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SUSTAINABLE MANAGEMENT OF OLIVE RAINFED ORCHARDS

BY THE INTRODUCTION OF LEGUMINOUS COVER CROPS

Carlos M. Correia^{1*}, Cátia Brito¹, Anabela Fernandes-Silva¹, Eunice A. Bacelar¹,

Berta M. Gonçalves¹, Helena Ferreira¹, Margarida Arrobas²,

José M. Moutinho-Pereira¹, Manuel A. Rodrigues²

¹CITAB, Universidade de Trás-os-Montes e Alto Douro, 5001-801 Vila Real, Portugal

²CIMO, Instituto Politécnico de Bragança, 5301-855, Bragança, Portugal

(Corresponding author*: ccorreia@utad.pt)

Abstract

The olive sector has a crucial economic, social, cultural and ecological relevance in the Mediterranean region, where tillage and herbicides application are still generalized practices. However, these practices oppose the recommendations of UE Common Agricultural Policy and thus, management methods that decrease requirement for agricultural chemicals are needed to reduce adverse environmental impacts. Moreover, they can play an important role in atmospheric CO₂ emission and fixation. We propose green manure legumes to reduce erosion, soil compaction and nitrate pollution, to improve biodiversity, soil carbon sink and fertility, to save fossil fuels and to increase yield and the safety and nutritional value of food products. The 3-year field study carried out in Northeast Portugal revealed that self-reseeding annual legumes is a promising strategy since increase the summer soil water content, the vegetative growth and the physiological tree performance, that was reflected in a 38% higher cumulative yield than tillage techniques.

Key words: Annual legumes, cover crops, *Olea europaea*, rainfed conditions, sustainability.

1. INTRODUCTION

In Mediterranean-type agroecosystems, olive (*Olea europaea* L.) has been traditionally grown in rainfed conditions, and is considered as a well-adapted crop, able to survive to periods of intense drought. Such success is mainly due to the high capacity of olive tree to develop anatomical (Bosabalidis and Kofidis, 2002; Bacelar *et al.*, 2004; Bacelar, *et al.*, 2007a; Guerfel *et al.*, 2007), physiological and biochemical drought resistance mechanisms (Gucci *et al.*, 1997; Chartzoulakis *et al.*, 1999; Giorio *et al.*, 1999; Bacelar *et al.*, 2007b). However, these adaptations imply a metabolic cost of construction and maintenance with negative consequences on growth and on yield performance of the tree.

Since Mediterranean basin is particularly vulnerable to present and future climate variability and climate change (Goubanova and Li, 2007), olive will experiment some hard changes in its environment that could be a problem for the species. An adjustment in orchard water use to prevailing conditions of water availability should be done by a careful and continuing management by attention to tree density, canopy size and condition of the ground cover (Connor, 2005). In particular, soil management practices can assist olive to survive and produce in water-limited environments, in order to ensure that the maximum amount of water can be stored during the rainfall season (Pinheiro, 2005) and to reduce water losses through evaporation. In addition, these practices contribute to control soil erosion (Vanwallegham *et al.*, 2010) and to preserve the organic matter levels. The introduction of

cover crops has shown advantages over the mobilization systems. Cover crops include those plants grown to protect and enrich the soil, and to control weeds (Cosimo *et al.*, 2003). The mobilization systems tend to leave the soil bare, so the soil is loosened due the torrential rains of autumn and winter that wash away the topsoil (Metzidakis *et al.*, 2008). This system also increase the water evaporation, promote deep soil compaction and cause superficial root system damage (Pastor, 2008), increase the organic matter oxidation, by providing oxygen to the microorganisms (Rodrigues *et al.*, 2010) and affect negatively the physical and chemical properties of the soil, such as soil organic matter content, available phosphorus, exchangeable potassium, cation exchange capacity and soil aggregate stability of the surface horizon (Metzidakis *et al.*, 2008). Cover cropping is the most suitable soil-management practice to protect the soil surface from erosion, to preserve the environment and to reduce production costs (Benites *et al.*, 2005). This practice increase water infiltration (Benites *et al.*, 2005; Pinheiro, 2005), soil aeration (Zdruli, 2004) and organic matter content (Pinheiro, 2005), improve soil structure and CO₂ sequestration from the atmosphere (Celano *et al.*, 2011) and promote the biodiversity in the agro-ecosystem (Benites *et al.*, 2005). Furthermore, because cover cropping helps to establish nutritional and biological equilibrium in the soil, the olive's physiological processes are stabilized and the performance of the olive and the fertility of the soil are enhanced (Cosimo *et al.*, 2003). Nevertheless, under Mediterranean rainfed conditions, the cover crop competition with olive trees for water and minerals must be avoided or minimized. Some annual legume species such as subterranean clover and other species with short growing cycle grown as cover crop can provide both these characteristics. They are comparatively less competitive for water than grasses and can fix atmospheric N₂. The available information on cover crop management in rainfed olive orchards is still scarce, making further research necessary. The aim of this work was to evaluate the benefits of cover crop in olive trees growing under Mediterranean conditions. We hypothesize that cover crop improve the soil water content and carbon assimilation and thus growth and crop yield. To test this hypothesis we evaluated soil and plant water status, gas exchange and chlorophyll fluorescence, vegetative growth and olive yield.

2.1. Plant material and experimental details

The experiment was carried out in Suçães, Mirandela (41° 29' N, 7° 15' W), in NE Portugal. The region benefits from a Mediterranean type climate with average annual temperature and precipitation of 14.3 °C and 509 mm, respectively. The commercial orchard was ~20 years old of cv. Cobrançosa and rainfed managed. The soil was a Leptosol derived from schist, sandy loam (13.1% clay, 26.6% silt, 60.3% sand) textured, pH(H₂O) 4.1, organic carbon (C) 7.9 g kg⁻¹ and extractable phosphorus (P) and potassium (K) (Egner-Riehm) 21.0 and 96.3 mg kg⁻¹, respectively. The farmer usually applied the fertilizers beneath the trees' canopy at rates equivalent to 60 kg N, P₂O₅ and K₂O hm⁻² and 15 g B per tree. Shortly before the trial started 1500 kg hm⁻² of lime (88% CaCO₃ and 5% MgCO₃) and 250 kg hm⁻² of superphosphate (18% P₂O₅) were applied.

Two ground-cover treatments were imposed in plots of 0.4 hm⁻². The treatments laid out were: ordinary fertilization and tillage techniques (T) used by local growers, which consisted of two tillage trips per year with a cultivator (in spring and early summer), as a control treatment, and a mixture of eleven annual (AL) pasture self-reseeding legumes (*Ornithopus compressus* L. cv. Charano; *Ornithopus sativus* Brot. cvs. Erica and Margurita; *Trifolium subterraneum* L. ssp *subterraneum* Katzn. and Morley cvs. Dalkeith, Seaton Park, Denmark and Nungarin; *Trifolium resupinatum* L. ssp *resupinatum* Gib and Belli cv. Prolific; *Trifolium incarnatum* L. cv. Contea; *Trifolium michelianum* Savi cv. Frontier; and *Biserrula pelecinus* L. cv. Mauro). Eight similar trees per treatment were pre-selected. This pre-selection of trees was performed in September 2009, before the ground-cover systems had been established and was based on the size and the similarity of the tree canopies. The objective was to reduce the experimental variability associated with the different size of the trees, a very common aspect of dry farmed orchards. The legume cover crop was sown in September 2009 at a rate of an eleventh of that recommended by each species if seeded alone in pure culture. The sowing

was done manually and the seeds of pasture legume species were incorporated in the soil by a roller. In early May the biomass of the cover crop was destroyed with a rotary slasher and left on the ground as a mulch. This would avoid the tilling of the soil and eliminate the consequent damage of the olive tree roots.

2.2. Soil water measurement

The soil water content (θ) was measured periodically, using a time-domain-reflectometry (TDR) tube probe (Trime-FM, Imko, Germany). In each treatment four trees were equipped with two access tube, one located 0.75 m apart tree trunk in the row direction and other in inter-row location, to a depth of 0.60 m. The measurements were taken at 0.20 m intervals. In the conventional tillage treatment, because the difficulty of adherence of the access tube to the soil as a result of tillage, the soil water content was determined gravimetrically in samples obtained at depths of 0–20, 20–40 and 40–60 cm using a 2.2 cm diameter manual probe. We verified that, in this soil, the TDR values underestimate soil moisture, so the calibration curve was taken to gravimetric method.

2.3. Leaf gas exchange, chlorophyll fluorescence and water potential measurements

Leaf gas exchange rates were measured with an infrared gas analyser (LC Pro+, ADC Bioscientific Ltd., UK), operating in the open mode. All measurements were carried out in the morning (9:00–11:00 h) on cloudless days on 2 fully expanded leaves of six trees per treatment. PPFD was always higher than 1000–1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, which is known to be over the saturation point in olive (Natali *et al.*, 1991). Net CO_2 assimilation rate (A), stomatal conductance (g_s), and the ratio of intercellular to atmospheric CO_2 concentration (C_i/C_a) was estimated according to von Caemmerer and Farquhar (1981). To eliminate possible effects of air humidity and temperature on transpiration, the intrinsic water use efficiency (WUE_i) was calculated as the ratio of A to g_s (Iacono *et al.*, 1998).

Chlorophyll *a* fluorescence features were measured *in situ* with a pulse-amplitude-modulated fluorimeter (FMS 2, Hansatech Instruments, Norfolk, England) on the same leaves and environmental conditions as described for gas exchange. Maximum quantum efficiency of photosystem II (PSII) was calculated as $F_v/F_m = (F_m - F_0)/F_m$ by measuring the fluorescence signal from a dark-adapted leaf when all reaction centers are open using a low intensity pulsed measuring light source (F_0) and during a pulse saturating light (0.7 s pulse of 15000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ of white light) when all reactions centers are closed (F_m). Leaves were dark-adapted for 30 min using dark-adapting leaf-clips for these measurements. Following F_v/F_m estimation, after a 20-s exposure to actinic light (1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$), light-adapted steady-state fluorescence yield (F_s) was averaged over 2.5 s, followed by exposure to saturating light (15000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for 0.7 s to establish F'_m . The sample was then shaded for 5 s with a far-red light source to determine F'_0 . From these measurements, several fluorescence attributes were calculated according to Bilger and Schreiber (1986) and Genty *et al.* (1989): photochemical quenching ($qP = (F'_m - F_s)/(F'_m - F'_0)$), non-photochemical quenching ($qN = (F_m - F'_m)/F'_m$), capture efficiency of excitation energy by open PSII reaction centres ($F'_v/F'_m = (F'_m - F'_0)/F'_m$) and efficiency of electron transport as a measure of the effective quantum yield of PSII ($\Phi_{\text{PSII}} = \Delta F/F'_m = (F'_m - F_s)/F'_m$). The apparent electron transport rate (ETR) was estimated as $\text{ETR} = (\Delta F/F'_m) \times \text{PPFD} \times 0.5 \times 0.84$, where PPFD is the photosynthetic photon flux density incident on the leaf, 0.5 is the factor that assumes equal distribution of energy between the two photosystems, and the leaf absorbance used was 0.84 because is the most common value for C_3 plants (Björkman and Demmig, 1987). The fraction of PPFD absorbed in PSII neither utilized in photochemistry nor dissipated thermally (PE), characterising an excess energy, was calculated as $\text{PE} = F'_v/F'_m \times (1 - qP)$, according to Demmig-Adams *et al.* (1996).

Measurements of midday shoot water potential (Ψ ; minimum daily values) were used to evaluate tree water status. A young leafy shoot per tree was collected, from a sunny position at the crown, from 6 replicate trees per treatment. After cutting, the small leafy shoot was immediately enclosed in a plastic bag to avoid any loss of water and quickly placed into the pressure chamber (model PMS 1000,

Oregon, Corvallis, USA). All measurements were completed within 1–3 minutes after excision of the leafy shoots from the plant, and were made in the shade. These all physiological measurements were performed during 2011, on April 18th, July 27th and September 13th.

2.4. Vegetative growth and crop yield

Plant heights, as well as longitudinal and transverse crown diameters were measured in 8 trees of each treatment, for estimating canopy volume, assuming an ellipsoid (Fernandes-Silva *et al.*, 2010). During the study, in order to safeguard the comparative results, the trees in all treatments were not pruned.

The crop yield was recorded from eight trees per treatment. The trees were harvested for four consecutive years, in late November of 2009, 2010, 2011 and 2012. The harvest of 2009 represents the year zero, since it was performed at the beginning of the experiment, before the effects of the treatments had manifested themselves. The harvest was performed by a trunk shaker head which detaches the olives which are collected by an associated inverted umbrella system.

2.5. Statistical analysis

Data analysis was carried out using the SuperANOVA (Abacus Concepts Inc., USA) software. A completely randomized design with a single factor (soil management) was used.

3. RESULTS

Soil water content (θ) varied between treatments and season, being lower in cover crop plot in April (30.7 and 57.6% in row and inter-row, respectively; Fig. 1). During the summer θ decreased sharply reaching the lower limit in tillage plot (0.064 and 0.051 $\text{m}^3 \text{m}^{-3}$, for row and inter-row, respectively), with major differences of 28.9% in row (September) and 56.8% in inter-row (July). Although the substantial differences in θ , Ψ values were similar between treatments (Fig. 2). Ψ decreased progressively with increasing seasonal drought, getting the lowest values late in the summer (about -3 MPa).

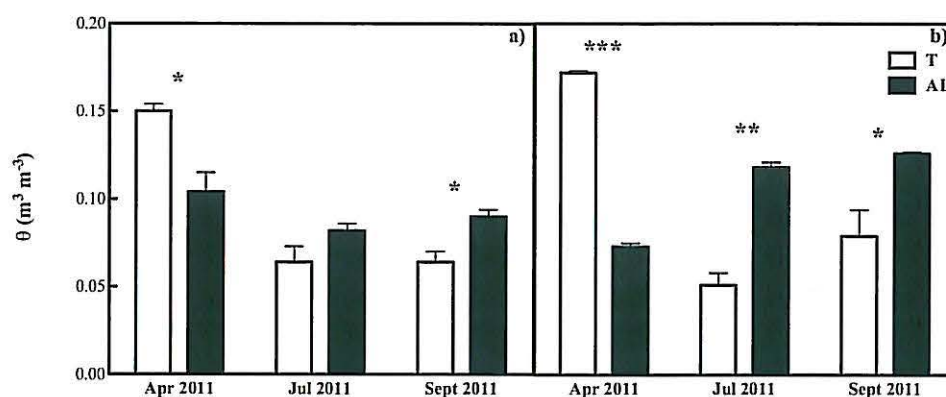


Figure 1. Seasonal variation of soil water content in the two treatments, on row (a) and inter-row (b). Each column represents the mean for the total soil profile and vertical bars represent the standard error of the mean. Within each date, the symbols *, ** and *** denotes significant differences at $P < 0.05$, < 0.01 and < 0.001 , respectively.

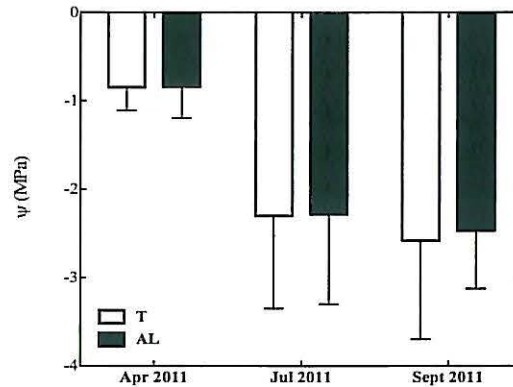


Figure 2. Seasonal variation of stem water potential in the two treatments. Each column represents the mean and vertical bars represent the standard error of the mean.

Significant effect of the type of soil management was observed on some leaf gas exchange (Fig. 3) and chlorophyll fluorescence (Fig. 4) variables in July and September, while no statistical differences were observed in April. P_n , g_s , Φ_{PSII} and ETR were higher in trees with cover crop, mainly in July. In addition, this treatment contributes to higher WUE_i in September and F'_v/F'_m and qP in July and, in opposition, to lower C_i/C_a and qN , in September and July, respectively. Seasonal variation data from April to summer month's showed significant increases of WUE_i , mainly in AL plot, and qN , and decreases of Φ_{PSII} , F'_v/F'_m , qP and ETR, mostly in trees subjected to conventional tillage. Furthermore, soil management system and season did not affect P_E and F_v/F_m (data not shown).

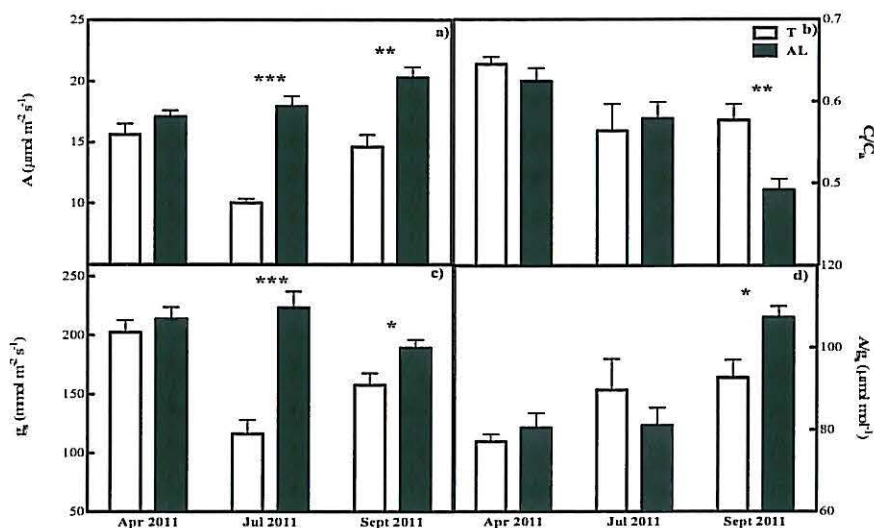


Figure 3. Seasonal variation of leaf net CO₂ assimilation rate (A), stomatal conductance (g_s), ratio of intercellular to atmospheric CO₂ concentration (C_i/C_a) and intrinsic water use efficiency (A/g_s) in the two treatments. Within each date, the symbols *, ** and *** denotes significant differences at $P < 0.05$, < 0.01 and < 0.001 , respectively.

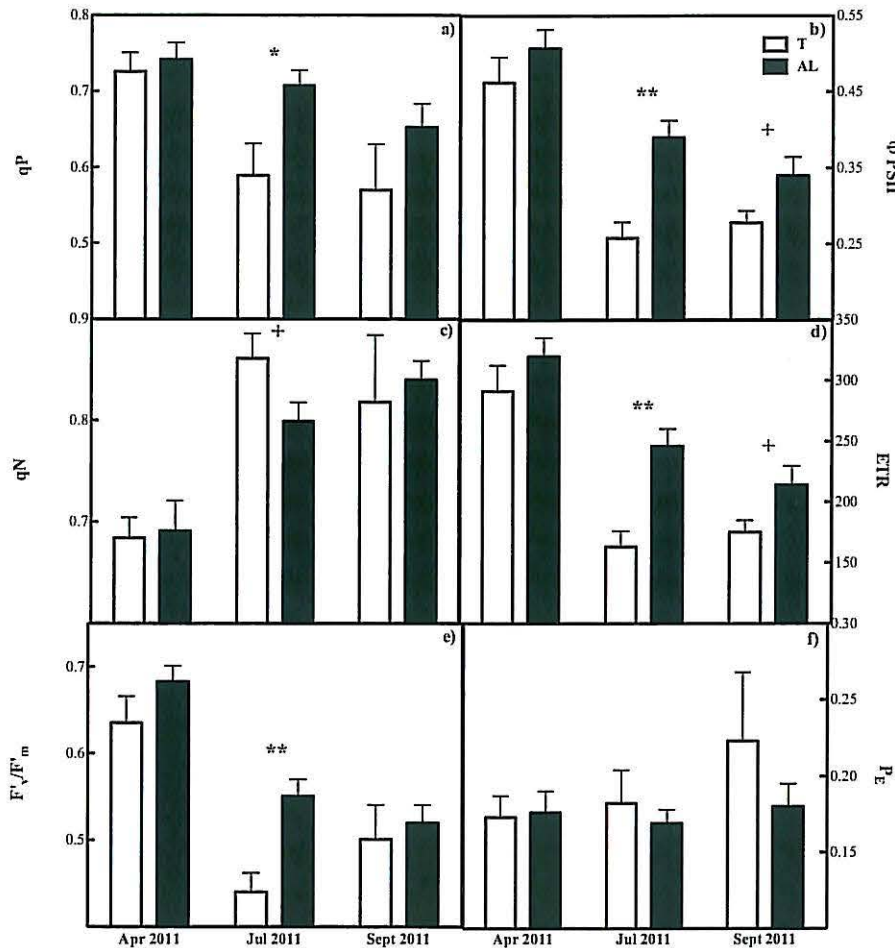


Figure 4. Seasonal variation of photochemical (qP) and non-photochemical (qN) quenching, capture efficiency of excitation energy by open PSII reaction centres (F'_{v}/F'_{m}), effective quantum yield of PSII (Φ_{PSII}), electron transport rate (ETR) and fraction of PPF absorbed in PSII neither utilized in photochemistry nor dissipated thermally (PE) in the two treatments. Each column represents the mean and vertical bars represent the standard error of the mean. Within each date, the symbols +, *, and ** denotes significant differences at $P < 0.1$, < 0.05 and < 0.01 , respectively.

At the start of the study, the tree crown size did not differ between treatments (data not shown). Plant canopy volumes increased progressively, with the largest increases in cover crop plot, where the canopy volume of trees reaches mean values of 22.6 against 18.9 m³ in tillage treatment. Relatively to the crop yield, the presence of legumes cover crop increased olive yield during the three seasons, with an accumulated value of 38% (Table 1).

Table 1. Variation of crop yield (kg tree⁻¹) in the two treatments in the period 2010-2012. Values are means \pm SE. Within each year, the symbols ⁺ and ^{**} denotes significant differences at $P < 0.1$ and < 0.01 , respectively.

Treatment	2010	2011	2012	3 years
T	20.9 \pm 2.0	13.1 \pm 2.2	4.4 \pm 0.4	38.4 \pm 3.6
AL	25.6 \pm 1.4	19.0 \pm 1.9	8.3 \pm 1.2	52.9 \pm 3.3
Significance	+	+	**	**

4. DISCUSSION

This study was conducted to analyze the effects of different soil management systems on olive tree orchards grown under rainfed conditions. Cover crop effects on soil water relationships were negative until the destruction of AL plants in the beginning of May, due to the transpiration by the cover crop. However, this result was not associated with consequences on tree physiological performance, since the soil water content was not critical to olive tree function. In any case, this may occur if the precipitation levels were unusually low during the previous winter and particularly in March and April, when the physiological activity of olive increases. Such situation may be highly detrimental on soils with low water-holding capacity or a root-restricting layer at a shallow depth (Waggoner and Mengel, 1988). An opposite and clearly positive response was observed in the critical drought summer months, since soil water content increased significantly in cover crop plot due to less runoff, greater infiltration and less evaporation when cover crop residues are retained on the soil surface (Unger and Vigil, 1998). Interestingly, θ in the row of AL plot was quite stable along the year and even higher in summer in inter-row, contrary to the tillage plot where θ in April is much higher than in summer. Furthermore, the summer θ values in the cover crop plot were of the same magnitude as obtained by Fernandes-Silva *et al.* (2010) in a study done in Mediterranean field conditions of continuous deficit irrigation correspondent to 30% of maximum crop evapotranspiration. In addition, Ψ did not fall below -3 MPa, which are in accordance with previous findings in others studies with irrigated trees (Fernández *et al.*, 1997; Tognetti *et al.*, 2004; Fernandes-Silva *et al.*, 2010).

Although the N released from cover crop residues had a slight but nevertheless positive effect on the olive yields and on tree crop growth (Rodrigues *et al.*, in press), we believe that the positive effects, here reported, on soil water content during the summer months had an important role for adequate tree physiological activity and thus on growth and crop yield. In fact, self-reseeding pasture cover crop had a good impact on the photosynthetic performance of olive trees, as confirmed by gas exchange and chlorophyll fluorescence responses. Gas exchange measurements showed increases in CO₂ assimilation rates in olive trees in AL plot that were associated with lower stomatal and non-stomatal limitations to photosynthesis. At the end of summer, as net photosynthesis was more benefited than stomatal conductance, WUE_i was higher in trees cultivated with the cover crop, indicating an optimization of carbon uptake *versus* water loss (Raven, 2002). Moreover, the reduction of g_s joined with comparable (July) or even higher (September) C_i/C_a indicates that non-stomatal components play an important role in limiting photosynthesis of olive trees submitted to tillage conditions. However, the C_i values should be treated with some caution, due to the non-uniform aperture of stomata over the leaf surface (patchiness). Under environmental stress conditions, the C_i calculated from gas exchange measurements can be overestimated and lead to erroneous conclusions of non-stomatal limitation of photosynthesis (Downton *et al.*, 1988). Nonetheless, our analysis is based on the assumption that patchy stomatal closure did not occur in mature olive leaves (Loreto and Sharkey, 1990; Nogués and Baker, 2000). Therefore, the present results of C_i should represent a limited capacity for CO₂ fixation by mesophyll cells (DaMatta *et al.*, 2002). Furthermore, our results showed that the reduction of g_s ,

and thus the restricted water loss through transpiration seemed to be effective in preventing large decreases in water potential and, thus, appeared to be the earliest response for dehydration avoidance in olive under tillage plot.

The analysis of chlorophyll fluorescence revealed that non-stomatal limitations were also responsible for the photosynthetic responses in the summer period. The data showed that total electron flow through PSII was inhibited in tillage treated plants, mainly in July, linked with reductions in $F'v/F'm$, qP , $\Phi PSII$ and ETR. Since $1-qP$ is a measure of the reduction state of the primary electron acceptor in PSII, Q_A (Schreiber *et al.*, 1994), it can be reasoned that the re-oxidation of Q_A was less effective and implies that a fraction of the PSII traps was closed during actinic PPFD (DaMatta *et al.*, 2002). Taking in account these results and the lower A in trees subjected to tillage we assume that a greater proportion of the absorbed PPFD was in excess of that required to support CO_2 fixation, and thus the above changes in PSII photochemistry may represent a down-regulation mechanism. These results, joined with the rise in qN in July (higher dissipation as heat) and the similar values of P_E suggested that such down-regulation was enough to match the decreased demand for electrons through $NADP^+$ consumption (DaMatta *et al.*, 2002). As a consequence, the F_v/F_m values were stable between treatments and within the physiological ranges for healthy leaves.

The improvement of soil water relationships and physiological activity of olive trees with cover crop had a positive effect on growth, which was reflected in a slight increase of canopy volume, and thus in higher radiation interception. For all these reasons, AL species contribute to increased olive fruit production. This result is in accordance with Pastor and Guerrero (1990), who found in a compilation of studies conducted in 15 years in Andalusia (Spain) that olive yield increase in non-tilled olive groves with respect to the tilled ones. However, as olive trees are alternate bearing species, the results of the next years are fundamental to better understand the effect of groundcovers on fruit yield.

5. CONCLUSIONS

Self-reseeding pasture legume species with short growing cycle grown as cover crop may be a promising strategy contributing to the sustainable management of olive orchards under rainfed conditions. In fact, the cover crop increased the soil water content during the summer drought period and improved the photosynthetic performance, contributing to increased growth and fruit yield. This is an interesting result since a sagacious soil management approach is essential for the olive industry due to the limited water resources available in the Mediterranean region.

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