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Legume living mulch for afforestation in agricultural land in Southern Spain

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ABSTRACT

Weed control is essential for a successful establishment and growth of tree seedlings in former agricultural land. Weed control methods are effective but can be costly in terms of time, damage to non-target vegetation, or increased soil erosion. Alternatively, some living mulches can exclude undesirable vegetation, protect the soil, compete minimally with associated trees, and supplement soil nitrogen, but there is a lack of knowledge on living mulch systems in Mediterranean afforestation. Thus, the objective of the present study was to evaluate the effects on Holm oak (*Quercus ilex* L.), mastic tree (*Pistacia lentiscus* L.), wild olive (*Olea europaea* L. var. *sylvestris* Brot.) and terebinth (*Pistacia terebinthus* L.) seedlings of wrinkled medick (*Medicago rugosa* Desr.) mulch. Survival, growth, photosynthesis, foliar nutrient and soil parameters were measured during the first year. 36 months after planting, seedlings in the living mulch had survival rates of between 60% for mastic tree and 8.3% for Holm oak, compared with survival rates of 70% in the mechanical treatment for mastic tree and 2% for Holm oak. Photosynthesis and foliar nutrient concentrations were improved by the living mulch treatment. The soil under the living mulch had higher CEC, soil organic matter levels and nitrogen content in comparison to the cultivated soil. The response of living mulch differs between species and environmental conditions but our study suggests a positive effect due to soil protection. Living mulch may be a promise alternative for use in Mediterranean afforestation programs.

1. Introduction

In afforestation of former agricultural fields, herbaceous weeds are known to compete with newly planted seedlings for water, nutrients, and light (Cogliastro et al., 1990). Silvicultural practices, that are often adopted for managing ground cover, can be classified as mechanical (tilling, mowing, and grazing), chemical (herbicide applications), and physical control (synthetic weed barriers and organic mulches), used alone or in combination with tree shelters (Dobois et al., 2000; Navarro Cerrillo et al., 2005). When compared with plantings without herbaceous weed control, treated forest plantations are commonly characterized by increased plant survival and enhanced growth (Navarro Cerrillo et al., 2005; Athy et al., 2006; South and Miller, 2007). These methods are all effective, but each has its disadvantages. Furthermore, soil left bare after tillage operations can increase surface exposed to erosion forces and facilitate loss of organic matter and eventual soil structure degradation (Pimentel et al., 1995). An alternative method of ground cover management is the use of living mulches.

Living mulch is a permanent cover crop that is planted in conjunction with a tree seedling for the purpose of suppressing weed growth while also protecting the soil. Living mulch has been recognized as a beneficial practice in forest plantations (van Sambeek et al., 1986; Alley et al., 1999). This weed control system improves soil structure, increases organic matter and humus in the soil, and promotes biological activity (Dupraz et al., 1997). Thus, the utility of living mulch has been assessed in various forestry applications, including the establishment of hardwood and conifer plantations (Alley et al., 1999). Ideal living mulch should provide sufficient ground coverage to exclude other plants and compete minimally with the tree seedlings for water and nutrients. Living mulch species must be selected in order to suppress weed growth but not compete excessively with the associated tree seedlings. There are some species with an adequate phenology and morphology such as grasses (*Lolium*, *Poa*, *Agrostis*, *Festuca*, etc.), and, in particular, legumes with a short or prostrate growth habit

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and dense foliage (*Trifolium*, *Lotus*, etc.) (van Sambeek et al., 1986; Navarro-Cerrillo and Saveedra, 1997). Legumes species have some advantages: they compete less for moisture during summer, they have less competitive root systems, a short growth characteristic, which prevents the living mulch from shading the tree seedling, dense foliage which protects the soil and suppresses weed growth, and the ability to improve soil fertility through symbiotic bacterial nitrogen fixation (Gordon and Wheeler, 1983).

A small number of studies have included the use of mulch with Mediterranean species (Dupraz et al., 1997; Navarro Cerrillo et al., 2005), but there is a lack of knowledge on living mulch systems in afforestation of Mediterranean species. Thus, the objective of the present study was to evaluate the effects on four Mediterranean species of wrinkled medick (*Medicago rugosa* Desr.) mulch on early survival, growth, photosynthesis, foliar nutrient composition and soil parameters.

2. Materials and methods

2.1. Experimental site

The experiment plot was located in Córdoba (Andalusia, Southern Spain) (coordinates 37°51′N and 4°48′E, 92 m altitude). Soil was classified as a flat loam Xerofluvents (www.soils.usda.gov) characterized by fine loamy soil textures and very slow permeability. The area has a dry Mediterranean climate with an average annual rainfall of 670 mm, with hot, dry summers and mild winters. The site has been periodically cropped and was left fallow during the 2001–2003 growing seasons, supporting a mixture of native and introduced herbaceous species associated with agricultural bottomlands. The study site was approximately 1500 m^2 in size and had a slope of less than 2°. The experiment was conducted from November 2004 to December 2005. Weather data at the site were obtained from Junta de Andalusia Automated Weather Network (www.ias.es). The 0.6-m annual air temperature during the study period averaged 18.6 °C, close to the 30-year average, 19.1 °C. The 2-m total rainfall between November 2004 and October 2005 was 384 mm, 43% lower with respect to the 30-year average (670 mm).

2.2. Site preparation, treatment establishment and experimental design

In November 2004, seedlings of holm oak (*Quercus ilex* L. subsp. *ballota* [Desf.] Samp.), mastic tree (*Pistacia lentiscus* L.), wild olive (*Olea europaea* L. var. *sylvestris* Brot.) and terebinth (*Pistacia terebinthus* L.) were planted at the site. The seedlings were obtained from a private nursery, and were supplied in 1000 cm^3 containers using coco fibre-peat-vermiculite (2:1:1 volume) as a substrate (Table 1).

The experiment was arranged as a factorial design with two levels of weed control (i.e. cultivation and living mulch), in a randomized complete block with 4 blocks and 20 replications per factorial combination, giving a total of 80 plants per treatment and species. Prior to the establishment of plots, the entire study area was ploughed and disked to kill existing vegetation and create a suitable seedbed. No fertilizer or soil amendments were added to the site at any time during the study. Lastly, the planting area was subsoiled, using a ripper with a single tine, to a depth of more than 60 cm. The planting was done by hand in a rectangular plot (10 m × 30 m) following a systematic spatial pattern distribution with a density of 3300 plants ha^{-1} (2 m × 1.5 m). The treatments imposed were:

1. Cultivation (CT) was carried out using two passes with a small rotary cultivator drawn by a 40 hp farm tractor to manage weeds (5-cm depth) twice during the growing season in May and October 2005.
2. A leguminous living mulch (LM) with *Medicago rugosa* Desr. was sown at a rate of 115 kg ha^{-1} in November 2004 using a Direttissima 250 no-tillage seeder (Gaspardo, Pordenone, Italy). All tree seedlings were established in complete competition with forages, i.e. no vegetation control was conducted around seedlings.

A blank treatment was not included as result of the very low survival observed in a previous study if compared with soil conservation techniques (Navarro Cerrillo et al., 2005).

2.3. Survival and morphological plant analysis

Survival, height and diameter were assessed every 3 months during the first year (February–November 2005) and in once per year in 2006 and 2007. A paint mark was placed on each stem approximately 3 cm above the ground line to ensure that diameter measurements were taken at the same point each time. Height was measured to the highest growing point.

Four plant leaf samples per treatment and species were collected in November 2005 by removing exposed mature leaves from the upper crown on each of the four species nearest the centre of each plot. All leaves were oven-dried at 80 °C for 48 h and ground through a 0.5 mm screen. Potassium, calcium and magnesium content were determined using a Varian SpectraAA-10 Atomic Absorption Spectrometer, while a Skalar segmented flow auto analyzer was used for colorimetric determination of phosphorus (880 nm). Nitrogen was determined by the micro-Kjeldahl method.

2.4. Gas exchange measurements

Three 1-year-old sun leaves of four plants per treatment were labelled and measured during 12 August 2005, taking care to always clamp the same leaf portion, corresponding to the central part across the rib. Between 6:00 and 18:00, net photosynthesis (P_{net}) was measured. An infrared gas analyzer (Ciras-1, PP systems, Hitchin, UK) equipped with a Parkinson leaf chamber was used to estimate net CO_2 assimilation rate at 350 mmol mol^{-1} CO_2 flow rate. Ambient radiation, humidity and temperature were used.

Table 1
Months of cultivation, height, diameter and dry weight of seedlings using mechanical soil treatment and ground cover of living mulch of wrinkled medick (*Medicago rugosa* Desr.) on plots in Córdoba (Southern Spain).

Species	Age (months)	Height (cm), N = 20	Ø (mm), N = 20	Dry weight (g), N = 10
Quercus ilex	20	75.6 ± 2.0	11.7 ± 0.4	105.0 ± 7.6
Olea europaea	16	130.5 ± 5.2	25.9 ± 12.7	102.1 ± 6.0
Pistacia lentiscus	16	76.7 ± 3.0	9.8 ± 0.2	40.5 ± 1.8
Pistacia terebinthus	18	65.5 ± 2.8	9.1 ± 0.2	19.2 ± 0.8

Mean ± 1 S.E.

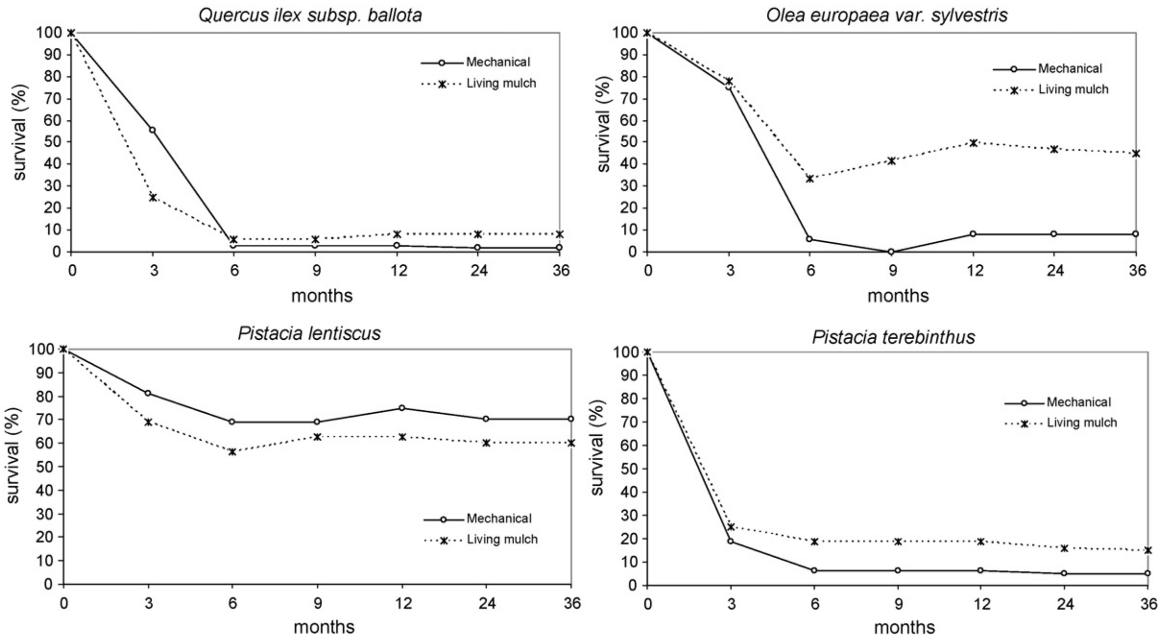

Fig. 1. Mean survival rate (%) of seedlings planted on mechanical and living mulch weed control treatment. Bars show 1 S.E.

Four plants per treatment were irrigated (10 l month^{-1} between March and October) as reference plants.

2.5. Soil measurements

Three soil samples per treatment were taken between depths of 15 and 30 cm during autumn 2005. Soil samples were double-bagged in sealable plastic bags and transported to the laboratory and air-dried for a few days, crushed, passed through a sieve of 2-mm mesh size and then stored separately for further analyses. The pH of the soil at different treatments was measured in the suspension of 1:5 (w/v) with the help of pH meter (Model EA940, Orion, USA) standardized with pH 4, 7 and 9.2 reference buffers. Total nitrogen content of the soil samples was determined by Gerhardt automatic analyzer (Model KB 8S, Germany), and extracted P_2O_5–P_{tot} by calcium acetate lactate (CAL) method. K^+, Mg^{2+} and Ca^{2+} were extracted from soil in ammonium acetate solution following repeated leaching procedure and then concentrations were determined using an Atomic Absorption Spectrophotometer (Model 2130, PerkinElmer, Inc., Norwalk, CT, USA). Cation exchange capacity (CEC) was determined using the equation $CEC = (K/780 + Ca/400 + Mg/240) + Factor$, where $Factor = (8 - pH_{buffer}) \times 8$ (Horwitz, 2000).

Volumetric soil water content during the trial period was measured at three random locations in each treatment using sensor (capacitance) measurements (ECH_2O System, WA, USA) connected to a data-logger (HOBO U12, Onset Computer Corporation, MA, USA) with a frequency of 15 min. Data were analyzed with the software Green Line (Onset Computer Corporation, MA, USA), and transformed into weekly averages.

2.6. Statistical analysis

All the variables were examined to ascertain that the variables were normally distributed and the variances were homogeneous. Prior to analysis, mean survival data were transformed into the arcsin of the square root of proportion survival. All these variables were analyzed with a Student's *t*-test ($P \leq 0.05$) (Quinn and

Table 2
Height and diameter of tree seedlings one and three year after plantation with mechanical soil treatment and ground cover of living mulch of wrinkled medick (*Medicago rugosa* Desr.) on plots in Córdoba (Southern Spain).

Treatment	Initial height (cm)	ΔHeight (cm)		Initial Ø (mm)	ΔØ (mm)	
	2004	2005	2007	2004	2005	2007
Quercus ilex						
Mechanical	78.8 (1.4)	−0.5 (2.1)*	22.7 (1.1)**	11.7 (0.4)	−1.4 (0.5)*	6.5 (1.6)
Living mulch	76.8 (1.5)	−3.8 (1.2)	18.5 (1.4)	11.5 (0.2)	0.03 (0.2)	7.3 (1.3)
Olea europaea						
Mechanical	130.0 (3.4)	−92.4 (17.4)*	21.9 (3.2)**	13.4 (0.2)	0.3 (0.3)	4.1 (0.7)**
Living mulch	135.8 (2.6)	−31.4 (10.5)	33.8 (2.6)	13.6 (0.2)	0.3 (0.1)	5.1 (0.5)
Pistacia terebinthus						
Mechanical	62.2 (2.0)	−10.2 (4.6)**	9.2 (3.1)	7.9 (0.2)	−0.4 (0.5)*	4.1 (1.1)
Living mulch	60.5 (2.2)	−8.7 (4.5)	11.1 (2.2)	8.5 (0.3)	−0.1 (0.6)	4.4 (0.9)
Pistacia lentiscus						
Mechanical	70.0 (2.5)	−13.2 (5.5)**	14.7 (3.7)**	8.3 (0.2)	1.1 (0.2)*	4.5 (1.1)*
Living mulch	69.6 (1.9)	−8.4 (5.1)	19.9 (3.9)	8.1 (0.4)	2.3 (0.6)	6.7 (0.9)

Mean ± 1 S.E. Level of significance: $^{**}P$-value <0.05; $^{*}P$-value <0.01.

Keough, 2002). Data were stored and processed by using Microsoft Excel 2000, and the descriptive statistical analysis of the data was done with SPSS v.13.0 software.

3. Results

3.1. Seedling performance

The weed control treatments had a different effect on survival depending on the species. 36 months after planting, seedlings in the living mulch had survival rates of between 60% for mastic tree and 8.3% for holm oak, compared with survival rates of 70% in the mechanical treatment for mastic tree and 2% for holm oak (Fig. 1). These differences were statistically significant at the end of the trial for Holm oak (arcsin transformation, $t = -11.36$; d.f. = 6; $P < 0.001$), wild olive (arcsin transformation, $t = -10.01$; d.f. = 6; $P < 0.001$), and terebinth (arcsin transformation, $t = -5.23$; d.f. = 6; $P < 0.001$) with living mulch, and mastic tree (arcsin transformation, $t = 3.1$; d.f. = 6; $P = 0.009$) in the mechanical treatment. On wild olive, living mulch increased survival by about 40% as compared with cultivated plants. However, in the rest of the species differences were less than 10%.

Mean height of plants showed a negative growth during the first year (Table 2). However, 3 years after plantation, both height and diameter growth were positive and significant differences were found between treatments. Significant differences between treatments regarding final height were found for Holm oak ($t = 12.71$; d.f. = 6; $P < 0.001$) with higher plants in the mechanical treatment, and wild olive ($t = -35.62$; d.f. = 6; $P < 0.001$) and mastic tree ($t = -4.71$; d.f. = 6; $P = 0.003$) in the living mulch treatment (Table 2). Diameter growth was less affected by the legume treatment (Table 2). The lowest values were obtained in mechanical treatment, and t-test revealed that the mechanical treatment was significantly different from the living mulch for mastic tree ($t = -12.24$; d.f. = 6; $P < 0.001$) and wild olive ($t = -10.81$; d.f. = 6; $P < 0.001$).

3.2. Foliar nutrient

The ground management treatments often had no significant effect on foliar nutrients. N concentrations in leaves of Holm oak plants grown in living mulch treatment were significantly higher as compared to those in cultivated soil ($t = -11.79$; d.f. = 6; $P < 0.001$) (Fig. 2) and Ca concentration decreased. For the remaining species the nutrient concentration did not differ significantly between different treatments (Fig. 2).

3.3. Gas exchange measurements

Fig. 3 shows the changes in CO_2 assimilation rate in the four species studied. CO_2 assimilation was maintained at a lower rate in the weed control treatments as compared to the irrigated reference plants. Compared to the irrigated plants, the plants had a relatively high CO_2 assimilation rate. For example, the maximum CO_2 assimilation rate for Holm oak, the irrigated reference plant, was 10.6 and 8.6 μmol m^{-2} s^{-1} in the living mulch treatment (Fig. 3). There were no marked differences between mechanical and living mulch weed control. However, CO_2 assimilation rate decreased more rapidly during Midday in the plants grown in the cultivated plot. Moreover, this decrease was much greater in Holm oak and wild olive compared to mastic tree and terebinth.

3.4. Soil features

The soil under the living mulch had higher CEC, soil organic matter levels and nitrogen content in comparison to the cultivated

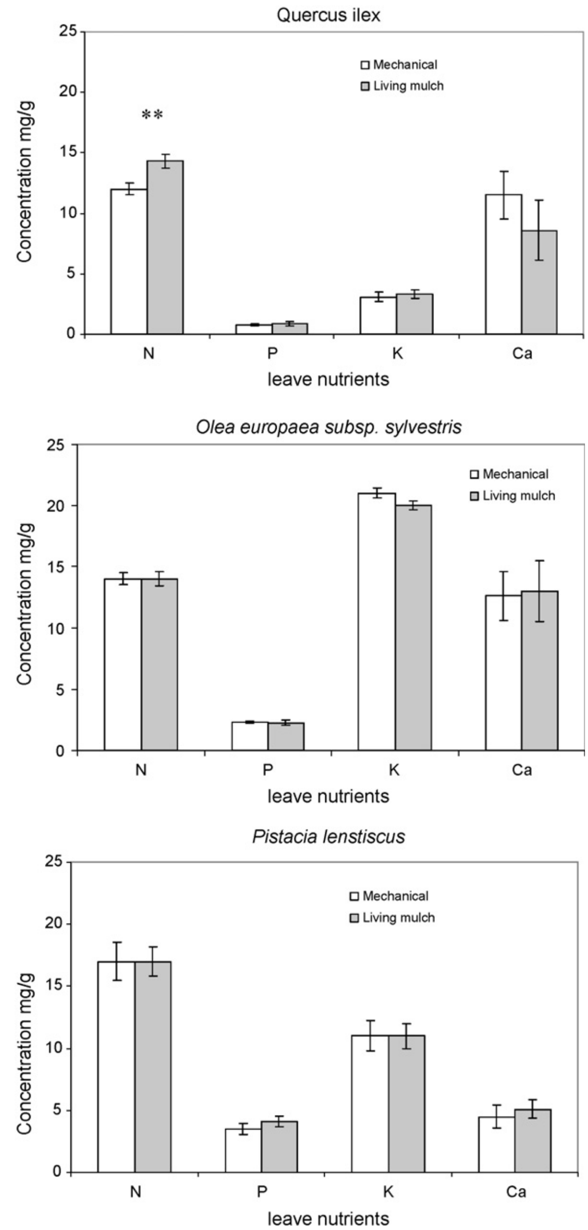

Fig. 2. Mean survival nutrient concentration on leaves (mg g^{-1}) on seedlings planted on mechanical and living mulch weed control treatment. Bars show 1 S.E.

Table 3
Selected physic-chemical properties of mechanical and living mulch soil of wrinkled medick (*Medicago rugosa* Desr.) on plots in Córdoba (Southern Spain).

Parameters	Mechanical	Living mulch	Reference soil
pH (1:5)	8.48 (0.01)	8.46 (0.05)	8.46
EC (meq/100 g)	9.19 (0.20)	10.45 (0.14)	10.21
Soil organic matter (%)	0.82 (0.02)	1.02 (0.03)***	0.88
Nitrogen (%)	0.06 (0.01)	0.07 (0.01)	0.06
P (mg kg^{-1})	6.5 (0.91)	11.52 (0.85)*	7.55
Ca (meq/100 g)[a]	5.51 (0.21)	5.77 (0.19)	5.49
Mg (meq/100 g)	2.94 (0.11)	3.84 (0.15)**	3.98
Na (meq/100 g)	0.26 (0.01)	0.27 (0.01)	0.29
K (meq/100 g)	0.46 (0.01)	0.56 (0.02)**	0.45

Mean ± 1 S.E., $N = 3$. Level of significance: **P-value <0.05; *P-value <0.01; ns = not significant.

[a] Ca, Mg, and K are exchangeable concentrations.

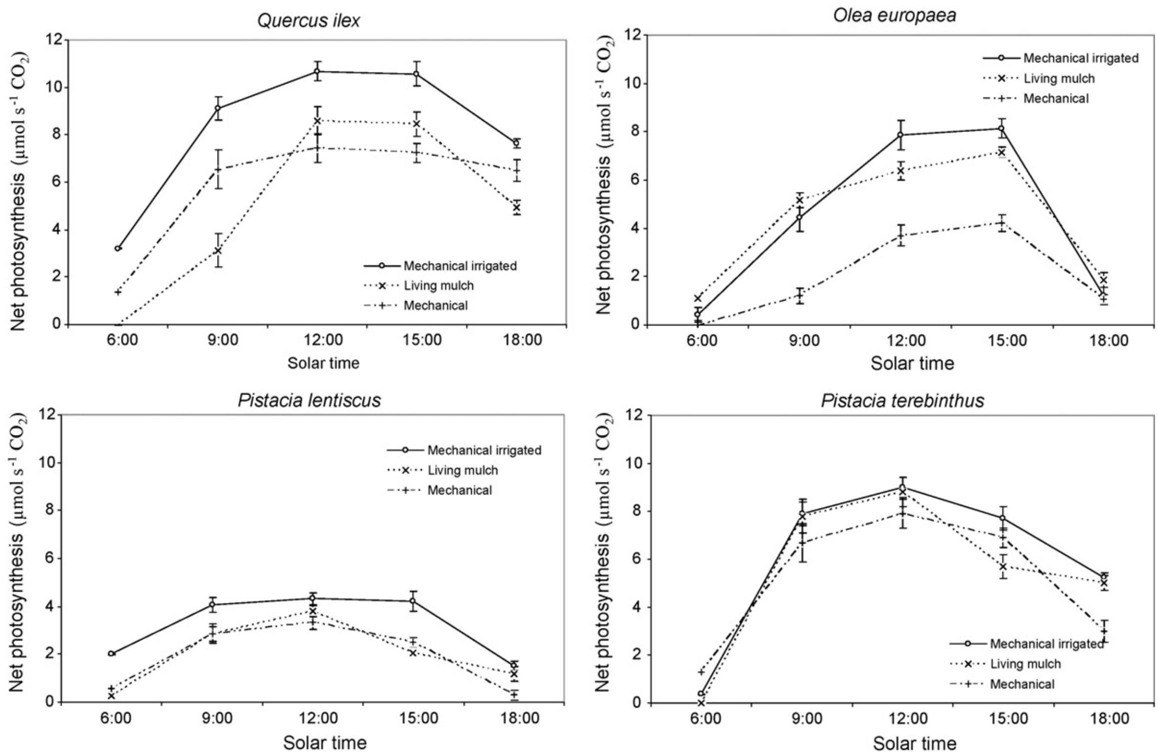

Fig. 3. Net photosynthesis (P_{net}) rate (μmol m^{-2} s^{-1}) of seedlings planted on mechanical and living mulch weed control treatment. Bars show 1 S.E. Mechanical irrigated plants were used as a reference value.

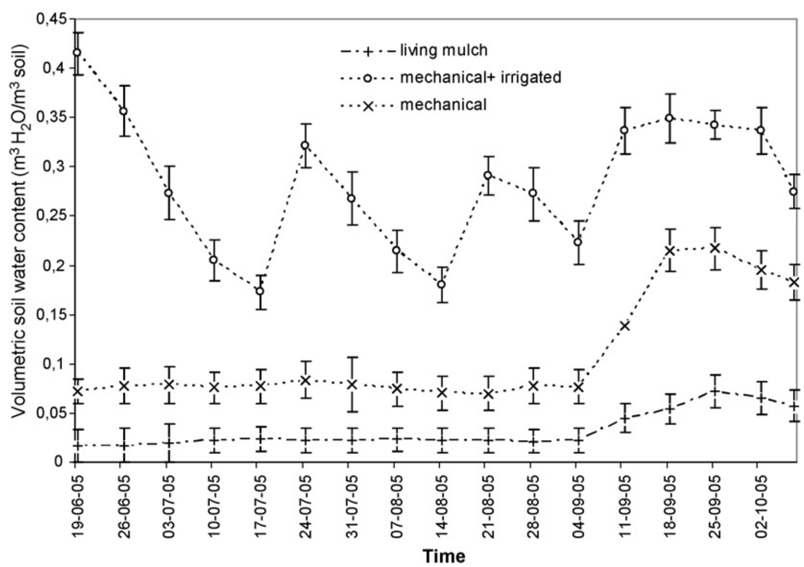

Fig. 4. Mean weekly volumetric soil water content between May and October 2005 for mechanical and living mulch. Bars show 1 S.E. Mechanical irrigated plants were used as a reference value.

soil (Table 3). The living mulch led to higher concentrations of nutrients (P, Ca, Mg, and K) as compared to cultivated soil. Living mulch consistently reduced soil water content during the summer (Fig. 4).

4. Discussion

A positive survival response to weed control is often associated with the control of a well-established and aggressive herbaceous layer in places prior to tillage (Devine et al., 2000; Navarro Cerrillo et al., 2005). In this study, a detectable mulching effect was observed with regard to survival, which may suggest that the intensity of weed control of living mulch may be sufficient under this condition.

Of the four species planted, *Q. ilex* had an extremely low survival by the end of the first growing season (2.7–8.3%). The mortality was higher than previously reported under these conditions (Navarro Cerrillo et al., 2005). *O. europaea* seedlings planted on living mulch treatment had a considerably higher survival (50%). *P. lentiscus* seedlings planted on both treatments had the highest survival (75–62.5%). These two species exhibited a good survivorship as its survival rates were higher than those in previous studies (Vilagrosa et al., 2003; Del Campo and Navarro-Cerrillo, 2004; Maestre et al., 2004), a desired outcome in many restoration projects. The results for Holm oak and terebinth were not clear due to the low survival levels. Although seedlings planted in a Mediterranean climate usually display a high mortality during the first growing season (Sánchez-Gómez et al., 2006), tree mortality may have increased due to the exceptionally low precipitation (e.g. irrigated reference plants survived 100%) as reported in other studies (Maestre et al., 2004). The months of November 2004 to October 2005 had 56% (384 mm) of the average precipitation (670 mm) in the area. The use of living mulch had a positive effect on survival, except for the mastic tree, and this is consistent with the previous literature indicating that mulches mainly have direct effects on early plantations (van Sambeek et al., 1986; Dupraz et al., 1997; Alley et al., 1999). Survival in mastic tree was high for both treatments showing the ability of this species to respond to Mediterranean conditions (Maestre et al., 2004; Sánchez-Gómez et al., 2006).

The effect of weed control treatments was often lowest in those variables related to growth. Different weed control treatments clearly did not favour different growth patterns. This suggests that the alteration of belowground resources related to weed control is not likely to have any great consequences on plant growth, as observed in previous studies (Navarro Cerrillo et al., 2005). During the first year, tree seedlings displayed a negative growth which actually shortened as the seasons progressed as has been reported in other studies with these species (Tsakaldimi et al., 2005). The reduction in seedling height may have been a response to the low growth habit of these species, which allows them to put more energy into diameter growth than height growth, particularly if long seedling stock is used (Navarro Cerrillo et al., 2006). Individual species grew at significantly different rates, but living mulch showed the lowest percent of height reduction (23% for *O. europaea*, 14% for *P. lentiscus* and 12% for *P. terebinthus*), whereas *Q. ilex* height growth reduction was greater on this treatment (4.9%). Legume mulch had a positive effect on the diameter growth of Holm oak and mastic tree seedling, suggesting that these species may respond positively to living mulch. This finding could complement the work of Smith et al. (2000), who found tree growth to be positively related to the width of mulch around its base.

Foliar levels of phosphorus and potassium were slightly higher on the living mulch than on the cultivated site; however, no differences due to cover type were found in any of the species. The relatively high foliar nitrogen levels even in the cultivated plots suggest that currently there is an adequate supply of available soil nitrogen at both sites and that the differences in Holm oak resulted from changes in soil nitrogen levels related to living mulch.

Appreciable photosynthetic stimulation at the living mulch site was found as compared to the cultivated site, which contrasts with the reduction in water availability on the mulched soil. Temporal variability in photosynthetic rates in Mediterranean species establishment has been shown in other trials (Ksontini et al., 1998; Martinez-Ferri et al., 2000; Vilagrosa et al., 2003). Other studies have suggested that there is no photosynthesis regulation on Mediterranean species after plantings when conditions were not significantly modified, which may reflect the need for more positive environmental alterations to observe large and frequent short-term CO_2 fluctuations (Vilagrosa et al., 2003). Among weed control systems under dry-conditions, the study of water availability, as in photosynthesis response, may be complex and poorer understood than expected (Maestre et al., 2004).

The soil analysis at the conclusion of the first growing season indicated that the treatments did significantly change soil qualities. Specifically, OM, N and other nutrients significantly increased in plots covered with living mulch when compared to those in cultivated plots. This can probably be attributed to the rapid decay of legume leaves and consequential release of minerals into the soil (van Sambeek et al., 1986; Hiltbrunner et al., 2007). CEC, a measurement of the soil's affinity for cations, increased significantly in the living mulch treatments. The predominance of a clay soil compounded with the increased OM input from the decaying leaves of legumes, which is also negatively charged (Raven et al., 1999), would increase the soil's ability to retain positively charged ions, thus raising the CEC value (Barton and Karathanasis, 1997). As predicted by other research (Pickering and Shepherd, 2000), soil pH was not significantly modified by the addition of mulch. Positive modification of soil properties due to mulching causes marked responses in plant development including increased growth (van Sambeek et al., 1986; Dupraz et al., 1997), and seedling survival (van Sambeek et al., 1986).

Contrary to our hypothesis, mulch had a consistent effect on soil moisture. In 2005, there was a significant difference throughout the spring and summer months when soil moisture of the living mulch plot was compared to that of the cultivated plot, although numerical differences were very small. This again can be attributed to the severe drought that occurred in 2005; there was so little moisture available that all the treatments were equally dry. The contradictory effect of a higher level of soil moisture and lower survival rates in the cultivated plot may explain the reduced differences in soil moisture between weed control treatments and an extremely low precipitation. Mediterranean species have shown their ability to explore deeper soil layers for water if their planting date is correctly selected and the seedlings are able to develop deep root systems (Lloret et al., 1999).

5. Conclusion

The use of living mulch had a positive effect on survival, except for the mastic tree, and this is consistent with the previous literature indicating that mulches mainly have direct effects on early plantings through modifying vegetation competition and soil parameters, particularly under persistent low soil moisture conditions. The removal of dominant agricultural weeds directly through the use of different weed control treatments may facilitate the early establishment of a wider array of both Mediterranean tree and shrub species, and this has important implications in afforestation programs. Living mulch should be considered as an alternative to either herbicide treatment or cultivation in some species (Holm oak, wild olive and terebinth). In areas where silvopastoral systems are one of the most promising forest alternatives, the creation of legume-natural grass pastures on afforested lands must be taken into consideration. Our results indicate a good feasibility for living mulch treatments in the early establishment of Mediterranean species. In the following years weed control could be restricted to around seedlings. An increase in survival of more than 40% in wild olive and 12% on terebinth appears to be an important threshold affecting the economic attractiveness of using these treatments in cropping lands in Mediterranean areas.

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