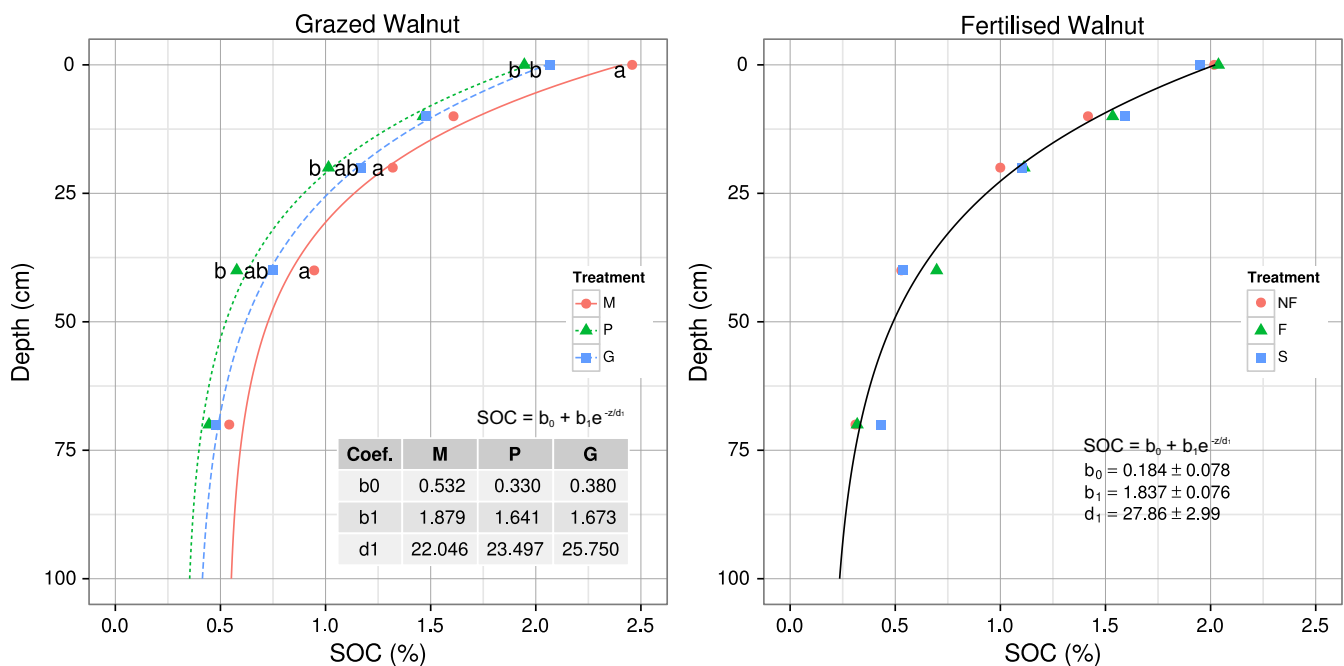


Fig. 1. Mean values \pm standard error of soil C content (SOC, %) under fertilisation treatments of control of herbaceous vegetation (Grazed Walnut) and fertilisation treatments (Fertilised Walnut). M: mowing; P: ploughing; G: grazing; NF: no fertilisation; F: inorganic fertilisation; S: legume sowing. Different letters indicate significant differences ($p < 0.05$) between treatments at each depth.

Table 3

Results of the Levenberg-Marquardt fit. Parameter estimations and standard errors are given for each experiment. R^2 are computed assuming normal distribution of residuals.

Parameter	Grazed Walnut			Fertilised Walnut
	Mowing	Ploughing	Grazing	All treatments
b_0	0.532 ± 0.171	0.33 ± 0.093	0.33 ± 0.093	0.184 ± 0.078
b_1	1.879 ± 0.187	1.64 ± 0.099	1.673 ± 0.055	1.837 ± 0.076
d_1	22.045 ± 5.697	23.49 ± 3.679	25.75 ± 2.175	27.86 ± 2.99
R^2	0.9842	0.9945	0.998	0.998

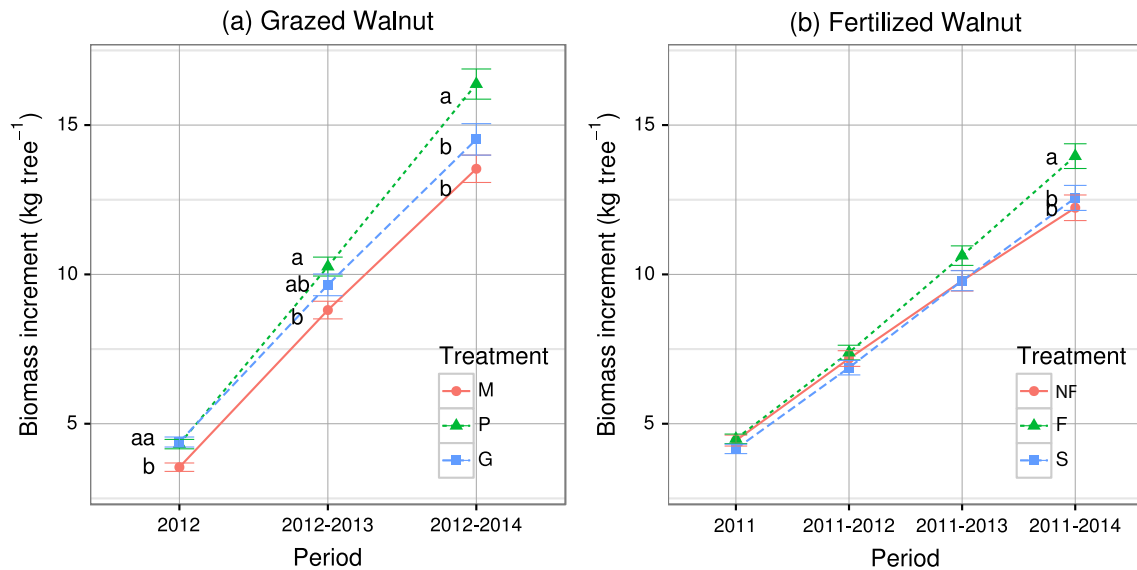


Fig. 2. Mean values \pm standard error of aboveground biomass increment (kg tree^{-1}) (a) under treatments of control of herbaceous vegetation (Grazed Walnut) and (b) under fertilisation treatments (Fertilised Walnut). M: mowing; P: ploughing; G: grazing; NF: no fertilisation; F: inorganic fertilisation; S: legume sowing. Different letters indicate significant differences ($p < 0.05$) between treatments in each period.

SOC content will be found. However, d_1 values were very similar among treatments.

3.2. Aboveground biomass

Fig. 2 shows that the highest increment of tree biomass were obtained with ploughing, where the effects increased with time ($16.37 \pm 0.50 \text{ kg aboveground biomass increment}$ since the beginning of the essay; $p < 0.001$), followed by grazing ($14.52 \pm 0.53 \text{ kg}$) and mowing ($13.54 \pm 0.46 \text{ kg}$).

In the Fertilised Walnut essay, the biomass increment detected with inorganic fertilisation ($13.96 \pm 0.41 \text{ kg}$ after three years) (Fig. 2) was significantly higher than the values observed with legume sowing ($12.56 \pm 0.41 \text{ kg}$) and no fertilisation treatment ($12.23 \pm 0.43 \text{ kg}$) ($p = 0.001$). Considering only the increment in the third year, trees over legumes improved tree growth ($2.77 \pm 0.11 \text{ kg}$) more than no fertilisation ($2.44 \pm 0.11 \text{ kg}$) but less than inorganic fertilisation ($3.33 \pm 0.18 \text{ kg}$) ($p < 0.001$).

3.3. Fine root biomass

When the distribution of pasture root weight in the soil profile (Fig. 3) is analysed, Grazed Walnut treatments can be ranked ($p < 0.001$) as follows: grazing (0.05 ± 0.01 – $0.31 \pm 0.05 \text{ kg m}^{-3}$), mowing (0.03 ± 0.01 – $0.16 \pm 0.03 \text{ kg m}^{-3}$) and ploughing (0.01 ± 0.00 – $0.05 \pm 0.01 \text{ kg m}^{-3}$). Differences between treatments were observed mainly in the upper 30 cm of the soil profile, where most pasture roots develop. In contrast, tree roots, which accounted for most of the root weight, were not significantly affected by the treatments. Consequently, no significant differences were detected in the whole root systems. In Fertilised Walnut, tree root biomass was not affected by treatments. Moreover, it seems that the weight of pasture roots under inorganic fertilisation and legume sowing were higher than in the no fertilisation treatment in the uppermost 30 cm, but there were no significant differences among Fertilised Walnut treatments. Overall, considering the whole root biomass of the upper metre of the soil profile, there were no significant differences among treatments in the two essays (data not shown).

3.4. Carbon stock of the system

Respect to the carbon stock of the system, in the Grazed Walnut essay (Fig. 4), mowing produced the highest SOC stock ($105.83 \text{ Mg ha}^{-1}$) and, then, the highest whole C stock ($132.05 \text{ Mg ha}^{-1}$), although this was only a tendency ($p = 0.09$). Mowing was followed by grazing ($116.49 \text{ Mg ha}^{-1}$) and ploughing ($107.62 \text{ Mg ha}^{-1}$), as grazing produced higher SOC stock (91.2 Mg ha^{-1}) than ploughing (81.49 Mg ha^{-1}). For the other parameters measured, no differences were observed among treatments for aboveground biomass (14.72 – 15.22 Mg ha^{-1}), thick roots (8.99 – 9.26 Mg ha^{-1}) or fine roots (1.58 – 1.74 Mg ha^{-1}).

In the other essay (Fertilised Walnut) (Fig. 5), legume sowing produced the highest C stock ($101.18 \text{ Mg ha}^{-1}$), similar to inorganic fertilisation ($100.35 \text{ Mg ha}^{-1}$), and SOC (82.64 Mg ha^{-1} and 81.4 Mg ha^{-1} , respectively). No fertilisation treatment recorded 91.26 Mg ha^{-1} .

In all cases, C stock in vegetation biomass (aboveground and belowground) with the three treatments were very similar. Improvement in tree growth was not observed in the representation of accumulated C of aboveground biomass because the different initial tree size masked the effect of treatments on trees. Carbon stock in aboveground biomass with legume treatment was slightly lower than under no fertilisation, due to the higher initial diameter of the no fertilised plots. Improved C stock with legumes is likely to continue, as legume implementation increased tree biomass in the final year and N supply by legumes occurs gradually.

4. Discussion

4.1. Response of SOC to management

Soil organic carbon storage to 1 m soil depth (73.1 – $105.83 \text{ Mg ha}^{-1}$) was similar to values reported in other agroforestry systems with deciduous species. Peichl et al. (2006), in an agroforestry system with hybrid poplar, found 79 Mg ha^{-1} . The values in this study are higher than the range detected by Howlett et al. (2011) (27 – 50 Mg ha^{-1}) in a cork oak dehesa in central western Spain (annual precipitation: 500 mm ; mean annual

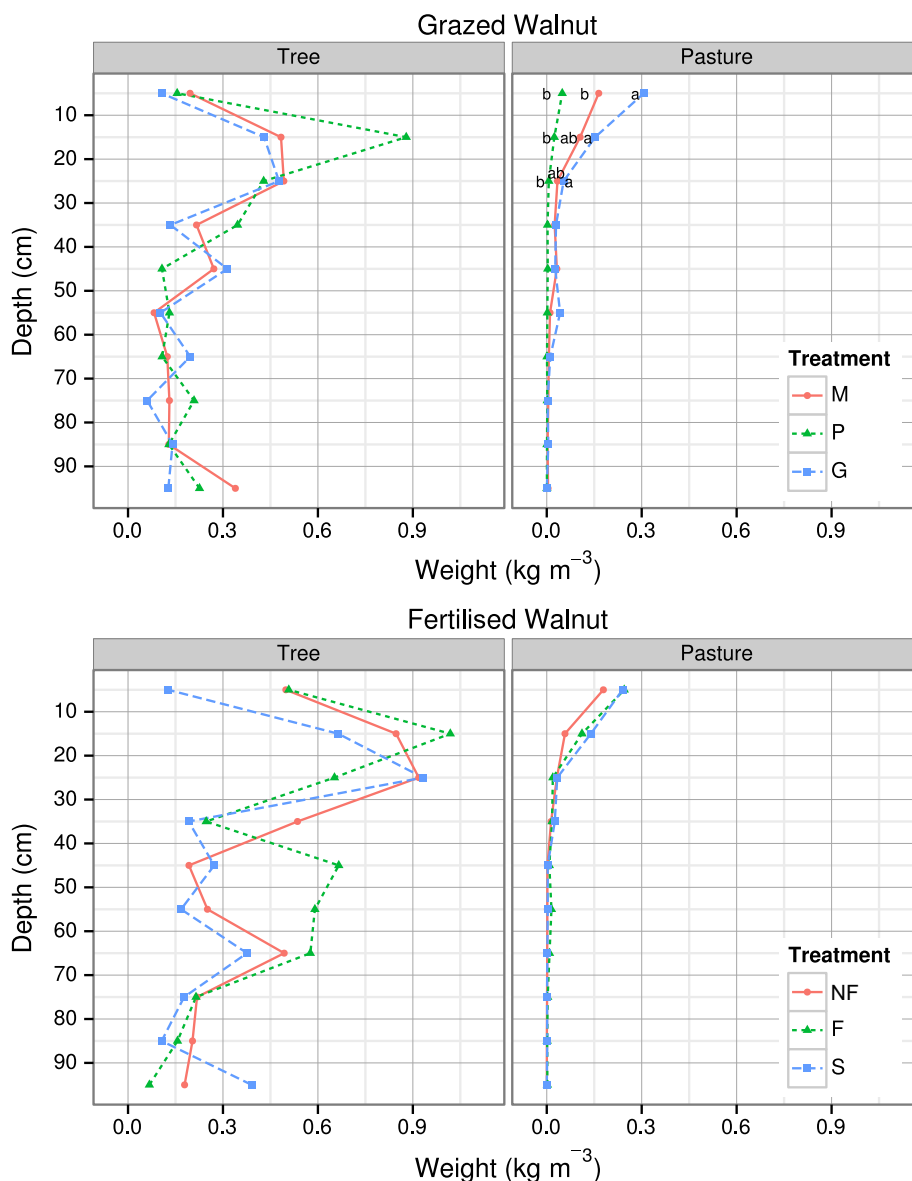


Fig. 3. Mean values \pm standard error of tree and pasture fine root weight (kg m^{-3}) at various depths under treatments of control of herbaceous vegetation (Grazed Walnut) and fertilisation treatments (Fertilised Walnut). M: mowing; P: ploughing; G: grazing; NF: no fertilisation; F: inorganic fertilisation; S: legume sowing. Different letters indicate significant differences ($p < 0.05$) between treatments at each depth.

temperature: 16.1°), because cooler and wetter climates such as the location of this experiment have greater potential to sequester C (Jobbágy and Jackson, 2000; Lal, 2005).

Mowing recorded the highest SOC content, due to incorporation of debris, followed by grazing and ploughing. Several authors (Jandl et al., 2007; Soussana and Lemaire, 2014; Triberti et al., 2016) noted that intensive site preparation, such as ploughing, can favour the loss of SOC because it stimulates decomposition of the forest floor and mineralisation of SOC, despite favouring biomass production, at least in the short term. The difference among treatments was maintained until 40–50 cm depth, even though the depth of ploughing was about 25 cm. Valboa et al. (2015) concluded that after five years of treatments, tillage at different depths affected SOC content below the bottom of the tilled layer. This was explained by the compacted layer produced by ploughing, with possible restriction of water vertical flow and root deepening.

Grazing produced similar SOC content to mowing. Earlier studies found divergent effects of grazing on SOC. McSherry and Ritchie (2013) showed that grazer effects are highly context-specific. Graz-

ing effects may shift from negative to positive with lower precipitation, increased fineness of soil texture, a transition from dominant grass species with C3 to C4 photosynthesis, and decreased grazing intensity. Soussana and Lemaire (2014) reported that at low stocking density, herbivores can enhance soil N cycling and net primary productivity, leading to increased soil C sequestration, which nonetheless declines at high stocking density. Thus in each unique environmental setting, a threshold level of grazing pressure intensification can be determined, above which any additional animal production would be associated with unacceptable environmental risks.

Soil organic carbon cycling is influenced by fertilisation in contrasting ways. Nitrogen fertilisation stimulates tree and pasture growth but the effect on the soil C pool is more complex. Several authors (O'Dea et al., 2015; Riggs et al., 2015) reported that N addition increases C sequestration in grasslands, especially on nutrient-limited sites (Jandl et al., 2007), because N fertilisation stimulates tree or pasture growth and increases C inputs into soils through litterfall and rhizodeposition. In some cases, N and P fertilisation may

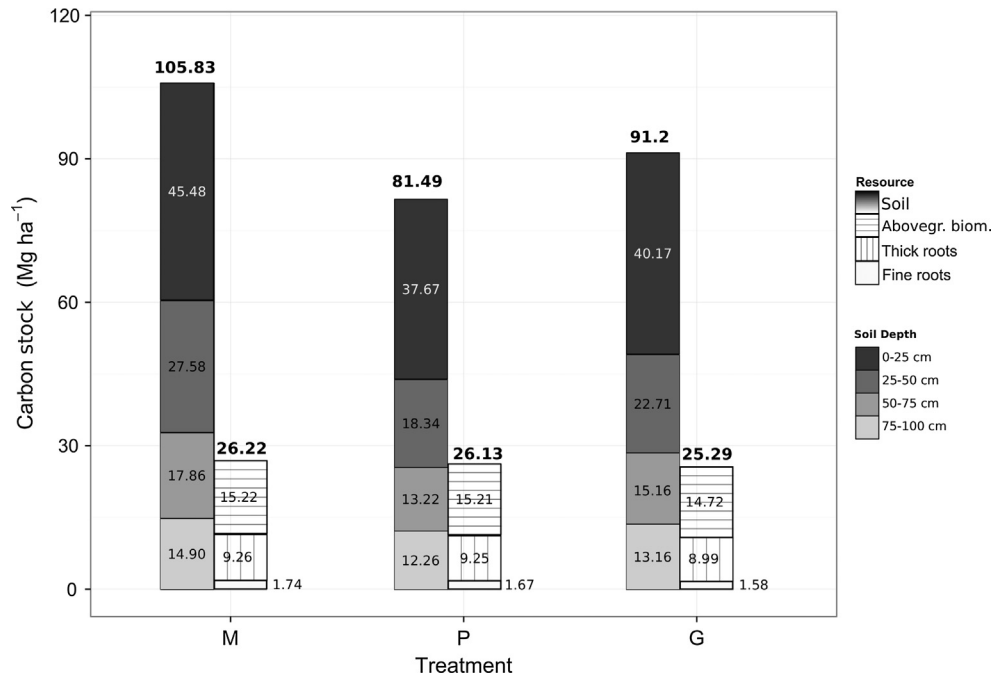


Fig. 4. Carbon stock (Mg C ha^{-1}) in soil, aboveground (Tree trunk and branches), and belowground biomass (Fine roots of herbaceous and trees; and Thick roots of trees) under treatments of control of herbaceous vegetation (Grazed Walnut). M: mowing; P: ploughing; G: grazing. Values above columns indicate total carbon stock of soil (0–100 cm depth) (left) and vegetation (right).

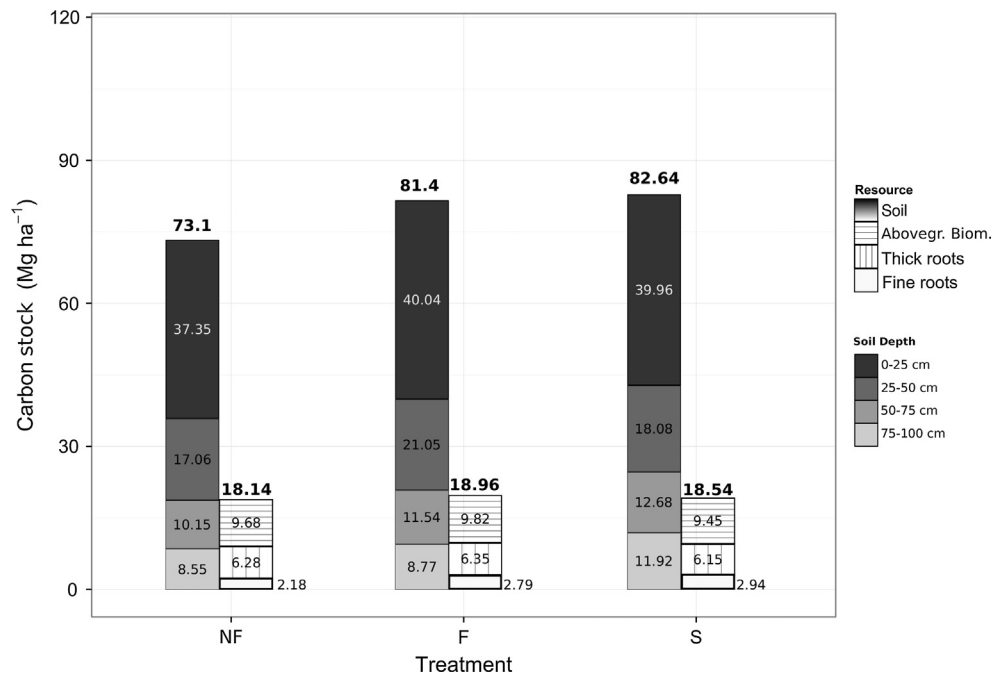


Fig. 5. Carbon stock (Mg C ha^{-1}) in soil, aboveground (Tree trunk and branches), and belowground biomass (Fine roots of herbaceous and trees; and Thick roots of trees) under fertilisation treatments. NF: no fertilisation; F: inorganic fertilisation; S: legume sowing. Values above columns indicate total carbon stock of soil (0–100 cm depth) (left) and vegetation (right).

decrease SOC content by increasing microbial activity and altering C substrate utilisation pattern of soil microbial communities through changes in plant biomass composition (Rumpel et al., 2015).

Legumes may increase the agroecosystem productivity by (1) increasing the available N-supply (26–50%) compared to cereal systems, thereby reducing the need for N fertiliser for subsequent crops, and (2) by increasing the quality of litter (Soussana and Lemaire, 2014; O'Dea et al., 2015). In a seven-year, experiment,

Cosentino et al. (2013), showed that in pastures initially sown with Italian ryegrass and later intercropped with subterranean clover, SOC content increased from 1.58% to 1.66%, whereas plots planted with durum wheat showed a slight decrease. In a long-term experiment, Triberti et al. (2016) reported that leguminous crops and inorganic fertilisation increased SOC content due to higher biomass production. The present study showed no differences among fertilisation treatments. McSherry and Ritchie (2013) concluded that short term studies (<20 years) may be less likely to detect differ-

ences in SOC content between treatments. However, legume sowing appears to offset SOC reduction due to mineralisation after ploughing before sowing (Zhang et al., 2007). Therefore, the influence of legumes is likely to improve over time, especially if they are managed as permanent grasslands.

4.2. System productivity: aboveground biomass

Tree growth was low due to high density (333 trees/ha). The variation of aboveground biomass observed among the treatments of grass understorey control indicated that herbaceous strata competed with mature trees for soil resources. The highest aboveground biomass increment for Grazed Walnut were obtained with ploughing, followed by grazing. The effects of grazing are likely to improve in the longer term due to livestock contribution of N and SOC, as soil C is a principal source of energy for nutrient recycling (Nave et al., 2010). In contrast, the aboveground biomass increment detected with ploughing is not expected to continue in the long term due to SOC content depletion.

Addition of N in inorganic form produced an increase in aboveground biomass compared to no fertilisation treatment. The improved C stock with legumes is likely to increase, as legume implementation started to enhance tree biomass in the final year, three years after sowing. After six years of legume sowing in a silvopastoral system under a *Pinus ponderosa* plantation, Dube et al. (2012) observed that the presence of clover in pasture alleys could enhance the biomass of trees and subsequently C stock. Similar results were reported by Sharrow et al. (1996), who analysed Douglas fir (*Pseudotsuga menziesii*)-sub clover (*Trifolium subterraneum*) silvopastoral systems and forest monocultures of the same species. In the case of inorganic fertilisation, the response was significant from the fourth year. Legume effect on trees is slower, as Triberti et al. (2016) observed. Long-term studies are required to quantify the effects of legumes. The increment in pasture production is not detrimental to tree biomass increment when the level of nutrients and water is sufficient for both strata.

4.3. Importance of fine roots as a carbon pool

Average fine root biomass (1.58–2.94 t m⁻³) was within the range reported by Noguchi et al. (2011) in a temperate climate (0.49–7.49 t m⁻³) and higher than the biomasses detected by Vogt et al. (1995) (0.60–1.65 t m⁻³). The greater development of pasture roots under grazing will allow more efficient use of nutrients and water by herbaceous strata and subsequently by the whole system. However, no significant response to fertilisation treatments was recorded. Several authors (Li et al., 2016; Triberti et al., 2016) noted that, in the medium term, the introduction of legumes can potentially play a significant role in increasing soil C storage by increasing aboveground rather than belowground biomass. In the longer term, belowground biomass is positively linked to SOC content, as found by Fornara and Tilman (2012) in a 27-year N fertilisation experiment conducted in a semi-arid system, because the root decomposition rate is relatively slower compared to decomposition of aboveground litter. In this experiment, the response of belowground biomass was limited, mainly for tree roots, followed by whole root weight. A possible reason for this discrepancy is that roots of adult trees (13 years old) are not affected by management treatments.

4.4. Potential of silvopastoral systems as carbon sinks: management implications

The results suggest that the largest C reservoir in all cases was SOC stock (76–82%). Consequently, positive or negative changes to soil C content, e.g. in response to N addition, could significantly

alter C stock in terrestrial ecosystems and have implications for the global climate in the future (Ciais et al., 2013; Riggs et al., 2015).

Soil organic carbon was concentrated at the surface, with 43% to 51% of total SOC located at 0–25 cm soil depth. Similar values were found by other authors (Jobbágy and Jackson, 2000; Alías et al., 2015; Ramesh et al., 2015). Rumpel et al. (2015) highlighted the lack of information about the impact of grassland management of C cycling at greater depths. In this study, roughly 50% of SOC stock was detected below 25 cm and 22–26% at 25–50 cm depth. Below this depth, SOC stock is much lower: 14–17% at 50–75 cm depth and 11–15% at 75–100 cm depth. Jobbágy and Jackson (2000) suggested that the relative distribution of SOC with depth has a stronger association with vegetation than with climate and is always deepest in shrublands, intermediate in grasslands and shallowest in forests. The data in this study (roughly 50% SOC stock in the first 25 cm) are within the range detected by these authors (from 29% in arid shrublands to 57% in cold humid forest).

Analysis of other C pools shows that SOC was followed by aboveground biomass, which supplied 9–14% of total C stock of the system. Ciais et al. (2013) reported that SOC contained two to five times as much C than aboveground biomass. Lal (2005) estimated that soil and aboveground parts hold roughly 60 and 30%, respectively, of the total C stored in tree-based land-use systems. Compared to the findings of other authors, the system in this study contained a lower proportion of C stock in aboveground biomass possibly due to the young age of the plantation.

The analyses indicated that 51–58% of the C stock in vegetation came from the aboveground biomass and the rest (42–49%) from the belowground, mostly thick roots. These values are higher than those reported by other authors (Montero et al., 2005; Alías et al., 2015), who considered that belowground biomass was 25–40% of the stock of aboveground biomass, although fine roots were not taken into account in these cases. In this study, fine roots comprised 15–32% of total belowground biomass but accounted for a very low proportion of total C (1.6–2.9%).

Total C stock in the system with the various treatments followed the same trend as SOC, as it was the largest portion of C accumulated in the system. Surprisingly, SOC had a higher response than biomass under the treatments, suggesting that SOC is more sensitive to management techniques. Avoiding soil disturbances is therefore important for the formation of stable organo-mineral complexes, which in turn are crucial elements in the process of C soil sequestration (Jandl et al., 2007). Mowing (to control competing understorey vegetation) and legume sowing (for fertilisation) were the treatments with highest C stocks, especially due to the SOC increment.

In conclusion, most of the C stocks was contained in SOC, at 50% in the surface soil layers (0–25 cm), followed by aboveground biomass. Grazing, as a method to control herbaceous vegetation, and legume implementation, for nitrogen supply, are suitable techniques to optimise soil C stocks and will also allow adequate tree growth in the long term.

Acknowledgements

The authors are grateful to Bosques Naturales S.A. for technical support. This study was funded by the Spanish Ministry of Science and Innovation (AGL2006-09435/FOR) and the European Union (Agforward, FP7-KBBE-2013-7-single-stage; No. 613520).

Appendix A.

See Appendix A–C.

Appendix A

Soil C content (SOC, %) under fertilisation treatments of control of herbaceous vegetation (Grazed Walnut) and fertilisation treatments (Fertilised Walnut) at different depths.

Essay	Depth (cm)	Treatment	Mean	Standard error
Grazed Walnut	0–10	Mowing	2.4584300	0.24181709
Grazed Walnut	0–10	Ploughing	1.8545665	0.18597698
Grazed Walnut	0–10	Grazing	2.0668987	0.14168004
Grazed Walnut	10–20	Mowing	1.6086620	0.09357652
Grazed Walnut	10–20	Ploughing	1.4646172	0.21653434
Grazed Walnut	10–20	Grazing	1.4780637	0.17717900
Grazed Walnut	20–30	Mowing	1.3196056	0.10866616
Grazed Walnut	20–30	Ploughing	1.0141145	0.20788194
Grazed Walnut	20–30	Grazing	1.1663766	0.13842982
Grazed Walnut	40–50	Mowing	0.9459590	0.14974452
Grazed Walnut	40–50	Ploughing	0.5776295	0.22803661
Grazed Walnut	40–50	Grazing	0.7506767	0.19077775
Grazed Walnut	70–80	Mowing	0.5418600	0.12010386
Grazed Walnut	70–80	Ploughing	0.4456690	0.11149693
Grazed Walnut	70–80	Grazing	0.4785383	0.09735518
Fertilised Walnut	0–10	No fertilisation	2.0171114	0.2090097
Fertilised Walnut	0–10	Inorganic fertilisation	2.0374130	0.2829314
Fertilised Walnut	0–10	Legume sowing	1.9484286	0.2585676
Fertilised Walnut	10–20	No fertilisation	1.4167633	0.1356864
Fertilised Walnut	10–20	Inorganic fertilisation	1.5342227	0.2374629
Fertilised Walnut	10–20	Legume sowing	1.5909091	0.1833283
Fertilised Walnut	20–30	No fertilisation	0.9996133	0.1400234
Fertilised Walnut	20–30	Inorganic fertilisation	1.1127224	0.2218989
Fertilised Walnut	20–30	Legume sowing	1.1026155	0.2147237
Fertilised Walnut	40–50	No fertilisation	0.5288090	0.1363698
Fertilised Walnut	40–50	Inorganic fertilisation	0.6970224	0.2117168
Fertilised Walnut	40–50	Legume sowing	0.5373339	0.1918014
Fertilised Walnut	70–80	No fertilisation	0.3108082	0.1535660
Fertilised Walnut	70–80	Inorganic fertilisation	0.3190255	0.1023511
Fertilised Walnut	70–80	Legume sowing	0.4334529	0.1665702

Appendix BAboveground biomass increment (kg tree⁻¹) under treatments of control of herbaceous vegetation (Grazed Walnut) and under fertilisation treatments (Fertilised Walnut) in different periods.

Essay	Treatment	Period	Mean	Standard error
Grazed Walnut	Mowing	2012	3.619740	0.1274667
Grazed Walnut	Mowing	2012–13	8.640558	0.2354585
Grazed Walnut	Mowing	2012–14	12.977273	0.3387458
Grazed Walnut	Ploughing	2012	4.563780	0.1303219
Grazed Walnut	Ploughing	2012–13	10.634641	0.2507343
Grazed Walnut	Ploughing	2012–14	16.086818	0.3758763
Grazed Walnut	Grazing	2012	4.533295	0.1369455
Grazed Walnut	Grazing	2012–13	9.718786	0.2748894
Grazed Walnut	Grazing	2012–14	14.348198	0.3787777
Grazed Walnut	No fertilisation	2011	5.749884	0.1849585
Grazed Walnut	No fertilisation	2011–12	9.201085	0.2483539
Grazed Walnut	No fertilisation	2011–13	12.390775	0.3133277
Grazed Walnut	No fertilisation	2011–14	15.268140	0.3706094
Fertilised Walnut	Inorganic fertilisation	2011	6.121508	0.2021876
Fertilised Walnut	Inorganic fertilisation	2011–12	9.900787	0.3015938
Fertilised Walnut	Inorganic fertilisation	2011–13	14.083770	0.4189777
Fertilised Walnut	Inorganic fertilisation	2011–14	18.256525	0.5528713
Fertilised Walnut	Legume sowing	2011	5.473811	0.1614221
Fertilised Walnut	Legume sowing	2011–12	8.934821	0.2211782
Fertilised Walnut	Legume sowing	2011–13	12.549837	0.2901294
Fertilised Walnut	Legume sowing	2011–14	15.945570	0.3466461

Appendix CTree and pasture fine root weight (kg m⁻³) at various depths (cm) under treatments of control of herbaceous vegetation (Grazed Walnut) and fertilisation treatments (Fertilised Walnut).

Essay	Treatment	Depth	Type	Mean	Stand. error
Grazed Walnut	Mowing	0–10	Tree	0.196000	0.079531
Grazed Walnut	Ploughing	0–10	Tree	0.155272	0.058443
Grazed Walnut	Grazing	0–10	Tree	0.106300	0.036108
Grazed Walnut	Mowing	10–20	Tree	0.483384	0.141118
Grazed Walnut	Ploughing	10–20	Tree	0.879100	0.214233
Grazed Walnut	Grazing	10–20	Tree	0.429909	0.136384
Grazed Walnut	Mowing	20–30	Tree	0.492333	0.116357

Appendix C (continued)

Essay	Treatment	Depth	Type	Mean	Stand. error
Grazed Walnut	Ploughing	20–30	Tree	0.428545	0.161207
Grazed Walnut	Grazing	20–30	Tree	0.477000	0.117437
Grazed Walnut	Mowing	30–40	Tree	0.216454	0.055445
Grazed Walnut	Ploughing	30–40	Tree	0.346071	0.085582
Grazed Walnut	Grazing	30–40	Tree	0.133916	0.038511
Grazed Walnut	Mowing	40–50	Tree	0.271181	0.080367
Grazed Walnut	Ploughing	40–50	Tree	0.107272	0.030304
Grazed Walnut	Grazing	40–50	Tree	0.313750	0.130900
Grazed Walnut	Mowing	50–60	Tree	0.082363	0.027454
Grazed Walnut	Ploughing	50–60	Tree	0.130923	0.080002
Grazed Walnut	Grazing	50–60	Tree	0.102416	0.028678
Grazed Walnut	Mowing	60–70	Tree	0.124100	0.043541
Grazed Walnut	Ploughing	60–70	Tree	0.107583	0.041561
Grazed Walnut	Grazing	60–70	Tree	0.196583	0.083841
Grazed Walnut	Mowing	70–80	Tree	0.130750	0.045155
Grazed Walnut	Ploughing	70–80	Tree	0.208666	0.096118
Grazed Walnut	Grazing	70–80	Tree	0.060500	0.026796
Grazed Walnut	Mowing	80–90	Tree	0.128916	0.074753
Grazed Walnut	Ploughing	80–90	Tree	0.129250	0.054641
Grazed Walnut	Grazing	80–90	Tree	0.140545	0.039390
Grazed Walnut	Mowing	90–100	Tree	0.339200	0.174191
Grazed Walnut	Ploughing	90–100	Tree	0.225916	0.096980
Grazed Walnut	Grazing	90–100	Tree	0.127363	0.046111
Grazed Walnut	Mowing	0–10	Pasture	0.164250	0.038669
Grazed Walnut	Ploughing	0–10	Pasture	0.048916	0.015643
Grazed Walnut	Grazing	0–10	Pasture	0.306636	0.070841
Grazed Walnut	Mowing	10–20	Pasture	0.105416	0.032798
Grazed Walnut	Ploughing	10–20	Pasture	0.023916	0.008660
Grazed Walnut	Grazing	10–20	Pasture	0.152909	0.065343
Grazed Walnut	Mowing	20–30	Pasture	0.033833	0.009909
Grazed Walnut	Ploughing	20–30	Pasture	0.006461	0.003306
Grazed Walnut	Grazing	20–30	Pasture	0.053363	0.018758
Grazed Walnut	Mowing	30–40	Pasture	0.025000	0.010798
Grazed Walnut	Ploughing	30–40	Pasture	0.002500	0.000645
Grazed Walnut	Grazing	30–40	Pasture	0.029250	0.014841
Grazed Walnut	Mowing	40–50	Pasture	0.031916	0.020966
Grazed Walnut	Ploughing	40–50	Pasture	0.003083	0.001703
Grazed Walnut	Grazing	40–50	Pasture	0.026750	0.010212
Grazed Walnut	Mowing	50–60	Pasture	0.010363	0.005322
Grazed Walnut	Ploughing	50–60	Pasture	0.001818	0.001181
Grazed Walnut	Grazing	50–60	Pasture	0.042166	0.016339
Grazed Walnut	Mowing	60–70	Pasture	0.006500	0.004534
Grazed Walnut	Ploughing	60–70	Pasture	0.000750	0.000304
Grazed Walnut	Grazing	60–70	Pasture	0.011250	0.006340
Grazed Walnut	Mowing	70–80	Pasture	0.004916	0.002800
Grazed Walnut	Ploughing	70–80	Pasture	0.001666	0.000898
Grazed Walnut	Grazing	70–80	Pasture	0.002500	0.001118
Grazed Walnut	Mowing	80–90	Pasture	0.002666	0.001350
Grazed Walnut	Ploughing	80–90	Pasture	0.000750	0.000350
Grazed Walnut	Grazing	80–90	Pasture	0.004909	0.002176
Grazed Walnut	Mowing	90–100	Pasture	0.004100	0.001846
Grazed Walnut	Ploughing	90–100	Pasture	0.000416	0.000336
Grazed Walnut	Grazing	90–100	Pasture	0.000181	0.000181
Fertilised Walnut	No fertilisation	0–10	Tree	0.498636	0.166357
Fertilised Walnut	Inorganic fertilisation	0–10	Tree	0.507916	0.219104
Fertilised Walnut	Legume sowing	0–10	Tree	0.126454	0.050018
Fertilised Walnut	No fertilisation	10–20	Tree	0.847000	0.198525
Fertilised Walnut	Inorganic fertilisation	10–20	Tree	1.018333	0.199594
Fertilised Walnut	Legume sowing	10–20	Tree	0.664000	0.275979
Fertilised Walnut	No fertilisation	20–30	Tree	0.918666	0.208092
Fertilised Walnut	Inorganic fertilisation	20–30	Tree	0.652333	0.214505
Fertilised Walnut	Legume sowing	20–30	Tree	0.932333	0.296725
Fertilised Walnut	No fertilisation	30–40	Tree	0.535583	0.220305
Fertilised Walnut	Inorganic fertilisation	30–40	Tree	0.247000	0.131772
Fertilised Walnut	Legume sowing	30–40	Tree	0.191454	0.029313
Fertilised Walnut	No fertilisation	40–50	Tree	0.192500	0.143888
Fertilised Walnut	Inorganic fertilisation	40–50	Tree	0.665916	0.253858
Fertilised Walnut	Legume sowing	40–50	Tree	0.272363	0.092345
Fertilised Walnut	No fertilisation	50–60	Tree	0.250916	0.102033
Fertilised Walnut	Inorganic fertilisation	50–60	Tree	0.590454	0.213015
Fertilised Walnut	Legume sowing	50–60	Tree	0.167666	0.066417
Fertilised Walnut	No fertilisation	60–70	Tree	0.493500	0.239965
Fertilised Walnut	Inorganic fertilisation	60–70	Tree	0.576090	0.265046
Fertilised Walnut	Legume sowing	60–70	Tree	0.375909	0.235738
Fertilised Walnut	No fertilisation	70–80	Tree	0.218000	0.064533

(continued on next page)

Appendix C (continued)

Essay	Treatment	Depth	Type	Mean	Stand. error
Fertilised Walnut	Inorganic fertilisation	70–80	Tree	0.214833	0.062647
Fertilised Walnut	Legume sowing	70–80	Tree	0.177181	0.076988
Fertilised Walnut	No fertilisation	80–90	Tree	0.203727	0.084286
Fertilised Walnut	Inorganic fertilisation	80–90	Tree	0.156300	0.117213
Fertilised Walnut	Legume sowing	80–90	Tree	0.106333	0.042063
Fertilised Walnut	No fertilisation	90–100	Tree	0.178727	0.093260
Fertilised Walnut	Inorganic fertilisation	90–100	Tree	0.067454	0.029917
Fertilised Walnut	Legume sowing	90–100	Tree	0.390700	0.172218
Fertilised Walnut	No fertilisation	0–10	Pasture	0.178909	0.040378
Fertilised Walnut	Inorganic fertilisation	0–10	Pasture	0.244818	0.070428
Fertilised Walnut	Legume sowing	0–10	Pasture	0.239636	0.054188
Fertilised Walnut	No fertilisation	10–20	Pasture	0.057583	0.016171
Fertilised Walnut	Inorganic fertilisation	10–20	Pasture	0.111333	0.046431
Fertilised Walnut	Legume sowing	10–20	Pasture	0.139500	0.049556
Fertilised Walnut	No fertilisation	20–30	Pasture	0.032000	0.015532
Fertilised Walnut	Inorganic fertilisation	20–30	Pasture	0.019166	0.007009
Fertilised Walnut	Legume sowing	20–30	Pasture	0.033100	0.013193
Fertilised Walnut	No fertilisation	30–40	Pasture	0.013750	0.006233
Fertilised Walnut	Inorganic fertilisation	30–40	Pasture	0.016727	0.005025
Fertilised Walnut	Legume sowing	30–40	Pasture	0.025363	0.012074
Fertilised Walnut	No fertilisation	40–50	Pasture	0.002166	0.001071
Fertilised Walnut	Inorganic fertilisation	40–50	Pasture	0.008090	0.003581
Fertilised Walnut	Legume sowing	40–50	Pasture	0.003181	0.001488
Fertilised Walnut	No fertilisation	50–60	Pasture	0.001900	0.001268
Fertilised Walnut	Inorganic fertilisation	50–60	Pasture	0.014833	0.006271
Fertilised Walnut	Legume sowing	50–60	Pasture	0.003100	0.002354
Fertilised Walnut	No fertilisation	60–70	Pasture	0.001500	0.000435
Fertilised Walnut	Inorganic fertilisation	60–70	Pasture	0.008727	0.003615
Fertilised Walnut	Legume sowing	60–70	Pasture	0.001909	0.001516
Fertilised Walnut	No fertilisation	70–80	Pasture	0.001000	0.000660
Fertilised Walnut	Inorganic fertilisation	70–80	Pasture	0.002916	0.001422
Fertilised Walnut	Legume sowing	70–80	Pasture	0.000909	0.000638
Fertilised Walnut	No fertilisation	80–90	Pasture	0.000272	0.000194
Fertilised Walnut	Inorganic fertilisation	80–90	Pasture	0.001400	0.001185
Fertilised Walnut	Legume sowing	80–90	Pasture	0.001777	0.000140
Fertilised Walnut	No fertilisation	90–100	Pasture	0.000272	0.000140
Fertilised Walnut	Inorganic fertilisation	90–100	Pasture	0.002000	0.002000
Fertilised Walnut	Legume sowing	90–100	Pasture	0.000100	0.000100

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