Short rotation coppice of leguminous tree *Leucaena* spp. improves soil fertility while producing high biomass yields in Mediterranean environment

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Abstract: The use of woody nitrogen-fixing plant species as multipurpose and energy crops aims to enhance biomass yield while improving soil properties. Yet, the effectiveness of this option is still under debate especially the use of short rotation cropping in water and nutrient limited environments. This study investigated whether short rotation coppicing of four taxa of multipurpose biomass woody legume *Leucaena* spp. can improve soil conditions when grown for biomass under a Mediterranean environment. Biomass yield, mineral composition and heat value of the biomass and the mineral and organic matter content of soil were evaluated. Under favorable growing conditions, woody dry biomass production was up to 29 Mg ha^{-1} year^{-1} with slight but significant differences between the four taxa that were tested. After 11 years of cropping, the soil showed higher fertility and microbial activity compared to the uncropped plot. *Leucaena* cultivation increased soil nitrogen by 35%, dehydrogenase activity by 98%, and organic matter and carbon content (by 41%). Annual cuttings resulted in the highest biomass production, followed by two and three year cuttings. The mineral composition and the calorific capacity of woody biomass make it suitable for commercial use as an energy source (generating on average 151 MWh ha^{-1} year^{-1}). In conclusion, short rotation coppicing of nitrogen-fixing woody species results in high biomass production rates with the restoration of degraded soils, constituting a sustainable agroforestry system for rural areas.

Keywords: nitrogen-fixing trees, energy use, pellets, litterfall, mineral nutrients.

1. Introduction

International commitments aimed at mitigating climate change lead to the promotion of clean and renewable energy sources, including biomass (United Nations, 2016). Worldwide energy production from biomass currently accounts for more than 60% of the renewable sources, with renewable sources contributing up to 19.3% of the global energy output (REN21, 2017). In this context, the use of lignocellulosic biomass, is steadily expanding. However, as the traditional agroforestry systems can not supply the growing demand, plantations of fast-growing woody species are necessary (Christersson and Verma, 2006; Bogdanski et al., 2010; IEA, 2016). Increasing the productivity of biomass crops would reduce the amount of land needed for biomass production and would increase the economic benefits of rural economies.

Lignocellulosic energy crops are expected to cover from 5 to 10% of the global forest area by 2050, mostly destined to marginal areas with degraded low fertility soils, and they will be required to provide a large amount of biomass in a cost-effective manner. Thus, it is necessary to evaluate the environmental impact of this intensive production system on already poor and degraded soils (Parrotta, 1999; Isaac et al. 2003; Vanguelova and Pitman, 2011; Lauri et al., 2014).

Currently, different plant species are used globally as short rotation energy crops (such as the genera *Populus*, *Salix*, *Eucalyptus*, *Miscanthus*, *Cynara*, *Robinia*, *Casuarina* and *Leucaena*) (Parrotta, 1999; Halford and Karp, 2011; Vanbeveren et al., 2017), some of which are considered multiple-purpose species providing numerous goods and services (e.g. forage, energy, soil restoration, pulp for the paper industry, and construction). In this regard, the incorporation of nitrogen-fixing plant species into energy crops, such as *Leucaena* spp., may represent an interesting option and, apart from the traditional multiple use of this genus, the extraction of new products by biorefinery techniques increases the possibility of biomass valorization (Feria et al., 2011; Loaiza et al., 2017). Nitrogen-fixing species are expected to improve soil fertility while enhancing crop productivity (Parrotta, 1999; Tadros et al., 2012; Conrad et al., 2017) and providing other ecosystem services, such as green fodder, soil restoration and carbon sequestration (Srinivasarao et al., 2014; Mukhopadhyay et al., 2016; Gusha et al., 2017). Moreover, they can contribute to decreased use of nitrogen (N) fertilizers, consequently reducing energy costs (Audsley, 2003; Giuntoli et al, 2017). *Leucaena leucocephala* and *Leucaena diversifolia*, for instance, are tropical woody legume species which are fast growing and well adapted to a wide range of climate and soil types, but mainly under non-arid subtropical climate (Bray and Sorensson, 1992; Parrotta, 1992, Zayed et al., 2018). *Leucaena leucocephala* is the *Leucaena* sp. most extensively cultivated worldwide, spreading throughout Latin America, Asia, Africa and Oceania, but, as far as we know, *Leucaena* sp. has not been widely used in commercial plantations under a Mediterranean climate. However, the global distribution of plantations and research plots also includes subtropical climate areas in Australia, Chile, South Africa and Spain (Zayed et al., 2018), many of them under a typical Mediterranean subtropical temperate climate (i.e. *Csa* climate in the Köppen-Geiger climate classification system) such as areas near the coast of the Iberian Peninsula, Italian Peninsula, Greece, Turkey and North Africa (Beck et al. 2018). In Europe alone, the degradation of farmland is such that 45% contains low organic matter content (< 2%), 15% presents problems due to excess of inorganic nitrogen fertilization, and more than 147 million hectares suffer from serious erosion problems (FAO, 2015). At least 20% of all these degraded lands are under a climate where *Leucaena* spp. can grow. As the *Leucaena* genus includes fast growing multiple-purpose species, the main impacts that could be expected on locations where *Leucaena* spp. are planted or have the potential to be planted for biomass production are: soil fertility recovery by improving organic matter and nitrogen content (Parrotta, 1999; Peng et al., 2013; Conrad et al., 2018), biofuel production for heat energy (Chaplot, 2014; Loaiza et al., 2017; Dalzell, 2019), soil erosion control and therefore reduced run-off and improved water quality (Shelton and Dalzell, 2007; Adhikary et al., 2017), and high quality fodder for ruminant livestock (Gusha et al., 2017; Leketa et al., 2019; Santana et al. 2019). All this can reactivate the rural economy while providing environmental benefits. In addition, cultivated *Leucaena* spp. can provide raw materials for wood and paper industries and for biorefineries (Malik et al., 2004; Feria et al., 2011; Sa´ad et al., 2019; Zayed et al., 2019), as well as restore contaminated soils (Mukhopadhyay et al., 2016). Finally, the ability of *Leucaena* spp. of fixing N and producing biomass for energy use contribute to reduce nitrogen fertilization and green-house gas emissions, and to improve carbon sequestration (Srinivasarao et al., 2014; Conrad et al., 2017, 2018). The latter, would be useful for European Union in order to comply with the promotion of the use of renewable energy (European Union, 2018), and with the 2015 Paris Agreement on Climate Change (United Nations Climate Change Conference, COP21).

Commercial *Leuceana* sp. plantations yield from 1 to 50 Mg ha^{-1} year^{-1} of dry biomass, depending on the growing conditions. Water stress, winter frosts and soil fertility have been identified as the main limiting factors for the growth and survival of these plantations (Parrotta, 1999; Tadros et al., 2012; Chaplot, 2014). However, as *Leucaena* spp. can become an invasive alien species in certain areas, it must be managed with caution (Chiou et al., 2016; Mello and Oliveira, 2016).

This study aims to evaluate whether short rotation coppicing of *Leucaena* spp*.* can produce a large amount of high quality biomass at the same time as improving soil conditions. It has been carried out in a Mediterranean environment. Soil properties were assessed under four commercial taxa of *Leucaena leococephala* (Lam.) De Wit and *Leucaena diversifolia* (Schlecht.) Benth. subjected to different biomass harvest frequencies. Different taxa were used because *Leucaena* species and varieties vary considerably in terms of tolerance to environmental stress and nutrient requirements (Foroughbakhch et al., 2007; Sangram and Keerthika, 2013; Katunga et al., 2014). The objectives of this study are: *i)* to assess plant growth and productivity of *Leucaena* spp. subjected to different short rotation coppice treatments in a Mediterranean environment; *ii)* to rate their suitability for use as energy crops; and *(iii)* to determine the evolution of soil conditions after a cropping period of 11 years.

2. Materials and Methods

2.1. Plant material and experimental design

Seeds of two *Leucaena* species were used (two provenances –`Honduras′, `India′– and one variety –`K360′– of *L. leucocephala*; and one provenance of *L. diversifolia* –`Hawaii′–), all of them being high productivity commercial taxa. At the beginning of March 2002, the seeds were soaked and inoculated with bacteria of the genus *Rhyzobium*. The following day, the seeds were sown in 300-cm^3 containers filled with *Spagnum* peat:vermiculite (3:1 volume ratio). After three months in a nursery, in the first week of June, the seedlings were planted in the field. The site was located in Huelva (Spain), which has a Mediterranean climate with mild winters and a summer drought period of 3–4 months (UTM zone 29S, X: 684824, Y: 4119171, 18 m a.s.l.). Mean temperature and annual rainfall in the area (based on data for the previous 20 years) were 16.2 ºC and 564 mm, respectively. The experimental design comprised a plot, divided in two parts, which were separated 150 m from each other. Plant growth and survival were evaluated in the two parts of the plot, while chemical parameters of the soil and plants were measured in one of them. Each one of the four taxa was replicated four times in each part. Each replicate consisted of two parallel rows, each containing eight plants, with a spacing of 0.6 m between plants and 1.8 m between lines (17.28 m^2); the crop density was 9259 plants ha^{-1} (Figure 1). Thus, the experimental design resulted in a total of 512 plants (4 taxa × 4 replicates × 16 plants × 2 parts). In the first part of the plot, two adjacent uncropped areas with natural vegetation (predominantly composed of clear scrubland containing species belonging to the genus *Cistus*) were selected as controls for soil sampling at the end of the assay (Figure 1).

[Figure 1 here]

The two plot parts showed similar physical-chemical soil properties. Soils were permeable with a sandy loam texture and were free of active limestone. At planting, the physical-chemical properties of the surface horizon (0–20 cm depth) of the first part of the plot were as follows: 76.5% as fine soil (< 2 mm particle-size) composed of 7.0% as clay, 7.5 % as silt and 85.5 % as sand, 1.1% organic matter (OM), pH = 6.0 (water pH; i.e. pH_{H2O}), EC = 3.94 mS m^{-1}, 360 mg kg^{-1} of N, 14.7 mg kg^{-1} of available P, 120 mg kg^{-1} of available K, and bulk density of 1.5 kg/dm^3. Soil preparation for planting consisted of the eradication of pre-existing vegetation, followed by a deep ripping at 50 cm and a shallow tillage. Since the viability of the *Leucaena*-nitrifying bacteria symbiosis was not assured because they were new species in this soil, and in order to facilitate plant growth during the first months after planting in this nutrient-poor soil, it was decided to apply a standard fertilization before planting with N (160 kg ha^{-1}), K (110 kg ha^{-1}), P (19 kg ha^{-1}) and Mg (12 kg ha^{-1}), equivalent to a 11–3–9 ratio fertilizer ($N–P_2O_5–K_2O$), plus 1,4 MgO. Thereafter, no fertilizer was added, and eight months after planting root nodules were observed in the field plot. Weeds were manually controlled during the first year. From the second year onwards, weed control was unnecessary because of the high cultivation density and the rapid growth of the new sprouts. Irrigation was applied only during the first three months after planting to ensure plant survival. Irrigation amounted to 54 mm (4.5 mm per week) from late June to mid-September. The adjacent unmanaged areas did not receive any agronomic inputs (irrigation, fertilization, weeds control, etc.), they were left to their natural development. Climatic conditions during the studied period are shown in Table 1.

[Table 1, here]

2.2. Shoot growth and biomass production

Height (*H*) and stem diameter (*D*, measured 7 cm above ground) were periodically measured in 12 plants per replicate. During the first three years of the study period, the effect of the short rotation cutting cycles was analyzed by measuring plant growth at one, two and three years after planting. We also measured growth in re-sprouted shoots one year after cutting. For this, the first year after planting plants in two replicates were cut, while plants in each of the remaining two replicates were cut the second and the third year, respectively (Figure 2). Therefore, during the first year there was no difference between cutting treatments, but they differed from the second year onwards. Each treatment is identified according to the following convention: $R_{i,j}S_k$ indicates age in years of the stump-roots (i), the number of harvests a stump has experienced (j) and the age of the aboveground plant part at the time of cutting (k). For example, for the first three years studied, the *111* treatment was composed by $R_{11}S_1 + R_{22}S_1 + R_{33}S_1$; the *21* treatment by $R_{21}R_2 + R_{32}S_1$, and the *3* treatment by $R_{31}S_3$.

Additionally, on a monthly basis, phenology was evaluated for each taxon and cutting treatment by recording the status of the sprouts (developing or resting) and the presence of flowers and fruits (developing or ripe). Furthermore, during the summer of the second and third cropping seasons (Figure 2), leaf area index (LAI) was measured using a portable radiometer (LP-80, Decagon Devices, Inc., WA, USA), predawn leaf water potential (Ψ) was determined using a Scholander pressure chamber (PMS Inc., Corvallis, OR, USA) and specific leaf area (SLA) was recorded (m^2 kg^{-1}).

[Figure 2 here]

After the third year, cultivation was extended for eight more years to determine the shoot sprouting vigor by measuring TDW production, and evaluate the effect of long-term cultivation on soil conditions. During that time, two replicates per plot were harvested every year and the other two were harvested every two years.

Biomass assessment was carried out during the winter season (February to March) by cutting and weighing shoots. Allometric equations were developed for those shoots that were not cut. These allometric equations related to the diameter of the base (D) and/or the height of the main stem (H) with the aboveground dry weight (Noulekoun et al., 2018). For this, stems with D ranging from 11 to 125 mm were used. Woody (W) and non-woody (NW) parts were oven-dried at 80 ºC and weighed to estimate dry weight (WDW and NWDW). Non-woody biomass was composed of leaves plus reproductive structures and twigs less than 5.0 mm in diameter. Total dry weight (TDW) was calculated as the sum of WDW and NWDW. A total of 16 different equations were tested (including linear, power, exponential, polynomial, logarithmic and quadratic) with D, H, and D^2 or combinations of these as predictor variables. Most of the equations yielded excellent results in terms of predicting aboveground dry weight. The power equation using D as a predictor showed a very good fit ($\mathrm{TDW} = a\,D^b$) and was thus used for estimating the weight of aboveground biomass ($\mathrm{TDW} = 3.803\,D^{1.664}$, $R^2 = 0.851$, $p < 0.001$, n = 48 for *L. diversifolia*; $\mathrm{TDW} = 3.357\,D^{1.614}$, $R^2 = 0.913$, $p < 0.001$, n = 68 for *L. leucocephala*; TDW is expressed in g and D in mm).

2.3. Physical-chemical characterization of plant and soil material

In addition to the initial soil sampling, soil from part one of the plot was also sampled in summer 11 years after planting. Two soil samples were taken between planted lines of each replicate using a soil core (2 samples × 4 replicates × 4 taxa = 32 soil samples). Samples were then separated into organic layer (i.e., litterfall) and two soil mineral layers (0–20 cm and 20–40 cm depth). Soil samples for each replica and layer were then pooled. We also sampled soils in the adjacent uncropped area of the plot (under natural vegetation) following the above-mentioned sampling protocol. The litter layer of the soil (O horizon) showed two visually different strata; an upper deposit composed of leaves, shoots and reproductive organs that were scarcely decomposed, and a lower stratum consisting of plant tissues which were partially or completely decomposed. The two strata were separated for thickness and dry weight quantification.

Litterfall was washed, oven-dried at 80 ºC for two days, ground and stored in sealed containers at room temperature (15–20 ºC). Soil samples were divided in two subsamples. One was used for physical-chemical analysis. For this, the subsample was dried at room temperature during two weeks and then milled and sieved (2-mm mesh). The other subsample was used for biochemical analysis, so the moisture content at the time of sampling was preserved. For this, subsamples were sieved (< 2 mm) and kept in the dark at a low temperature (4 ºC). Nutrient content of litterfall as well as physical-chemical and biochemical properties of soil were determined using standardized methods (Table 2).

Regarding the aboveground plant biomass, four shoots per replica were sampled, separated into leaves and woody stems, and samples from each replica then pooled for analysis. They were also washed, oven-dried at 80 ºC, ground and stored in sealed containers at room temperature (15–20 ºC), and their physical-chemical properties analyzed (Table 2). Woody material was ground (Woodstock 3PH, Smartec®, Italy) and sieved to a particle size of 0.2 to 5.0 mm in order to create homogenous samples. Then, wood pellets were manufactured using a pelleting press (PLT-400, Smartec®, Italy). The sample (i.e., sawdust) moisture content was set to 128 g kg^{-1} with a bulk density of 258 kg m^{-3} and an operating temperature ranging between 95 and 105 ºC. The die channels had a diameter of 6 mm; the first part had a cone-shaped opening 3.5 mm deep and 70º angles; the active part was 28 mm long, so the compression ratio was 4.67. Afterwards, the mechanical durability, moisture content, length and diameter, and the bulk density of the pellets were determined according to methods described in ISO 17225-2:2014.

[Table 2 here]

2.4. Data analysis

Linear mixed models were used to evaluate plant growth (*D* and *H*) and dry weight (TDW, WDW and NWDW) during the first three years after planting. The year (*Y*), cutting treatment (*CT*) and taxa (*T*) were considered as fixed effects in the model. The triple and the pairwise interactions between main effects were also included as fixed effects in the model. We considered replicate unit as random term in the model to account for non-independency among observations within the same individual (i.e. repeated measurements) (Zuur et al. 2009). An autoregressive correlation structure to remove the first-order autocorrelation between observations was also used (Pinheiro et al. 2018). Analogously, we used the same model approach to evaluate TDW for the period between the fourth and the eleventh year after planting. Since we only considered two cutting treatments (annual and biennial), we evaluated TDW in the fifth, seventh, ninth and eleventh years after planting. To do this, we calculated TDW as Mg ha^{-1} year^{-1} by averaging biennial and annual production for the evaluated period (i.e. every two years). The percentages of variance explained by fixed and random effects were evaluated following Nakagawa and Schielzeth (2013). Soil parameters were analyzed by a two-way ANOVA but considering cultivation treatment (*CU*: natural vegetation, *L. diversifolia*, and each of the three taxa of *L. leucocephala*) and soil layer (*SO*: 0–20 cm and 20-40 cm) as fixed effects, and also their interaction ($CU \times SO$). Nutrient content of litterfall and leaf samples were analyzed by a two-way ANOVA considering cutting treatment (*CT*), the taxa (*T*) and $CT \times T$ as fixed effects. Significant differences were established at $p < 0.05$. To evaluate pairwise comparisons between cutting treatments and taxa, the Tukey HSD test was used. All statistical analyses were performed using the R statistical free software and the SPSS 19.0 software (IBM® SPSS Statistics®).

3. Results and Discussion

3.1. Plant growth and biomass production

A significant effect of the pairwise interactions $CT \times Y$ and $T \times Y$ on total dry weight (TDW) was found (Table 3). TDW increased over the study period more intensively in the *111* cutting treatment than in the *21* and *3* treatments and, regarding taxa, the greatest increase in TDW corresponded to *L. leucocephala* `India´ while the smallest to *L. leucocephala* `Honduras´ (Table 4, Fig. 3). Analogously, WDW and NWDW showed similar results than TDW (Tables 3 and 4). Therefore, both *Leucaena* species showed a good adaptation to the environmental conditions of the experimental plantation as demonstrated by the high survival (> 98.5%) and growth rate. The different biomass components (woody, non-woody and total dry weights) were significantly correlated ($p < 0.001$). The correlation equation for the two species as a whole and for stems with $D > 13$ mm was WDW = 0.7768 TDW - 7.1565 (r = 0.99, n = 84). It is noteworthy that both *Leucaena* sp. showed a high fruit production after two years (up to 10.5% of the total dry weight). Interestingly, the appearance of reproductive organs in *L. leucocephala* occurred just five months after planting and after harvesting, and six months for *L. diversifolia*. Therefore, coppicing could enhance vegetative growth in the subsequent rotation cycle by keeping the plants in a partially juvenile stage, for at least 5–6 months, during which the production of reproductive organs was reduced. The ratio of woody to non-woody dry weight (WDW/NWDW) increased significantly from 2.5 in the $R_{1,1}S_1$ treatment, just at the end of the first year, (equivalent to 71.4% WDW/TDW) to 3.0–3.5 for the rest of cutting treatments (75.3–77.6% WDW/TDW).

[Table 3 here]

[Table 4 here]

[Figure 3 here]

Biomass production of the sprouts one year after cutting ($R_{2.2}S_1$, $R_{3.3}S_1$ and $R_{3.2}S_1$) was always greater than that was cut every two or three years ($R_{2.1}S_2$ and $R_{3.1}S_3$), with slight but significant differences between the taxa (Table 3, Figure 3). In addition, the sum of the biomass harvested annually exceeded that produced by the longer two and three year rotations (Figure 3). The biomass yield in our study is within the highest production range reported under favorable growing conditions for *Leucaena* spp. (Parrotta, 1999; Prasad et al., 2011; Chaplot, 2014; Fagbenro et al., 2015). The first year ($R_{1.1}S_1$), the biomass production of *L. leucocephala* was 72% greater than that of *L. diversifolia*, even if the production of the first year for the two species was significantly lower than that of the subsequent annual rotations (i.e., about 75% lower than that of $R_{2.2}S_1$ and $R_{3.3}S_1$, if measured in Mg ha^{-1}). This might be related to a growth slowdown during the first months of life due to planting shock (Parrotta, 1992). Annual rotations ($R_{i.j}S_1$) produced shoots of a smaller diameter compared to biennial or triennial rotations ($R_{2,1}S_2$ and $R_{3,1}S_3$, respectively) (Table 3, Figure 4). In spite of smaller diameter, annual rotation resulted in greater biomass production per plant because of a large number of sprouts

(1.01 for *L. diversifolia* and 1.96 for *L. leucodephala* in the first cutting, but 4–9 in the second and 5–12 thereafter for both species; only diameters greater than 15 mm were counted), in contrast to biennial and triennial rotations (1.41 for *L. diversifolia* and 2.26 for *L. leucocephala* in their first cutting). Consequently, coppicing reinvigorated plant growth and biomass production after each harvest probably due to the breakdown of apical dominance and the subsequent development of numerous new sprouts from adventitious buds that retained their juvenile stage for a few months. The hormonal imbalance caused by cutting the aboveground biomass and the production of a greater proportion of earlywood vessels in the new sprouts might be behind this response (Dillen et al., 2011; Sjölund and Jump, 2013). It seems that coppicing *Leucaena* spp. the first year after planting produce this result, and its effect was repeated in the following two years. In addition, the proliferation of thinner stems in annual rotations, with higher density and less branching, facilitate the mechanization of the harvest (Vanbeveren et al., 2017).

The annual biomass yielded in our study equals the highest yields reported in the Iberian Peninsula with other lignocellulosic crops, such as kenaf, *Miscanthus* or *Eucalyptus* (Fernández et al., 2018), and exceeds those reported for other tree crops, such as poplar, black locust or willow (around 20 Mg ha^{-1} year^{-1}) (Sixto et al., 2015). The role of *Leucaena* as a nitrogen-fixing species, capable of fixing much more than 100 kg ha^{-1} year^{-1} of N (Högberg and Kvarnström, 1982; Conrad et al., 2017; Conrad et al., 2018), combined with their multiple uses, make it an interesting alternative short rotation energy crop, at least for degraded soils and until these increase their fertility. It was reported that *L. leucocephala* is capable of fixing more than 500 kg ha^{-1} year^{-1} of N (Duke and duCellier, 1993), and it has been estimated that 73% of *L. leucocephala* N can come from atmospheric N_2-fixation (Conrad et al. 2018). As a result, the nitrogen fixed can contribute to decrease the use of inorganic N-fertilizers and, consequently, to reduce the high economic and energy costs related to their production (Giuntoli et al., 2017). Taking into account an average TDW production of ca. 36 Mg ha^{-1} year^{-1} (annual cuttings, Table 4), and the nutrient contents in Table 5, then about 515 kg ha^{-1} year^{-1} of N are required to support plant growth, of which 376 kg ha^{-1} year^{-1} could be fixed by *Leucaena-Rhizobium* root nodules. As a consequence, according to Audsley et al. (2003) and Giuntoli et al. (2017), the 376 kg of mineral N-fertilizer saved means saving 5602 kWh ha^{-1} year^{-1} during manufacturing, and reducing the emission of 1718 kg ha^{-1} year^{-1} of CO_2 into the atmosphere during manufacturing and transportation (i.e. 14.9 kWh and 4.57 kg of CO_2 per kilogram of N).

Biomass production from the fourth to the eleventh year followed the same pattern reported for the first three years. Significant effects of cutting treatment (CT, $p < 0.001$) and the interaction between cutting treatment and year ($CT \times Y$, $p = 0{,}001$) when evaluating TDW from the fourth to the eleventh year after planting were found (Table A.1). Annual rotation ($R_{i.j}S_1$) showed higher TDW than biennial rotation ($R_{i.j}S_2$): predicted mean (SE) were 38.8 (0.3) and 22.0 (0.4), respectively (Table A.2). TDW decreased with years after planting in both rotation treatments but more pronounced in biennials (predicted slopes (SE) were -0.07 (0.10) and -0.63 (0.16), respectively). This trend was similar for all study taxa, yet *L. leucocephala* `India´ yielded significantly more biomass ($p = 0.008$) than *L. leucocephala* `Honduras´(predicted mean (SE) were 32.0 (1.6) and 29.2 (1.6), respectively (Tables A.1 and A.2). Therefore, from the fourth to the eleventh year, annual rotations also produced more biomass than biennials, so that not only environmental conditions or genotype affect production, but also the harvesting frequency (Guidi Nissin et al., 2018; Kulig et al., 2019). At the end of the study period, we did not observe a decrease in the vigor of sprouts, despite some stumps having up to 10 cuttings. The more harvests can be obtained from a vigorous stump, the longer the cultivation cycle will be. This obviously delays the stump removal process and replanting costs (Dillen et al., 2011). Also, we did not observe significant differences in the vigor of the sprouts between replicates or between cultivation treatments.

Plant height was significantly influenced by the pairwise interactions $T \times CT$ and $T \times Y$ (Table 3). Plant height was higher in the *111* cutting treatment for *L. leucocephala* `India´ and `K360´, but in the case of *L. diversifolia* height was higher in the *3* treatment compared to *111* treatment (Fig. 4). In general, for *111* treatment, plant height was higher in *L. leucocephala* `India´ followed by the other two *L. leucocepha* taxa with *L. diversifolia* showing lower height value, while for *3* and *21* treatments *L. diversifolia* stood out over *L. leucosephala*. In addition, during the second and third year of study, height growth was significantly higher in *L. leucocephala* `India´ than in *L. diversifolia* (estimated slopes (SE) were 69.1 (4.1) and 52.5 (4.1) cm year$^{-1}$, respectively), but *L. leucocephala* `Honduras´and `K360´ (estimated slopes 65.5 (4.1) and 58.5 (4.0) cm year^{-1}, respectively) did not differ significantly from the previous two taxa. Plant diameter was significantly influenced by the pairwise interactions $T \times CT$ and $CT \times Y$ (Table 3). Plant diameter was significantly lower in the *111* cutting treatment for *L. diversifolia* than in the *21* and *3* treatments. However, significant differences in plant diameter between cutting treatments were not found for any of the *L. leucocephala* taxa (Fig. 4). Plant diameter was generally greater in the *3* cutting treatment also showing a greater diameter increment during the second and third year of study than *21* and *111* treatments (estimated slopes 6.1 (0.5), 2.3 (0.5) and 1.5 (0.3) cm year^{-1}, respectively).

[Figure 4 here]

Regarding phenology, both species showed sprouting and growth of buds throughout the year. Maximum shoot growth was observed when maximum and minimum daily temperatures were over 20 ºC and 12 ºC, respectively. Analogously, when daily maximum and minimum temperatures were below 17 ºC and 5 ºC, respectively, no shoot growth was reported. Fine twigs (≤ 1 cm diameter) and young annual sprouts were severely affected by temperatures below -4 ºC. The LAI did not display significant differences between species, yet significant differences were detected for the cutting treatment ($p = 0.001$) with values of 3.17 (0.09) for $R_{i.j}S_1$ and 4.00 (0.10) for $R_{2.1}S_2$ and $R_{3.1}S_3$. This range of LAI values is similar to those reported for other fast growth species, such as corn or aspen, since LAI is related to the exchange of energy and carbon dioxide between the crop and the atmosphere, and to the biomass production (Gower et al., 1999). No significant differences between taxa or cutting treatments were reported for SLA, which averaged 16.54 (0.55) m^2 kg^{-1}.

The predawn xylem water potential of the one-year-old shoots (1st and 3rd replicates) and the two- or three-year-old shoots (2nd and 4th replicates) were not significantly different between taxa, yet showed significant differences according to the cutting treatment. Predawn water potential in summer for annual re-sprouts was always higher than -1.6 MPa, with a value of -1.1 MPa until the end of July. In contrast, the two- and three-year-old shoots showed a predawn water potential of -1.5 MPa at the end of July, reaching minimum values around -2.0 (0.3) MPa at the beginning of September, which resulted in partial leaf abscission. *Leucaena* species are moderately tolerant to water stress (Natarajan and Paliwal, 1995; Chen et al., 2012). However, at least for *Leucaena leucocephala*, when predawn leaf water potential is below -1.0 MPa, gas exchange is abruptly limited, and plant survival is jeopardized when it drops below -2.2 MPa (Mrema et al., 1997; Liang and Zhang, 1999). The different tolerance to water stress of the annual re-sprouted shoots compared to the two- or three-year-old shoots could be due to a lower soil water consumption of the harvested replicates because of a lower LAI and the time required for the new sprouted shoots to reach the same size of the two- or three-year-old shoots (on average it took 3–4 months and needed temperatures above 15 ºC). Other experimental plantations in the area under more severe rainfed conditions showed lower growth and even plant mortality during summer drought, which might indicate that the present trial comprised a moderate rainfed situation with a slight drought period only in summer. The low drought and cold tolerance of these species probably reduces their invasive nature in the Mediterranean environment.

3.2. Physical-chemical properties of biomass

No significant differences between taxa ($p > 0.100$) were observed for dry basis heating values, ash content (Table 5), wood density and bark percentage. However, we found significant differences ($p < 0.05$) between the thin woody fraction (stems and branches < 25 mm of diameter) and the thickest fraction (> 50 mm of diameter); mean values (SE) for wood density were 0.60 (0.03) and 0.74 (0.05) kg/dm^3, respectively, and for bark percentage were 30.0 (2.4) and 12.0 (2.5) %, respectively. The calorific capacity of both species is high enough for commercial use as an energy source (Mainoo and Ulzen-Appiah, 1996; Feria et al., 2011; Fernández et al., 2015). Average annual production of 25 to 35 Mg ha^{-1} year^{-1} considering only the woody dry biomass (excluding the first year after planting), would result in 126 to 176 MWh ha^{-1} year^{-1}, when based on the lower heating value. Moreover, the non-woody dry biomass (25% of TDW) would contribute an additional 42 to 59 MWh ha^{-1} year^{-1}.

The mineral nutrient content of leaves, woody fraction and litterfall was not significantly different between species (except for specific cases of K and Cl, Table 5). The four taxa studied showed high nutrient contents, especially N, according to other studies (Parrotta, 1999; Fagbenro et al., 2015) which should be taken into account in the management of plantations because of the ecological value of the biogeochemical cycling of nutrients. For example, in the same way as mentioned above, an annual average extraction of 29 Mg ha^{-1} year^{-1} of woody dry biomass (WDW) implies the removal of 249, 23, 310, 160, and 64 kg ha^{-1} year^{-1} of N, P, K, Ca and Mg, respectively. However, simply by leaving the non-woody biomass (NWDW ≈ 7.25 Mg ha^{-1} year^{-1}) in the field after harvest the nutrients not removed would represent 228, 23, 106, 109 and 23 kg ha^{-1} year^{-1} of N, P, K, Ca and Mg, respectively, which are recycled and added to the nutrients contained in the litterfall. As a consequence, managers may take it into account for plantation management, both for the economic and environmental aspects related to the use of inorganic fertilizers, while at the same time extracting the best quality biomass for energy use (the woody biomass), since high nutrient content, mainly N, S, K and Cl but also Ca and Mg, are responsible for many undesirable reactions in combustion furnaces and power boilers (Jenkins et al., 1998).

[Table 5 here]

The production of pellets from the ground woody tissue had an efficiency of 99% (i.e. pellet to sawdust dry weight ratio after palletization). The pellets had the following characteristics: diameter = 6.02 (0.01) mm, length = 19.9 (0.4) mm, bulk density = 636 (5) kg m^{-3}, particle density = 1325 (15) kg m^{-3} dry weight basis, mechanical durability = 96.3 (1.2) %, and moisture content = 6.1 (0.2) %. However, the slightly high ash, Cl, N and S content (Table 5) could limit their application for domestic furnaces of a small size, yet not for industrial uses, according to the international standards for the pellets trade (TRADERSbiomass, 2020). The resulting pellets had hardwood characteristics and were of better quality than pellets from non-woody species. Even so, the transformation of *Leucaena* biomass into pellets confers an added value for commercialization, although the pellet quality provided by debarked coniferous wood will never be achieved, as it is known that chemical composition (i.e. lignin content, and the presence of hydrophobic extractives), as well as temperature and pelletizing pressure, have a significant influence on the bonding quality between biomass particles during the pelletizing process (Holm et al., 2006; Stelte et al., 2011). In addition, the energy use of the pellets can be improved if biomass is debarked before production. For instance, the ash content of bark 7.6 (0.3) % was much higher than that of debarked wood, which was 1.3 (0.3) %. Moreover, considering that non-woody material comprises around 25% of the total dry weight, other possible uses could be considered, such as forage or composting for fertilization, given their high N, amino acid and protein content (Isaac et al., 2003; Tadros et al., 2012; Tang et al., 2013; Gusha et al., 2017). Nonetheless, this last issue needs further study.

3.3. Soil properties

After 11 years of short rotation cropping, on the one hand, the litter layers of the soil (O horizon) showed no significant differences between taxa; the thickness of the top layer was 1.62 (0.15) cm, while that of the lower stratum was 2.97 (0.25) cm, corresponding to dry weights of 1.55 (0.12) kg m^{-2} and 6.12 (0.66) kg m^{-2}, respectively. Thus, after 11 years, 77 Mg ha^{-1} of dry matter (7.0 Mg ha^{-1} $year^{-1}$) and 2.07 Mg ha^{-1} of N (i.e. 188 kg ha^{-1} $year^{-1}$ of N, apart from the N harvested with the biomass) had accumulated in the upper part of the mineral soil. This accumulation of litterfall is much greater than in forests of temperate zones (Andivia et al., 2016) and close to the amount existing in tropical forests (Cassart et al., 2017), and was a consequence of the high productivity of this crop. The nutrients contained in the litter represent a reservoir that will slowly be incorporated into the soil (Sandhu et al., 1990; Parrotta, 1999; Gnahoua et al., 2013) which, together with the contributions from the fine roots (Jha and Prasad Mohapatra, 2010), will maintain productivity of the site. The mineral soil layer showed higher levels of organic matter, water-soluble organic C, N and dehydrogenase activity after 11 years of a *Leucaena* biomass production system, when compared with the conditions prior to establishing the system, and when compared to adjacent uncropped areas (Table 6), being representative of an increase in soil fertility due to cultivation (Mrema et al., 1997; Peng et al., 2013; Conrad et al., 2017). This positive effect of woody energy crops on soils have been also reported for other species (Ramesh et al., 2013; Fernández et al. 2018). Therefore, leaf litter decomposition, root turnover and microbial activity prevented soil degradation and, despite the continuous cropping and biomass extraction, soil fertility was improved.

The increase of N in the soil (0–40 cm depth) compared to the uncropped areas ranged from 75 to 104 kg ha^{-1} $year^{-1}$, which entails economic and environmental benefits, as discussed above. Similarly, the organic C increase ranged from 1.2 to 2.3 Mg ha^{-1} $year^{-1}$, therefore acting as a sink for carbon (equivalent to 4.4 – 8.4 Mg ha^{-1} $year^{-1}$ of CO_2), which allows mitigating the effects of climate change. This C sequestration capacity obtained for short rotation *Leucaena* plantation is within the usual high range of other plantations, 0.28–1.39 Mg ha^{-1} year^{-1} (Conrad et al., 2017; Campos et al., 2020), although some agroforestry systems have a huge potential of C sequestration to the extent of 10 Mg ha^{-1} year^{-1} in short rotation plantations when organic amendments are added (Srinivasarao et al., 2014). The importance of the increase in dehydrogenase activity is that it is a distinctive feature of soil degradation metabolic pathways as it is associated with intracellular metabolism of living cells and, therefore, is a good indicator of total microbial activity (Peng et al., 2013; Mukhopadhyay et al., 2016). The increase in the activity of this enzyme is an unequivocal sign of the soil improvement, which will lead to further growth of the plantation.

The other soil properties analyzed during the period of the present study (pH, EC, urease and β-glucosidase activities) did not change significantly ($0.148 < p < 0.506$). The average values (SE) at 0–20 cm depth of pH [KCl], pH [H2O], EC, available P and K, urease activity and β-glucosidase activity for the soil treatments as a whole were 5.01 (0.16), 5.72 (0.25), 4.8 (1.5) mS m^{-1}, 13.1 (2.4) mg kg^{-1}, 110 (15) mg kg^{-1}, 0.70 (0.10) μmoles g^{-1} h^{-1} of N-NH$_4$ and 51.3 (8.6) μg g^{-1} h^{-1} of PNP, respectively. Although no symptoms of leaf mineral deficiencies were observed throughout the study period, it should be studied if some of the main nutrients, for example P and K, should be applied to the soil to compensate for the exports in the harvested biomass and to prevent any reduction in biomass production. In addition, we did not find any visual symptoms of soil erosion in cultivated plots during the study period or plants of the cultivated taxa invading nearby areas of natural vegetation.

[Table 6 here]

4. Conclusions

Short rotation cropping legume species of the genus *Leucaena*, harvested annually can produce up to 39 Mg ha^{-1} year^{-1} of total dry biomass, which can generate up to 201 MWh ha^{-1} year^{-1} energy. Moreover, after 11 years of cropping, the nitrogen (≥ 35 %) and organic matter (≥ 41 %) content and dehydrogenase activity (≥ 98 %) of the 0–20 cm layer of mineral soil increased compared to uncropped areas and to the original soil. Therefore, soil fertility and carbon sequestration were improved using short rotation cropping of *Leucaena* species. Consequently, cultivation and gains may be compatible with the management of this multipurpose species in a sustainable agroforestry system.

Appendix

[Table A.1 here]

[Table A.2 here]

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Table 1: Climatic conditions in the area during the study period.

Climatic variable	**Mean (standard error)**
Absolute maximum temperature (ºC)	43.8
Warmest month's average maximum temperature (ºC)	31.3 (0.8)
Average annual temperature (ºC)	16.7 (0.3)
Coldest month's average minimum temperature (ºC)	4.5 (1.7)
Absolute minimum temperature (ºC)	-5.4
Annual precipitation (mm)	556 (197)
Summer precipitation from June to August (mm)	16 (11)
Annual reference crop evapotranspiration, ETo (mm)	1109 (30)

Table 2: Physical-chemical properties of litterfall, soil and plant material determined, together with brief explanation of the technical methods and instruments used.

Sample	Property	Technical method	Observations
Litterfall, soil and plant	Total N	Kjedahl method (auto-analyser Bran+Luebbe®, Mod. AIII).	
Litterfall and plant	C, S	Elemental analyzer (Thermo Scientific™ FLASH 2000).	
Litterfall, soil and plant	Ca, P, K, Mg	ICP-OES (Thermo Jarrell Ash Corporation). Olsen method for available P in soil.	After undertaking the total extraction with HNO_3. Available K in soil after extraction with ammonium acetate.
Soil	pH, Electrical conductivity (EC)	Multiparameter bench (PC 80, XS® Instruments)	pH: with distilled water (pH [H_2O]) and also in basis of a KCl solution (pH [KCl]). Volume fraction 1:2.5 for pH and 1:5 for EC.
Soil	Oxidizable organic matter fraction (OM)	Walkley and Black method.	
Soil	Water-soluble carbon (WC)	Combustion catalytic oxidation (Carbon TOC-VCSH, Shimadzu®).	By extraction with Milli-Q water following a 1:10 (soil to water) ratio.
Soil (0-20 cm layer)	β-glucosidase activity (β-gluc), dehydrogenase activity (Dhe), urease activity (Urease)	Standardized methods for these biochemical properties (Tabatabai, 1994).	β-gluc (p-nitrophenol content). Dhe (iodonitrotetrazolium formazan content). Urease (N-NH_4 content).
Plant	Cl	Photometer (HI 83200, Hanna® Instruments).	After undertaking the total extraction with HNO_3.
Plant	Ash	Muffle oven	550 ºC (ISO 18122:2015).
Plant	Heating values (constant volume)	According to the European standard EN 14918:2011 for solid biofuels using an automatic isoperibol calorimeter (Parr 6300®)	- Cold milling in order to avoid chemical structure disruptions. - Crushed to a powdered form and sieved through a 1 mm size mesh. - Data referred to a dry basis (moisture-free) after oven-drying at 105 ºC (ISO 18134-3:2015).

Table 3: *F* and *p* values (in parentheses) for the fixed effects of the linear mixed models to evaluate height (*H*), stem diameter (*D*), as well as total (TDW), woody (WDW) and non-woody (NWDW) dry weight, over the first three years after planting. Marginal and conditional R^2 for each model is also shown. Effects: *Y*, year; *CT*, cutting treatment; *T*, taxa.

	H (cm)	***D*** (mm)	**TDW** (Mg ha^{-1})	**WDW** (Mg ha^{-1})	**NWDW** (Mg ha^{-1})
Y	224.0 (<0.001)	152.4 (<0.001)	224.0 (<0.001)	227.5 (<0.001)	198.3 (<0.001)
CT	5.1 (0.080)	10.5 (0.026)	9.3 (0.031)	4.5 (0.095)	7.0 (0.049)
T	7.7 (<0.001)	4.6 (0.003)	4.4 (0.020)	3.4 (0.046)	5.9 (0.007)
CT × *Y*	0.4 (0.633)	32.6 (<0.001)	6.7 (0.002)	4.2 (0.021)	14.6 (<0.001)
T × *Y*	3.4 (0.018)	0.7 (0.521)	3.3 (0.026)	3.4 (0.025)	3.0 (0.040)
CT × *T*	16.8 (<0.001)	10.7 (<0.001)	1.3 (0.310)	0.8 (0.560)	2.0 (0.126)
CT × *T* × *Y*	1.2 (0.395)	1.1 (0.421)	0.9 (0.483)	1.0 (0.457)	0.8 (0.576)
R^2-*marginal*	0.566	0.324	0.591	0.576	0.573
R^2-*conditional*	0.579	0.426	0.717	0.712	0.720

Table 4: Estimated year slope [mean (standard error)] for each cutting treatment and taxa of the linear mixed models to evaluate accumulated total (TDW), woody (WDW) and non-woody (NWDW) dry weight over the first three years after planting. Different letters depict significant differences ($p < 0.05$) between cutting treatments and taxa, respectively. Cutting treatments: *111* ($R_{11}S_1 + R_{22}S_1 + R_{33}S_1$), *21* ($R_{21}S_2 + R_{32}S_1$) and *3* ($R_{31}S_3$). Taxa: *Ld*, *Leucaena diversfolia*; *Ll*, *Leucaena leucocephala*.

	TDW (Mg ha^{-1} year^{-1})	**WDW** (Mg ha^{-1} year^{-1})	**NWDW** (Mg ha^{-1} year^{-1})
Cutting treatment			
111	35.7 (1.3) a	25.0 (0.9) a	9.8 (0.4) a
21	21.1 (1.8) b	15.6 (1.3) b	5.4 (0.5) b
3	10.1 (1.8) c	7.9 (1.3) c	2.1 (0.5) c
Taxa			
Ld	22.2 (1.9) b	15.8 (1.3) b	6.0 (0.6) b
Ll \`Honduras´	15.3 (1.9) c	10.8 (1.3) c	4.1 (0.6) c
Ll \`India´	29.7 (1.9) a	20.9 (1.3) a	8.4 (0.6) a
Ll \`K360´	22.0 (1.9) bc	15.8 (1.3) b	5.9 (0.6) bc

Table 5: Chemical and energetic properties [mean (SE)], expressed on a dry weight basis, for the different samples (leaves, woody tissues [stem and branches with bark] and litterfall) for the four taxa of *Leucaena* as a whole.

	Leaves	**Woody tissues**	**Litterfall**
C (%)	44.94 (0.52)	44.98 (0.78)	42.63 (0.84)
N (%)	3.14 (0.19)	0.86 (0.12)	2.67 (0.25)
P (%)	0.32 (0.05)	0.08 (0.02)	0.13 (0.02)
K (%)	1.47 (0.12)	1.07 (0.08)	0.32 (0.11)*
Ca (%)	1.50 (0.16)	0.55 (0.20)	1.47 (0.44)
Mg (%)	0.32 (0.02)	0.22 (0.02)	0.20 (0.01)
S (%)	0.28 (0.05)	0.09 (0.03)	0.45 (0.05)
Cl (%)	0.27 (0.03)*	0.08 (0.02)	*n.a.*
HHV (MJ/kg)	19.7 (0.2)	19.4 (0.2)	*n.a.*
LHV (MJ/kg)	18.4 (0.2)	18.1 (0.2)	*n.a.*
Ash (%)	10.5 (0.3)	1.89 (0.03)	*n.a.*

Asterisks indicate significant differences ($p < 0.05$) among taxa, with *L. diversifolia* (*Ld*) being significantly different from the three taxa of *L. leucocephala* (*Ll*): K = 0.45 (0.08) % for *Ld*, and K = 0.18 (0.07) % for *Ll* as a whole; Cl = 0.23 (0.02) % for *Ld*, and Cl = 0.33 (0.02) % for *Ll* as a whole. *n.a.*: not analyzed.

Table 6: Soil characteristics [mean (SE)] at two depths (*SOD*, 0–20 cm and 20–40 cm). Different letters within a row denote significant differences ($p < 0.05$) between cultivation treatments (*CU*). OM: organic matter; WC: water-soluble carbon; Dhe: dehydrogenase activity (µg g^{-1} h^{-1} of INTF).

Parameter	***Soil Depth (SOD)***	***Cultivation treatment (CU)***(1)			***Significance level (p)***		
		Uncultivated	***Ld***	***Ll***(2)	***CU***	***SOD***	***CU x SOD***
OM (%)	*0-20 cm*	1.03 (0.12) a	1.85 (0.06) b	1.45 (0.21) b	**< 0.001**	0.110	0.866
	20-40 cm	0.66 (0.09) a	1.70 (0.15) b	1.21 (0.18) b			
WC (mg kg^{-1})	*0-20 cm*	139 (20) a	186 (13) b	172 (22) ab	**0.004**	0.745	0.104
	20-40 cm	112 (22)	146 (10)	117 (16)			
N (mg kg^{-1})	*0-20 cm*	390 (76) a	625 (50) b	525 (77) ab	**0.012**	0.060	0.787
	20-40 cm	230 (32) a	493 (20) b	455 (68) b			
Dhe	*0-20 cm*	0.66 (0.04) a	1.40 (0.21) b	1.31 (0.10) b	**0.015**		

(1) Five cultivation treatments were used (*CU*): uncultivated (natural vegetation), cultivated under *L. diversifolia* (*Ld*), and three treatments cultivated under each *L. leucocephala* taxon (*Ll*).

(2) As *L. leucocepahala* taxa did not differ significantly between each other, the mean values for the three taxa as a whole are shown.

Table A.1: *F* and *p* values (in parentheses) for the fixed effects of the linear mixed models to evaluate total dry weight (TDW) for the period between the fourth and the eleventh year after planting. Marginal and conditional R^2 for each model is also shown. Effects: *Y*, year; *CT*, cutting treatment; *T*, taxa

	TDW (Mg ha^{-1} year^{-1})
Y	3.7 (0.059)
CT	1203.3 (<0.001)
T	5.0 (0.008)
CT × *Y*	13.0 (0.001)
T × *Y*	0.2 (0.995)
CT × *T*	0.2 (0.929)
CT × *T* × *Y*	0.1 (0.998)
R^2-*marginal*	0.907
R^2-*conditional*	0.907

Table A.2: Estimated total dry biomass [mean (standard error)] for each cutting treatment and taxa evaluated by the linear mixed models from the fourth to the eleventh year after planting. Different letters depict significant differences ($p < 0.05$) between cutting treatments and taxa, respectively. Cutting treatments: *annual* ($R_{i,j}S_1$) and biennial ($R_{i,j}S_2$). Taxa: *Ld*, *Leucaena diversfolia*; *Ll*, *Leucaena leucocephala*.

	TDW (Mg ha^{-1} year^{-1})
Cutting treatment	
annual	38.8 (0.3) a
biennial	22.0 (0.4) b
Taxa	
Ld	30.3 (1.6) ab
Ll `Honduras´	29.2 (1.6) b
Ll `India´	32.0 (1.6) a
Ll `K360´	30.3 (1.6) ab

Figures captions

Figure 1: Experimental design of the plot containing two parts, and a detailed description of the experimental unit (replicate). The numbers **1**, **2**, **3** and **4** refer to the taxa (1: *Ld*, 2: *LlH*, 3: *LlI*, 4: *LlK*); and **b** are the border lines, which were composed by the same *Leucaena* taxa. *Ld*: *Leucaena diversifolia*; *Ll*: *Leucaena leucocephala* (LlH, `Honduras´; LlI, `India´; LlK , `K360´).

Figure 2: Measurement and harvesting dates during the first three cropping years. The measurements of height (*H*) and stem diameter at the base (*D*) of all the experimental units were carried out in late February to early March, just before each harvest. The experimental units harvested every year after the *D* and *H* measurements are indicated. Measured parameters in summer: LAI, leaf area index; SLA, specific leaf area; Ψ, predawn leaf water potential.

Figure 3: Total dry biomass (TDW ± SE) accumulated throughout the first three years of the study for *L. diversifolia* and *L. leucocephala*. (*Left*) Accumulated biomass for the four taxa as a whole if cutting takes place only once in the indicated years (*3*, $R_{31}S_3$), annually (*111*, $R_{11}S_1 + R_{22}S_1 + R_{33}S_1$), and assuming annual cutting the 3rd year ($R_{32}S_1$) after a biennial cutting the first time ($R_{21}S_2$) (*21*, $R_{2.1}S_2 + R_{32}S_1$). Different letters indicate significant differences between the cutting treatments ($p = 0.031$). (*Right*) Accumulated biomass of the four *Leucaena* taxa for the *111* cutting treatment. Different letters indicate significant differences between taxa ($p < 0.001$) at the end of the third year after planting. *Ld*: *Leucaena diversifolia*; *Ll*: *Leucaena leucocephala* (LlH, `Honduras´; LlI, `India´; LlK, `K360´).

Figure 4: Boxplot of plant height (*H*) and stem diameter (*D*) for each taxa and cutting treatment throughout the first three years of the study. Taxa: *Ld* (*L. diversifolia*), *Ll* (*L. leucocephala*) being the three taxa, `Honduras´ (LlH), `India´ (LlI) and `K360´ (LlK). Cutting treatments: *111* [$R_{11}S_1 + R_{22}S_1 + R_{33}S_1$], *21* [$R_{21}S_2 + R_{32}S_1$] and *3* [$R_{31}S_3$]. Boxes are 95% and 5% percentile values, while the solid lines indicate the median. Different letters depict significant differences ($p < 0.05$) between cutting treatment for each taxa.

Figure 1.

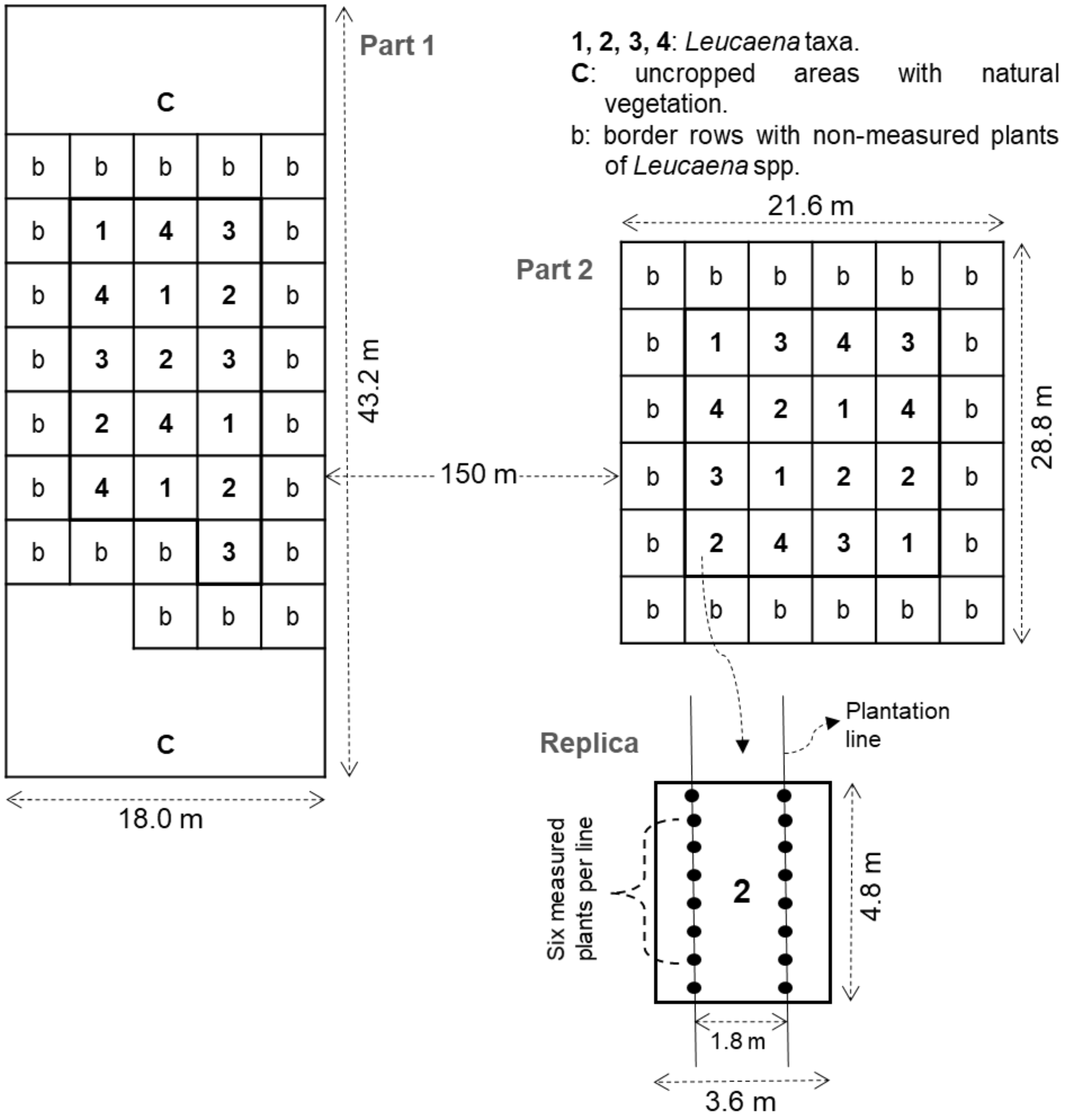

Part 1
C
b b b b b
b 1 4 3 b
b 4 1 2 b
b 3 2 3 b
b 2 4 1 b
b 4 1 2 b
b b b 3 b
b b b
C
43.2 m
18.0 m
150 m
1, 2, 3, 4: *Leucaena* taxa.
C: uncropped areas with natural vegetation.
b: border rows with non-measured plants of *Leucaena* spp.
21.6 m
Part 2
b b b b b b
b 1 3 4 3 b
b 4 2 1 4 b
b 3 1 2 2 b
b 2 4 3 1 b
b b b b b b
28.8 m
Replica
Plantation line
Six measured plants per line
2
4.8 m
1.8 m
3.6 m

Figure 2

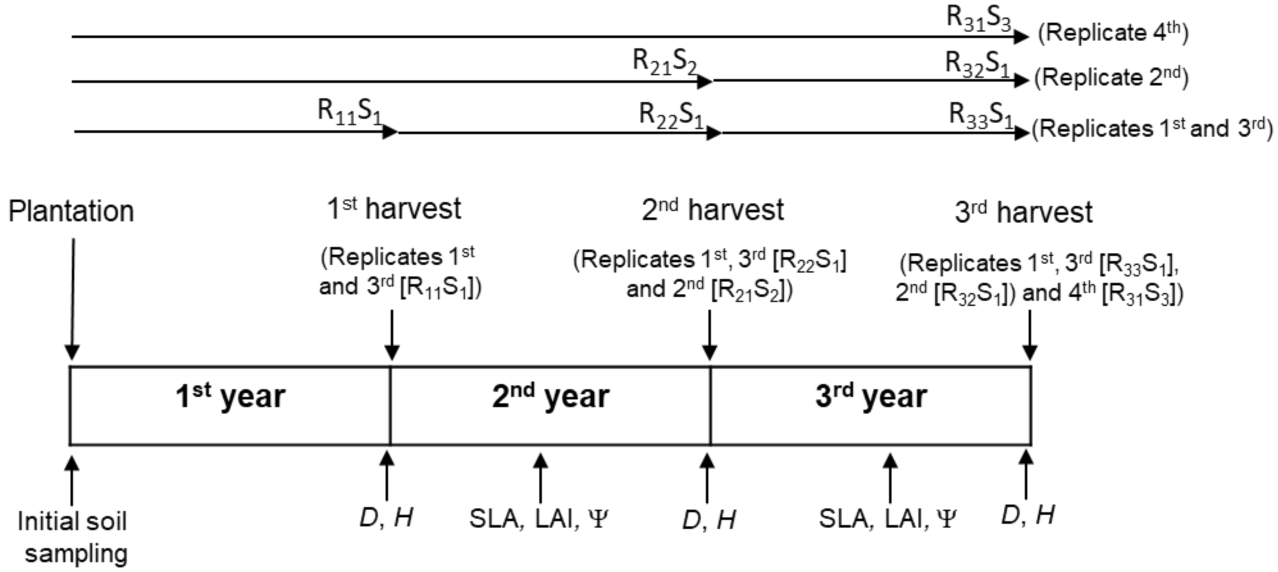

$R_{31}S_3$ (Replicate 4th)
$R_{21}S_2$
$R_{32}S_1$ (Replicate 2nd)
$R_{11}S_1$
$R_{22}S_1$
$R_{33}S_1$ (Replicates 1st and 3rd)
Plantation
1st harvest
(Replicates 1st and 3rd [$R_{11}S_1$])
2nd harvest
(Replicates 1st, 3rd [$R_{22}S_1$] and 2nd [$R_{21}S_2$])
3rd harvest
(Replicates 1st, 3rd [$R_{33}S_1$], 2nd [$R_{32}S_1$]) and 4th [$R_{31}S_3$])
1st year
2nd year
3rd year
Initial soil sampling
D, H
SLA, LAI, Ψ
D, H
SLA, LAI, Ψ
D, H

Figure 3

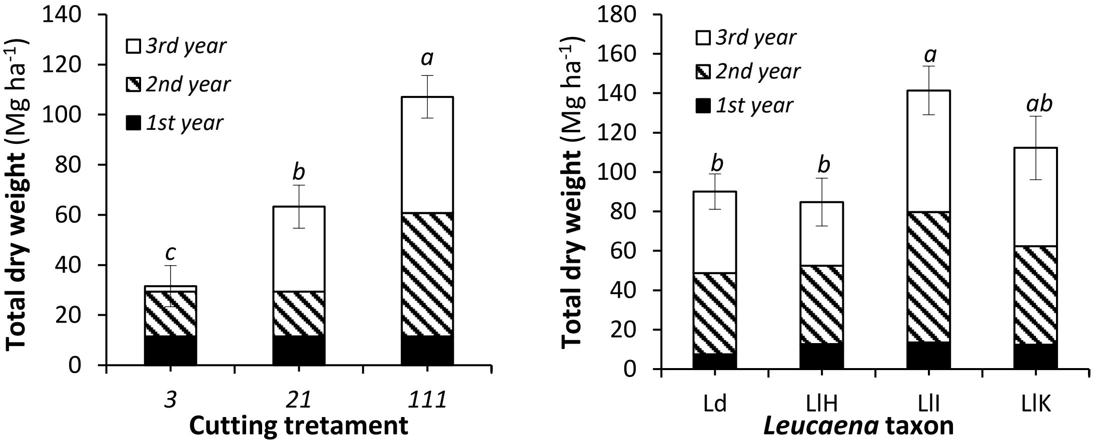
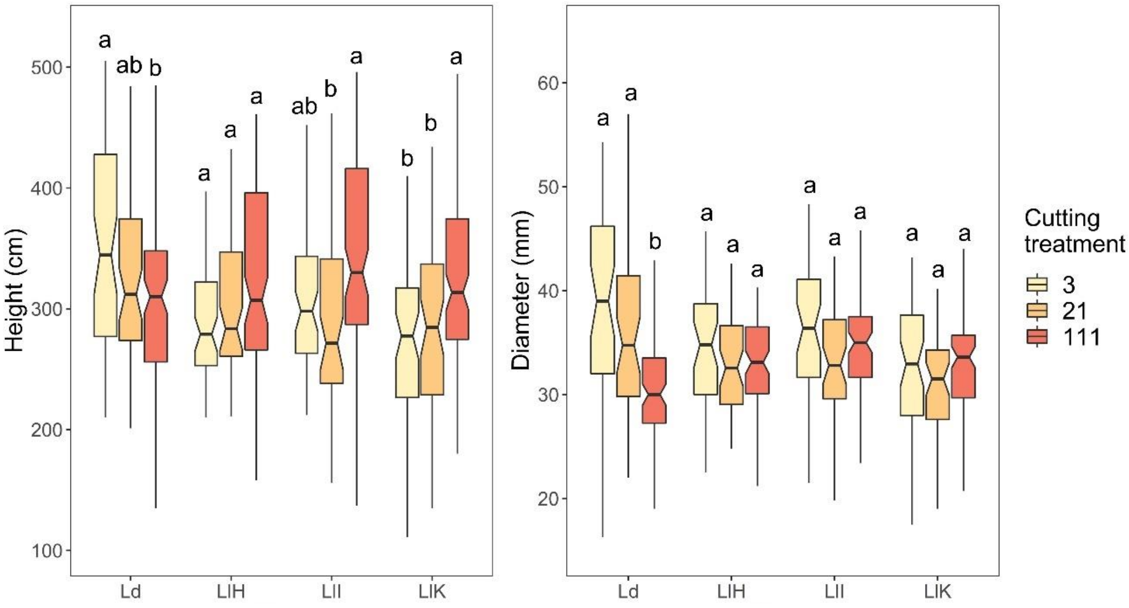

Total dry weight (Mg ha^{-1})
140
120
100
80
60
40
20
0
3rd year
2nd year
1st year
c
b
a
3
21
111
Cutting tretament
Total dry weight (Mg ha^{-1})
180
160
140
120
100
80
60
40
20
0
3rd year
2nd year
1st year
b
b
a
ab
Ld
LlH
LlI
LlK
Leucaena taxon

Figure 4


Height (cm)
100
200
300
400
500
a
ab
b
a
a
a
ab
b
a
b
b
a
Ld
LlH
LlI
LlK
Diameter (mm)
20
30
40
50
60
a
a
b
a
a
a
a
a
a
a
a
a
Ld
LlH
LlI
LlK
Cutting treatment
3
21
111