



## Net ecosystem CO<sub>2</sub> exchange in an irrigated olive orchard of SE Spain: Influence of weed cover



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### ABSTRACT

No-till management and the establishment of plant cover have been implemented in olive crops in recent years in order to prevent soil erosion and increase soil organic carbon. However, the effect of these conservation practices on the net CO<sub>2</sub> exchange at the ecosystem scale has not been explored so far. In this study, we analyze the influence of resident vegetation cover (hereafter weeds) on the net ecosystem CO<sub>2</sub> exchange (NEE) in an irrigated olive orchard located in Jaén (SE Spain) by using the eddy covariance technique. NEE was measured in the olive orchard under two treatments, one with weed cover in the alleys from autumn to spring, and another where weed growth was avoided by the application of a glyphosate herbicide. Our study demonstrates that the presence of weeds in the alleys increased carbon assimilation in the weed-cover treatment during the weed growing period (from December to April). However, the net ecosystem CO<sub>2</sub> uptake decreased in the weed-cover treatment during late spring (May and June), after weeds were cut and left on the soil, compared to the weed-free treatment, probably due to an increase in soil respiration. On an annual basis, weed removal decreased net carbon uptake by 50% compared to the weed-cover treatment. The annual NEE was  $-140 \text{ g C m}^{-2} \text{ y}^{-1}$  in the weed-cover treatment and  $-70 \text{ g C m}^{-2} \text{ y}^{-1}$  in the weed-free treatment. In summary, our study demonstrates that, during the first year of differential treatment, maintenance of weed cover in olive groves has a positive effect on CO<sub>2</sub> uptake and enhances the capacity of the agro-system to act as a net CO<sub>2</sub> sink.

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### 1. Introduction

Soil cultivation and anthropogenic climate change have caused a great impact on the global soil carbon (C) cycle over the last century. Inadequate management of agricultural land has led to accelerated rates of soil erosion and has exposed trapped C to decomposition, accelerating mineralization of soil organic carbon (SOC; Lal, 2004). As a consequence, these practices have modified gains and losses of soil C, altering the natural C balance and increasing greenhouse gas emissions (Aguilera et al., 2015; Amundson et al., 2015). Some estimates point to global SOC losses by agricultural erosion of  $404 \text{ Tg C y}^{-1}$  (Doetterl et al., 2012) and to global C releases to the atmosphere associated with erosion that

range from 0.8 to  $1.2 \text{ Gt C y}^{-1}$  (Lal, 2003). These C emissions are equivalent to 12% of global C emissions by fossil fuels and industry ( $9.80 \text{ Gt C}$  in 2014; Le Quéré et al., 2015). Therefore, the application of sustainable practices aimed to increase C sequestration in agriculture has become a relevant subject of interest. This can be especially important in Spain, where SOC contents lower than 1% are frequent, mostly in southern areas and agricultural soils (Rodríguez Martín et al., 2016).

Olive trees (*Olea europaea L.*) are one of the most important crops in the Mediterranean basin, where they cover around 9.5 Mha and account for 98% of the world's olive cultivation area (Repullo-Ruibérriz de Torres et al., 2012). The largest area dedicated to this crop is found in Spain, where it occupies 2.6 Mha and represents 72% of world's olive production (data for 2013–2014; IOOC, 2015). Around 60% (1.5 Mha) of the olive cultivation in Spain is located in Andalusia (southern Iberian

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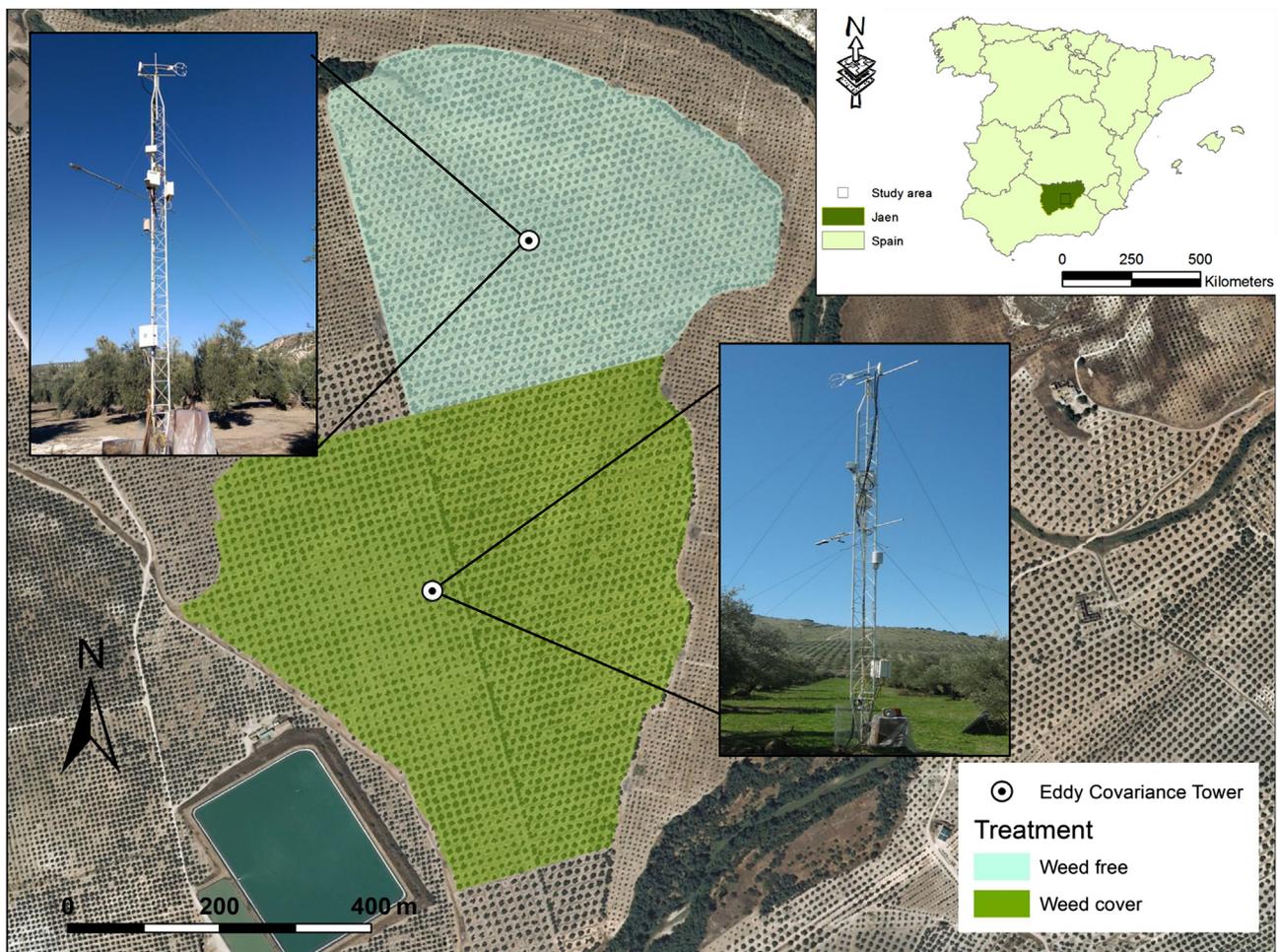
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Peninsula; [MAGRAMA, 2012](#)). Thus, olive groves represent an important agricultural system in this region due to its environmental, social and economic benefits. However, olive groves are subject to several environmental problems due to inadequate conventional soil-management practices such as intensive tillage and overgrazing, which have caused high runoff and erosion rates, high soil and SOC losses, and the loss of soil fertility ([Álvarez et al., 2007](#); [Francia Martínez et al., 2006](#); [Martínez-Mena et al., 2008](#); [Gómez et al., 2009](#)). In order to mitigate these problems, research has been carried out in recent decades to improve soil management practices, and prevent the mineralization of organic matter and the loss of soil structure and fertility ([FAO, 2004](#)).

One of the most widespread conservation practices applied in olive-grove plantations has been the maintenance of spontaneous resident vegetation cover (hereafter “weeds”) in the alleys from autumn to spring ([Marquez-Garcia et al., 2013](#); [Nieto et al., 2013](#)). Weed covers, in addition to protecting the soil against erosion, offer a number of well-known benefits for soil properties: improvement of soil physicochemical properties ([Ramos et al., 2010](#)); increases in the interception and storage of rainfall water, as well as in soil water content and water availability in deep soil ([Celano et al., 2011](#); [Palese et al., 2014](#)); increases in atmospheric C fixation and SOC content, thereby improving soil structure and fertility ([Hernández et al., 2005](#); [Castro et al., 2008](#); [Gómez et al., 2009](#); [Repullo-Ruibérriz de Torres et al., 2012](#); [Marquez-Garcia et al., 2013](#); [Soriano et al., 2014](#); [Herencia, 2015](#)); and increased

biodiversity ([Plaza-Bonilla et al., 2015](#)). In this regard, some estimates point to SOC increases between 44% and 85% in topsoil (0–15 cm) in olive groves after 100 years of cover crop management ([Nieto et al., 2013](#)), and preliminary estimations suggest an increase in soil C sequestration of around  $1 \text{ ton C ha}^{-1} \text{ y}^{-1}$  in olive orchards under Mediterranean conditions due to the adoption of plant covers ([Vicente-Vicente et al., 2016](#)). Thus, agricultural systems can function as C sinks if adequate management practices are applied.

Although numerous studies have examined the effect of weed cover on soil properties and SOC content, little research has been focused on their effect on soil  $\text{CO}_2$  fluxes or how they affect the ecosystem C balance in olive orchards. Indeed, few studies have reported information on  $\text{CO}_2$  fluxes from olive groves or quantified the ecosystem C uptake accounting for total  $\text{CO}_2$  inputs and outputs (see [Testi et al., 2008](#); [Nardino et al., 2013](#)). So far, most  $\text{CO}_2$  exchange measurements have been conducted at the tree ([Villalobos et al., 2012](#); [Pérez-Priego et al., 2010](#)) and soil levels ([Bertolla et al., 2014](#)) by using chambers, and soil  $\text{CO}_2$  emissions have been also estimated via modelling approaches ([Nieto et al., 2010](#)). In the absence of weed cover, net ecosystem  $\text{CO}_2$  exchange (NEE) from olive groves will result from the balance between  $\text{CO}_2$  inputs by tree photosynthesis and  $\text{CO}_2$  outputs by aboveground autotrophic respiration (olive leaves, trunks and branches), belowground autotrophic respiration (olive roots) and heterotrophic soil respiration. However, in the presence of weeds, it is



**Fig. 1.** Location of the olive orchard and picture of the eddy tower installed at each treatment considered at this site: maintenance of spontaneous weeds (weed cover) and weed removal by application of an herbicide (weed free). Points indicate the location of the eddy covariance towers. The colored area indicates the fetch for each treatment.

necessary to account for CO<sub>2</sub> uptake via weed photosynthesis and CO<sub>2</sub> emissions via weed and weed-covered soil respiration for quantification of NEE. Knowledge of how conservation *versus* traditional practices may affect the net CO<sub>2</sub> uptake in olive groves is lacking and this information is necessary to elucidate the role that these practices play in C sequestration and thus, their potential regarding climate change mitigation.

Non-destructive, ecosystem-scale and long-term measurements of NEE are possible thanks to the technological development of robust tools such as the eddy covariance (EC) technique (Dabberdt et al., 1993; Baldocchi, 2003). While this technique has been used to characterize CO<sub>2</sub> and water vapor exchanges in natural (Baldocchi et al., 2001; Reichstein et al., 2007) as well as agricultural ecosystems under differing management (Baker and Griffis, 2005; Chi et al., 2016), its application to agricultural systems such as olive orchards is practically non-existent. Some reasons for the absence of information on these widespread crops in the Mediterranean region are: i) the steep slopes where these crops are usually located, which complicate the implementation of these micrometeorological techniques; and ii) the intensive management including irrigation, fertilization and pruning, which reduces stress for water, nutrients or light, and strongly modifies CO<sub>2</sub> exchanges compared to other Mediterranean ecosystems or rainfed crops (Testi et al., 2008; Nardino et al., 2013). Therefore, quantification of CO<sub>2</sub> exchange in olive groves at the ecosystem scale is necessary to understand how they contribute to the C balance and how different management practices can amplify or diminish their capacity to act as sinks of CO<sub>2</sub>. To our knowledge, only a few studies have measured NEE in olive groves at ecosystem scale using the eddy covariance technique (Testi et al., 2008; Nardino et al., 2013; López-Bernal et al., 2015). However, these studies were conducted either during short time periods or in young olive orchards, and none analyzed the influence of no-till practices such as maintenance of plant cover on the ecosystem C uptake.

In this study, we measure NEE in an irrigated mature olive orchard of SE Spain under two management regimes, maintenance of weed cover and weed suppression, using the eddy covariance technique. The objective of this study was two-fold: i) to characterize monthly and annual patterns of NEE in an irrigated, mature olive orchard; and ii) to analyze the effect of weed cover on the ecosystem C uptake in olive groves as compared to management for weed suppression.

## 2. Materials and methods

### 2.1. Study site

This research has been conducted in “Cortijo Guadiana” (37°54′39.30″N, 3°13′42.40″W), an irrigated olive (*Olea europaea* L., “Arbequina”) orchard in Úbeda (Jaén, Spain), which belongs to the oil group “Castillo de Canena, S.L.” (Fig. 1). The site is situated at 370 m above sea level. The climate is Mediterranean, with a mean annual temperature of 16 °C, a mean annual precipitation of 495 mm, and a mean annual potential evapotranspiration (calculated using the Penman-Monteith equation) of 1220 mm (from 15-year records at the Agroclimatic Station of Úbeda, Junta de Andalucía, <http://www.juntadeandalucia.es/agriculturaypesca/ifapa/ria/servlet/FrontController>). Predominant winds come from the northwest during the day and from the south and southeast at night. The farmland has a total extension of 1500 ha, but our experiment was developed in a flat area, where two homogeneous plots were delimited of 29.3 ha (weed cover) and 20.2 ha (weed free). Soil organic matter content is 2.9% from 0 to 5 cm and 2.4% from 5 to 15 cm. Soil texture is clay loam, with 24% sand, 32% silt and 44% clay. Trees are irrigated by drip 3 times a week from

February to October, at a rate of 32 L h<sup>-1</sup> per tree for 8 h (at night). Within irrigation, 40 g of NPK fertilizer per tree is applied together with water (0.156 g NPK L<sup>-1</sup> water, every irrigation night). The olive trees are 80 years old with a 7 × 7 m spacing between them (204 trees ha<sup>-1</sup>) and tree height is approximately 4 m. The Plant Area Index (PAI) of trees was determined from the indirect measurement of the gap fraction using upward hemispheric images taken with a 4.5 mm F2.8 EX DC HSM circular fisheye lens (Sigma Corporation of America). Images were processed with the software CAN-EYE v6.1 (INRA-CSE, Avignon). PAI of the trees (corrected by clumping effect) was 8.13 ± 0.83 m<sup>2</sup> vegetation/m<sup>2</sup> ground surface.

In the two areas selected in the olive orchard (Fig. 1), two treatments were applied: 1) weed-free treatment, in which a glyphosate-based herbicide was applied to avoid spontaneous weed growth (September 2014), and 2) weed-cover treatment, which is the management commonly applied in the orchard and consists of maintenance of spontaneous weed cover in the alleys from autumn to spring. In spring (29–30 April), weeds were mechanically whacked and left on the surface to avoid excessive water consumption and competition for water with trees.

### 2.2. Eddy covariance and meteorological and soil measurements

During the hydrological year 2014 (October)–2015 (September), fluxes of CO<sub>2</sub> and latent (LE) and sensible (H) heat have been determined from fast-response (10 Hz) instruments mounted atop 10 m-towers, one in each treatment (Fig. 1). The towers were placed in the center of each treatment and separated by about 500 m to avoid interference from one treatment to another. Wind vector components and sonic temperature were measured by three-axis sonic anemometers (CSAT-3, Campbell Scientific, Logan, UT, USA; hereafter CSI), while densities of CO<sub>2</sub> and H<sub>2</sub>O, together with temperature and pressure, were measured by enclosed path infrared gas analyzers (IRGA, Li-Cor 7200; Lincoln, NE, USA). The stainless steel intake tubes are 1 m in length and have outside diameters of 6.35 mm. Flow rates are 15 L min<sup>-1</sup> and pass through 2 μm filters that reduce dust entering the gas analyzer optical cell. Calibrations of the IRGAs were done every six months using an ultra-high purity N<sub>2</sub> zero gas, and a 500 ppm CO<sub>2</sub> span gas (in N<sub>2</sub>). High-speed (10-Hz) mixing ratios of CO<sub>2</sub> and water vapor (calculated from the IRGAs measurements), wind vector components and sonic temperatures were registered in LI-7550 Analyzer Interface Units.

At each treatment, additional instrumentation measures environmental and soil states. Air temperature and humidity were measured at 6 m by a thermo-hygrometer (HC2S3, Rotronic AG, Bassersdorf, Switzerland), from which vapor pressure deficit (VPDs) was calculated. Incoming and outgoing short-wave and long-wave radiation components were measured by a 4-component radiometer (CNR-4, Kipp & Zonen, Delft, Netherlands), installed at 2 m from the mast at a height of 7 m, allowing the determination of net radiation (R<sub>n</sub>) and albedo. Incident and reflected photosynthetic photon flux densities (PPFDs) were measured using photodiodes at 7 m (Li-190, Li-Cor, Lincoln, NE, USA). To monitor the temporal evolution of soil moisture, soil water content (SWC) was measured in an alley of each treatment using two soil moisture probes installed at 0.10 m depth (CS616, CSI). On each treatment, two thermocouples (TCAV, CSI) measured soil temperatures at 0.04 m soil depth and two heat flux plates (HFPO1, Hukseflux, Delft, the Netherlands) were inserted at 0.08 m. Environmental and soil measurements were stored as 30 min averages by a datalogger (CR3000, CSI). Finally, precipitation data were obtained from the Úbeda Agroclimatic Station of the Junta de Andalucía (<http://www.juntadeandalucia.es/agriculturaypesca/ifapa/ria/servlet/FrontController>) located at 7 km from our study site.

### 2.3. Eddy data processing and statistical analysis

Fluxes of CO<sub>2</sub> (NEE) and LE and H fluxes were calculated on half-hour bases using the EddyPro 5.2.0 software (LI-COR Inc., Lincoln, Nebraska, USA). Raw 10-Hz data were filtered for spikes and compensation for time lags between the air sampling point and the analyzer was done by maximizing the correlation between vertical wind speed and mixing ratios of CO<sub>2</sub> and water vapor. Half-hour covariances between the vertical wind component and CO<sub>2</sub>, water vapor and sonic temperature were calculated using block averaging, double coordinate rotations and spectral corrections for high frequency range (Moncrieff et al., 1997). Without the spectral correction, the CO<sub>2</sub> fluxes were, on average for the whole study period, 7% and 8% less for the weed-cover and weed-free treatments, respectively, than the corrected CO<sub>2</sub> fluxes.

Due to high power requirements by the air pump, the system suffered frequent energy losses that caused data gaps, mainly during nighttime. During May and September, continuous data losses were found from 4 am to 7 am in the weed-free treatment due to energy loss. Nighttime fluxes measured during weak turbulence were rejected by filtering with a friction velocity ( $u_*$ ) below 0.15 m s<sup>-1</sup> (Reichstein et al., 2005). In addition, data quality check of the half-hourly NEE, H<sub>2</sub>O flux and sensible heat flux (H) was applied by filtering according to the following parameters: 1) For CO<sub>2</sub>: i) quality of data = 0 or 1 (Mauder and Foken, 2004); ii) CO<sub>2</sub> variance < 50 ppm<sup>2</sup>; iii) -12° < pitch < 12°; iv) -4 < skewness < 4; v) Kurtosis < 10; 2) For H<sub>2</sub>O: i) quality of data = 0 or 1; ii) H<sub>2</sub>O variance < 0.5 ppt<sup>2</sup>; iii) -10° < pitch < 10°; iv) -4 < skewness < 4; v) Kurtosis < 9; and for H: i) quality of data = 0 or 1; ii) H variance < 2 (W m<sup>-2</sup>)<sup>2</sup>; iii) -10° < pitch < 10°; iv) -4 < skewness < 4; v) Kurtosis < 9. Data gaps due to environmental conditions, instrument malfunction and nighttime low turbulence led to a data coverage of 41% in the weed-cover treatment (69% during daytime and 22% during nighttime), and 38% in the weed-free treatment (62% during daytime and 22% during nighttime). Data losses are frequent in eddy covariance studies (data gap average 35%, see Falge et al., 2001). Despite high data gaps in our site, most data losses occurred during night when GPP is absent and Reco is generally low, and low friction velocities ( $u_* < 0.15$ ) lead to not valid CO<sub>2</sub> fluxes during many nighttime periods. Thus, frequent data losses during nighttime at our site likely had little influence on monthly and annual CO<sub>2</sub> budgets. Data gaps were filled using the marginal distribution sampling technique described by Reichstein et al. (2005). This technique also calculates uncertainties for actual measurements by simulating gaps and applying the gap-filling procedure. Twice the standard deviation of sums of the 30-min uncertainties derived from the gap-filling procedure was considered as our NEE error for the different time periods we analyzed (monthly and annual NEE). Positive values of NEE indicate net CO<sub>2</sub> release to the atmosphere, while negative values represent net CO<sub>2</sub> uptake. Half-hour NEE values were integrated to obtain C exchange (g C m<sup>-2</sup>) at daily, monthly and annual scales.

An estimation of the flux footprint during daytime and nighttime periods was determined using the method described by Kljun et al. (2004) to verify that fluxes originated from well within the fetch (higher than 200 m from the tower). Daytime periods were defined when net radiation was higher than 10 W m<sup>-2</sup>.

Flux partitioning into Gross Primary Production (GPP) and Ecosystem Respiration (Reco) was performed according to the method by Reichstein et al. (2005) and Lasslop et al. (2010). However, unexpected seasonal behavior and unreliable estimations of annual GPP and Reco were obtained for both treatments, suggesting these partitioning methods are not suitable for application at our site. For this reason, the light-response curves were used to model GPP and Reco. The rectangular hyperbolic

light-response function (Falge et al., 2001) was applied to monthly averages of 30-min daytime data, and monthly parameterization coefficients were obtained according to the equation:

$$NEE = \frac{-b_1 \times PPF D}{b_2 + PPF D} + b_3 \quad (1)$$

where PPF D is the incident photosynthetic photon flux density, the coefficient  $b_1$  is the maximum gross primary production (GPP<sub>max</sub>,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ );  $b_2$  represents the level of PPF D for which GPP is half of GPP<sub>max</sub> ( $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ); and the parameter  $b_3$  represents the ecosystem respiration (Reco,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). For determination of the parameterization coefficients, only measured data (quality = 0 or 1) until noon was considered in order to account for the effect of PPF D on NEE and avoid the effect of high VPD on NEE. In order to fit the data to the light-response model described in equation 1, we firstly generate the initial values (uniform distribution) of the model coefficients randomly by delimiting realistic ranges for every coefficient: 0–50 for  $b_1$ , 0–2000  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  for  $b_2$  and 0–20  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  for  $b_3$ . These initial values are necessary to start the fitting procedure, which consists of calculating the nonlinear (weighted) least-squares estimates of the parameters of the non-linear model (Eq. (1)). We used R software version 3.1.3 (R Development Core Team, 2015) to perform this analysis. Coefficients were considered significant when  $p < 0.05$ .

To assess the accuracy of the eddy covariance measurements, we analyzed linear regressions between the sum of latent heat (LE) and sensible heat (H) versus net radiation (R<sub>n</sub>) minus soil heat flux (G, calculated as the sum of the soil heat flux at 0.08 m and the heat storage term (Q) in the 0–0.08 m soil depth):  $R_n - G = LE + H$ . We determined the energy balance closure using 30-min time series of R<sub>n</sub>, H, LE and G for the period between April and June. This period was selected in order to account for the period of maximum weed growth and the later period when weeds were cut, and also because there were simultaneous measurements of soil heat flux, soil temperature, and soil water content at both treatments.

### 2.4. Soil CO<sub>2</sub> efflux measurements

In addition to the eddy covariance measurements, soil CO<sub>2</sub> effluxes were measured in cylindrical PVC collars (10 cm diameter × 5 cm height) inserted into the soil in the alleys of the two treatments. Five collars were inserted per treatment and the soil CO<sub>2</sub> efflux was measured at midday, once a month from March to July, with a manual chamber system model EGM-4/SRC-1 (PP-Systems, Hitchin, UK). Each collar was measured three times and the average was used as the soil CO<sub>2</sub> efflux of the plot. The flux was determined from the slope of the CO<sub>2</sub> molar fraction (referenced to dry air) measured every 5 s during 120 s after chamber closure and was corrected for atmospheric pressure and the chamber air temperature. Significant differences ( $P < 0.05$ ) in soil CO<sub>2</sub> efflux between the two treatments (weed-covered soil and weed-free soil) were analyzed using a one-way ANOVA. Analyses were conducted using R software version 3.1.3 (R Development Core Team, 2015).

### 2.5. Weed biomass and weed organic carbon determination

Weed sampling was conducted at the beginning of April (before weed whacking) in order to quantify aboveground weed biomass and the organic C input contributed by weed biomass. Five square plots of 0.5 m × 0.5 m (0.25 m<sup>2</sup>) were selected and weeds were cut and harvested for determination of dry weight. Organic C released by weeds was determined using the Walkley and Black method modified by Mingorance et al. (2007). Samples of 30 mg of plant

material were weighed and 5 mL of potassium dichromate and 7.5 mL of sulfuric acid were added. After digestion at 155 °C for 30 min, 10 mL of distilled water was added and absorbance was measured at 600 nm in a spectrophotometer. The organic C content was determined from the calibration curve built with increasing concentrations of glucose.

## 2.6. Crop productivity quantification

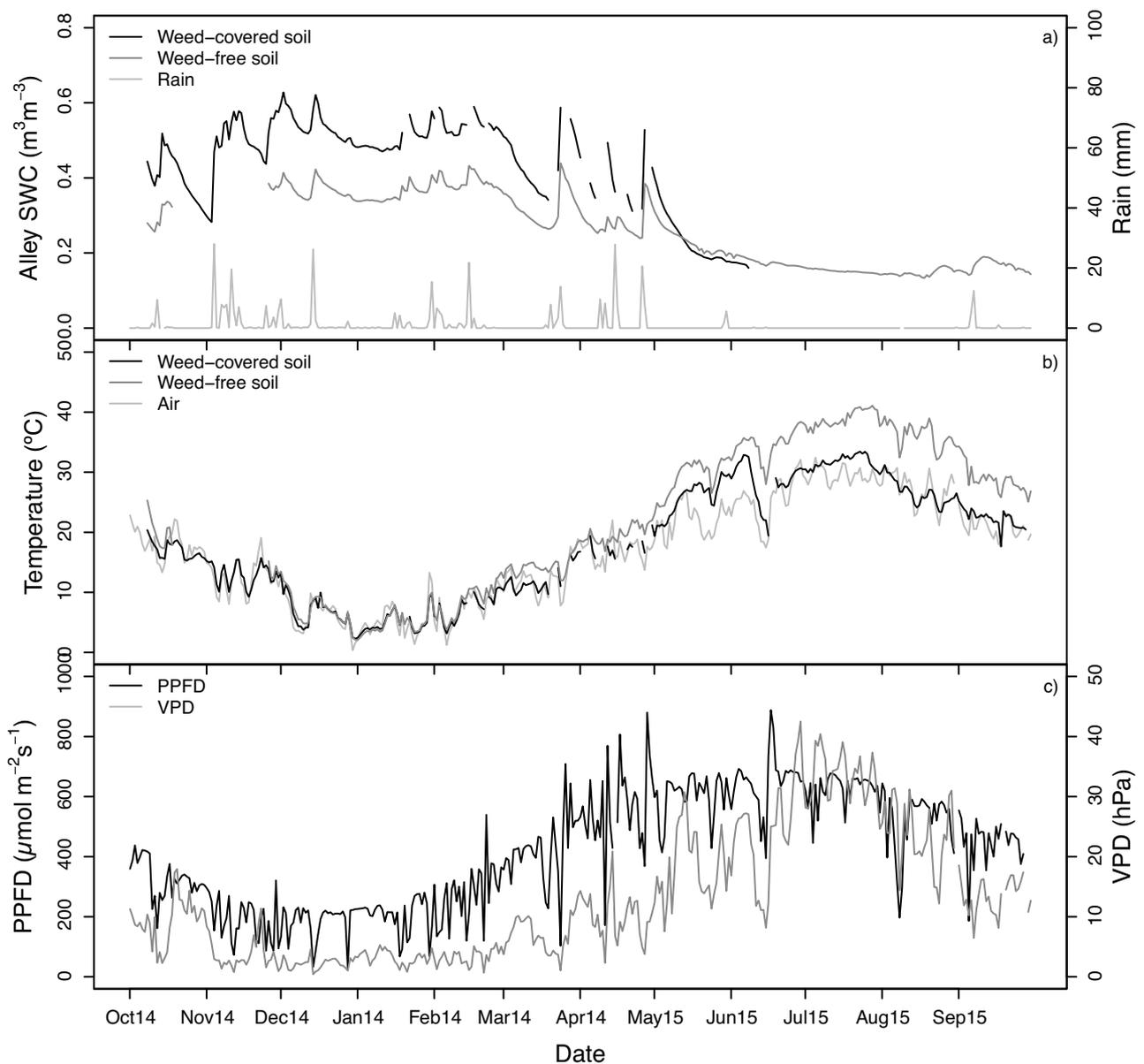
Olive harvesting was carried out in December 2015. Wooden sticks and a trunk-shaker machine were used to dislodge olives from 14 trees selected randomly at each treatment. Olives were collected on nets placed on the ground and then weighed. Samples of olives were transported to the laboratory and dried in an oven at 60 °C in order to determine the dry weight. From this value, we calculated the average crop productivity for each treatment, expressed as kilograms of olives per tree, as well as the C export by

olive yield in  $\text{g C m}^{-2}$  by using the relation:  $1 \text{ g dry matter} = 0.4782 \text{ g of C}$  (Palese et al., 2013).

## 3. Results

### 3.1. Meteorological conditions and soil variables

Meteorological conditions and evolution of soil variables in the two treatments during the study year are shown in Fig. 2. Annual rainfall during the study year was 381 mm, mainly concentrated from November to April, and lower than the climatological average for this site (495 mm; Fig. 2a). The mean annual temperature was 17 °C, and the maximum and minimum average daily temperatures were 32.4 °C (in July) and 0.4 °C (in December) (Fig. 2b). The maximum and minimum averaged daily values of VPD were 42.5 hPa and 0.4 hPa, recorded at the end of June and in December, respectively. PPFD was the highest during the dry season.



**Fig. 2.** Daily averages of environmental and soil variables during the hydrological year: a) Soil water content (SWC,  $\text{m}^3 \text{m}^{-3}$ ; average of the two moisture probes) measured in an alley at 0.10 m in the weed-covered and weed-free soil, and rain (mm) during the studied period. b) Air temperature (data average of the two treatments) and soil temperature (°C) in the weed-covered and weed-free soil (average of the two thermocouples). c) Photosynthetic photon flux density (PPFD,  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and vapor pressure deficit (VPD, hPa) (data average of the two treatments).

Maximum averaged daily PPFD was  $888 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  in June and minimum daily value was  $30 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  in December (Fig. 2c). There were large differences in alley SWC between the soils with and without weed covers (Fig. 2a) (standard deviation of SWC at each treatment was very low, with average values for the whole period of 0.03 and maximum and minimum values of 0.05 and 0.004, respectively). During wet periods, SWC was up to  $0.2 \text{ m}^3 \text{ m}^{-3}$  higher in the weed-cover than in the weed-free soil. However, during the dry soil period, soil moisture was similar for both soils. There were also marked differences in soil temperature between treatments (Fig. 2b). From October to March, soil temperature was similar at both treatments and strongly coupled with air temperature. However, during spring (April, May and June) and summer (July, August, September) months, the temperature was higher in the soil with no weeds, reaching daily averages up to  $13^\circ\text{C}$  above air temperature and  $12^\circ\text{C}$  above the weed-cover soil temperature.

### 3.2. Validity of eddy measurements: flux footprint analysis and energy balance closure

The footprint analysis showed that upwind distances contributing to the measured  $\text{CO}_2$  flux were in all cases within the fetch for each treatment. The median of the  $x_{.90}$  (distance from anemometer delimiting 90% of the flux) during the studied period was 164 m in the weed-cover treatment and 172 m in the weed-free treatment at night, and much less during daytime.

Regarding the energy balance closure, results were similar at both treatments. The closure deficit was 27% in the weed-cover treatment and 29% in the weed-free treatment, with  $R^2$  of 0.90 and 0.87, respectively. The energy balance closure improved at both treatments when only the drier period from May to June was considered, with closure deficits of 26% and 23% and  $R^2$  of 0.91 and 0.90 at the weed-cover and weed-free treatments, respectively.

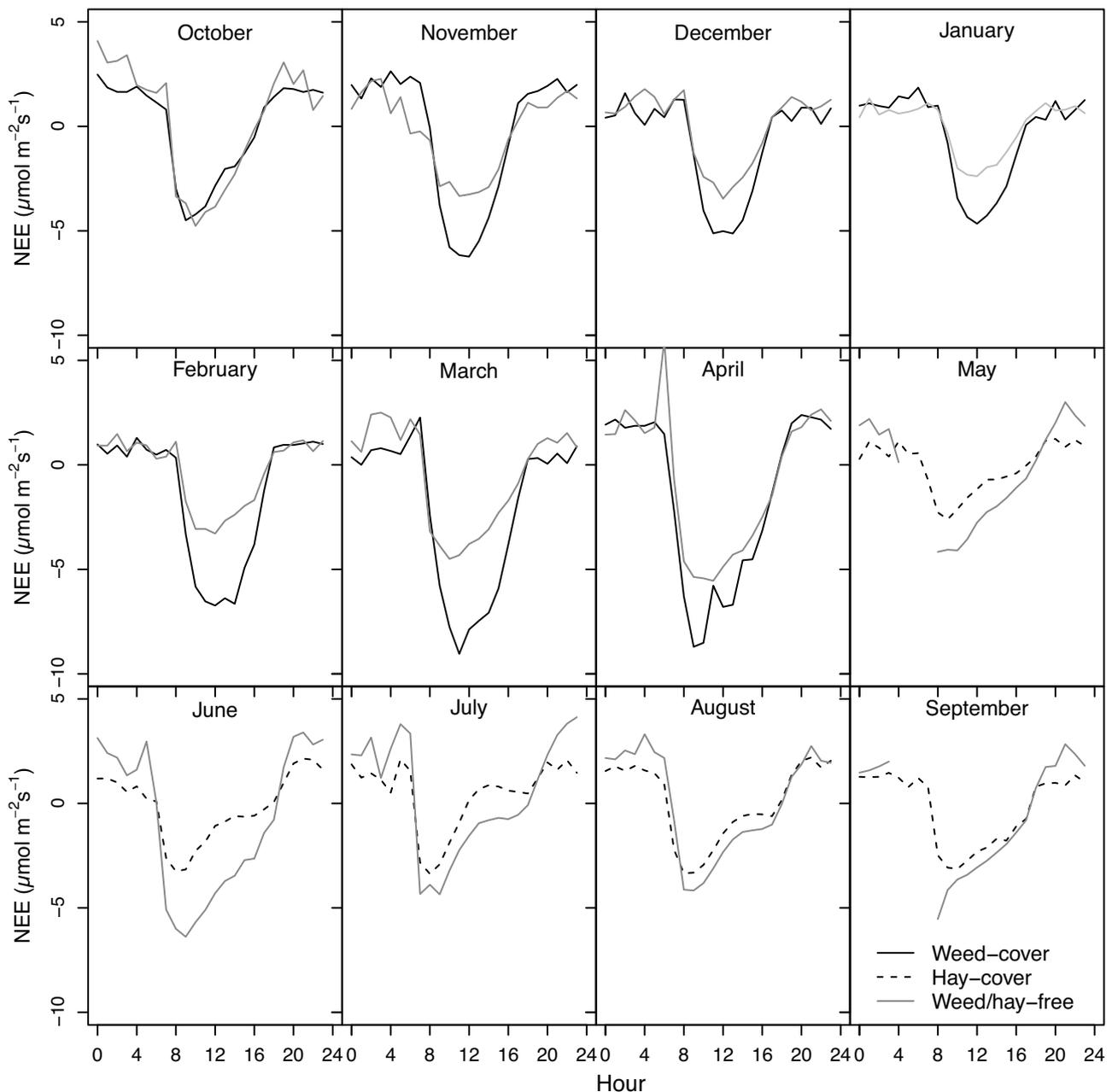


Fig. 3. Average monthly diurnal trends in net ecosystem  $\text{CO}_2$  exchange (NEE) in the two treatments. Monthly averages were calculated from measured hourly data of NEE.

### 3.3. Temporal variability of NEE between treatments

For both treatments, as expected, monthly diurnal curves of NEE showed positive values at night and increasingly negative values after sunrise as incoming solar radiation increased, up to a maximum after which NEE increases, then reaching positive values after sunset (Fig. 3). In addition, a change is observed throughout the year in the time of day when the maximum net CO<sub>2</sub> uptake occurs. While the highest values of net CO<sub>2</sub> uptake occurred at midday (12 pm–1 pm, solar hour) during autumn and winter, maximum CO<sub>2</sub> uptake occurred at earlier hours in spring (10 am–11 am, s.h.) and summer (8 am–9 am, s.h.; Fig. 3).

Thus, despite irrigation, some controlling effects of VPD were found in diurnal trends of NEE. Fig. 4 shows monthly diurnal trends of PPFD, VPD and NEE at both treatments during the growth period in March and the hot dry period in August. During the growth period and under low water stress (maximum monthly diurnal VPD was 16 hPa), NEE was strongly coupled with light intensity, and maximum net CO<sub>2</sub> uptake coincided with maximum light intensity (maximum monthly diurnal PPFD was 1280  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ ; Fig. 4a and c). By contrast these variables showed lags during periods of high water stress (maximum monthly diurnal VPD in August was 41 hPa), when net CO<sub>2</sub> uptake peaked several hours before the time of maximum light intensity (maximum monthly diurnal PPFD was 1630  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ ; Fig. 4b and d). This net CO<sub>2</sub> uptake peak usually occurred before the time of maximum VPD in both periods (low and high water stress), but the delay between both was greater during periods of high water stress (Fig. 4b and d). It can be also seen that during the growth period, net CO<sub>2</sub> uptake was much higher in the weed-cover (maximum monthly diurnal net CO<sub>2</sub> uptake was

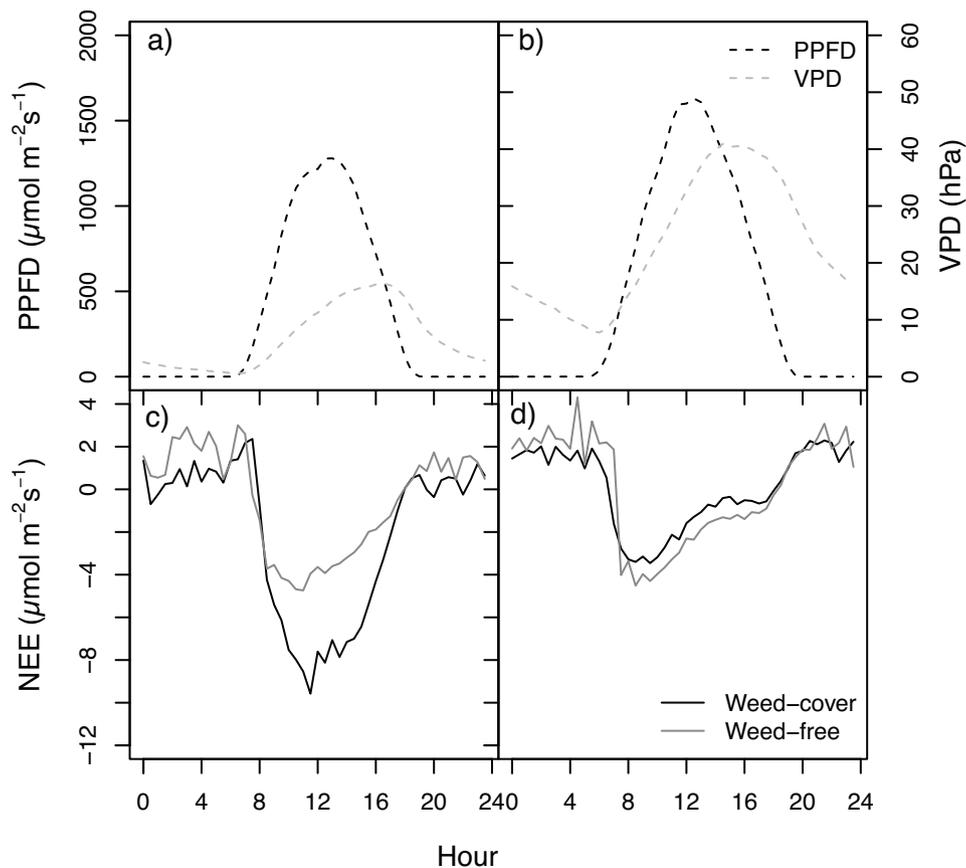
**Table 1**

Monthly NEE (gap-filled data) (and error) for each month at the two treatments.

	Monthly NEE ( $\text{g C m}^{-2}$ )	
	Weed-cover	Weed-free
Oct	3.68 (3.63)	4.59 (3.92)
Nov	-7.30 (3.88)	-5.17 (2.94)
Dec	-35.61(3.48)	-5.51 (2.74)
Jan	-18.06 (3.10)	-17.74 (1.80)
Feb	-36.66 (4.98)	-9.07 (2.26)
Mar	-74.43 (6.83)	-28.09 (3.61)
Apr	-26.76 (6.06)	-19.40 (4.90)
May	7.43 (2.43)	-14.85 (4.18)
Jun	2.49 (2.91)	-21.84 (5.78)
Jul	25.51 (3.17)	23.26 (4.87)
Aug	6.82 (3.00)	6.91 (4.02)
Sep	12.71 (3.52)	16.16 (4.26)

$-9.6 \mu\text{mol m}^{-2}\text{s}^{-1}$ ) than in the weed-free treatment (maximum monthly diurnal net CO<sub>2</sub> uptake was  $-4.7 \mu\text{mol m}^{-2}\text{s}^{-1}$ ), but both showed similar NEE during August when weeds had been already cut (maximum monthly diurnal net CO<sub>2</sub> uptake was  $-3.5$  and  $-4.5 \mu\text{mol m}^{-2}\text{s}^{-1}$ , respectively; Fig. 4c and d).

Throughout the year, important differences were observed in monthly NEE between the two treatments. Results show that NEE was similar in the two treatments in the initial conditions (October), when there were no weeds in either of the two treatments (Fig. 3). Both treatments showed positive values during this month, indicating a net CO<sub>2</sub> emission to the atmosphere (Table 1). For this period, daily NEE values ranged from  $-0.69$  to  $1.26 \text{ g C m}^{-2}$  and from  $-1.07$  to  $1.07 \text{ g C m}^{-2}$  in the weed-cover and



**Fig. 4.** Monthly diurnal trend of PPFD and VPD during March (a) and August (b) and monthly diurnal trend of NEE in both treatments during March (c) and August (d). Monthly averages were calculated from measured half-hourly data of NEE.

weed-free treatment, with daily averages of 0.12 and 0.15 g C m<sup>-2</sup>, respectively.

From November to April, negative monthly values of NEE were found at both treatments, indicating net CO<sub>2</sub> uptake (Table 1). However, as weeds grew, C uptake was much higher in the weed-cover than the weed-free treatment (Fig. 3), with the maximum difference in March. During this month, NEE in the weed-cover treatment was up to -15.5 μmol m<sup>-2</sup> s<sup>-1</sup> and daily NEE ranged from -3.49 to 0.06 g C m<sup>-2</sup>, with an average value of -2.40 g C m<sup>-2</sup>, while in the weed-free treatment, NEE was up to -11.0 μmol m<sup>-2</sup> s<sup>-1</sup> and daily NEE ranged from -2.44 to 0.10 g C m<sup>-2</sup>, with an average value of -0.91 g C m<sup>-2</sup>.

In April, weeds reached their maximum size. Average above-ground weed biomass was 220 ± 58 g m<sup>-2</sup>, of which 36% was organic C content (79.4 g OC m<sup>-2</sup>). During this month, although net CO<sub>2</sub> uptake was still higher in the weed-cover treatment, NEE values became more similar at both treatments (Fig. 3). Daily NEE ranged from -2.92 to 1.20 g C m<sup>-2</sup> in the weed-cover treatment, with an average of -0.89 g C m<sup>-2</sup>, while daily NEE ranged from -1.49 to 0.50 g C m<sup>-2</sup> in the weed-free treatment, with an average of -0.65 g C m<sup>-2</sup>. This period coincided with the highest soil CO<sub>2</sub> efflux recorded in the soil covered by weeds at midday (Fig. 5). From March to May, the soil CO<sub>2</sub> efflux was significantly higher in the weed-covered than weed-free soil. However, differences were especially marked in April, when the soil CO<sub>2</sub> efflux in the weed-covered soil was up to 5.6 times higher than in the weed-free soil.

At the end of April, weeds were cut and left on the soil to allow weed residues (hereafter, 'hay') to decompose and incorporate into the soil. From May to June, contrary to the pattern observed during the weed growth period, net CO<sub>2</sub> assimilation in the hay-free treatment surpassed that observed in the hay-cover treatment (Fig. 3). Negative monthly values of NEE (net CO<sub>2</sub> uptake) were obtained for the hay-free treatment, whereas the hay-cover showed positive values (net CO<sub>2</sub> emission to the atmosphere; Table 1). Daily average NEE in the hay-cover and hay-free treatments were, respectively, 0.24 and -0.48 g C m<sup>-2</sup> in May, and 0.08 and -0.73 g C m<sup>-2</sup> in June.

During the summer months (July to September), both treatments showed similar and positive monthly values of NEE (Table 1). Average daily values in July, August and September were 0.82, 0.22 and 0.42 g C m<sup>-2</sup> in the hay-cover treatment, and 0.75, 0.22 and 0.54 g C m<sup>-2</sup> in the hay-free treatment. Soil respiration rates measured during the dry season (June and July) were low (Fig. 5) and soils both with and without hay showed similar respiration rates (average soil respiration rate was 0.87 ± 0.17 μmol m<sup>-2</sup> s<sup>-1</sup> in

the soil covered by hay, and 0.78 ± 0.02 μmol m<sup>-2</sup> s<sup>-1</sup> in the bare soil).

#### 3.4. Functional relationships between environmental variables and NEE

The light-response curves showed a significant relationship between NEE and PPFD during winter and early spring (Table 2), whereas no significant relationship was found during the dry period. Consequently, significant parameterized coefficients of monthly GPP<sub>max</sub> and R<sub>eco</sub> were generally obtained from the light-response equation for both treatments during winter and early spring (Table 2), but no significant values were obtained for either of them during summer. Modeled values of R<sub>eco</sub> were higher in the weed-cover than in the weed-free treatment (with the exception of February, where modeled R<sub>eco</sub> was unexpectedly higher in the latter), and modeled GPP<sub>max</sub> was up to 4.3 times higher in the weed-cover than in the weed-free treatment (maximum modeled GPP<sub>max</sub> was -28.30 and -15.15 μmol m<sup>-2</sup> s<sup>-1</sup>, respectively). Also, a better relationship between NEE and PPFD was found during the weed growth period (from December to March) in the weed-cover treatment compared to the weed-free treatment. Concretely, in March when GPP<sub>max</sub> was highest in the weed-cover treatment, a better fit was found in this treatment (R<sup>2</sup> = 0.98) compared to the weed-free treatment (R<sup>2</sup> = 0.71). For the same PPFD level (1216 μmol photons m<sup>-2</sup> s<sup>-1</sup> at noon), maximum net CO<sub>2</sub> assimilation was double in the weed-cover than of the weed-free treatment (Fig. 6).

Nevertheless, a worse fit was observed for the weed-cover treatment during April and May, when weeds were cut and net CO<sub>2</sub> fixation decreased in the hay-cover treatment. During these months, significant values of GPP<sub>max</sub> were obtained in the hay-free treatment, but no significant values of GPP<sub>max</sub> or R<sub>eco</sub> were found in the hay-cover treatment.

Contrary to expectations, no significant relationship was found between nighttime NEE and temperature either at daily or monthly scales. Nighttime NEE was low at both treatments during the study period, with averages of 0.77 and 0.85 g C m<sup>-2</sup> night<sup>-1</sup> in the weed-cover and weed-free treatments, respectively. Nonetheless, we could observe some seasonal trends in nighttime NEE related to temperature for both treatments. Nighttime NEE was low from December to March (average nighttime NEE was 0.29 and 0.42 g C m<sup>-2</sup> night<sup>-1</sup> in the weed-cover and weed-free treatments, respectively), coinciding with periods of low air temperature (average nighttime temperature was 5.4 °C, and ranged from 2.5 °C in January to 9.0 °C in March), while higher values of nighttime NEE were found from April to November (average nighttime NEE was 1.01 and 1.11 g C m<sup>-2</sup> night<sup>-1</sup> in the weed-cover and weed-free treatments, respectively), coinciding with periods of higher air temperatures (average nighttime temperature was 18.5 °C, and ranged from 11.2 °C in November to 24.8 °C in July).

#### 3.5. Annual NEE and olive productivity between treatments

Although higher net CO<sub>2</sub> emissions were found during late spring in the weed-cover treatment compared to the weed-free treatment, a positive effect of weed cover was found in annual net ecosystem exchange. The cumulative NEE values during the study year (Fig. 7) resulted in an annual NEE value of -140 ± 20 g C m<sup>-2</sup> in the weed-cover and -70 ± 10 g C m<sup>-2</sup> in the weed-free treatment. Thus, although the weed-free treatment acted as a net C sink during longer period (from November to June) than the weed-cover treatment (from November to April), the higher assimilation rate in the latter during the weed growth period was able to offset the higher emissions found in this treatment during late spring. As a result, annual net ecosystem C uptake was reduced by 50% in the

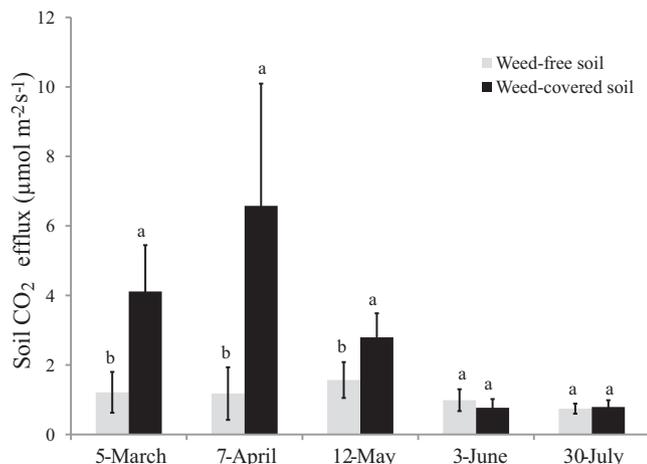


Fig. 5. Soil CO<sub>2</sub> efflux (mean ± sd, n = 5) measured at midday in the alleys of the two treatments.

**Table 2**

Coefficients for  $GPP_{max}$  ( $b_1$ ),  $R_{eco}$  ( $b_3$ ) and PPFD when  $GPP_{max}$  was half ( $b_2$ ) obtained by applying the Falge et al. (2001) equation using monthly averages of daytime 30-min data until noon for each month. The coefficient of determination of the relationship between NEE and PPFD is also shown. Only months with at least one significant parameterization coefficient are shown.

		Weed-cover			Weed-free		
		Estimate	Standard error	p value	Estimate	Standard error	p value
December	$b_1$	19.3	2.9	$p < 0.001$	11.3	6.6	0.138
	$b_2$	757.7	272.3	0.032	654.6	1012.1	0.542
	$b_3$	4.2	0.5	$p < 0.001$	2.8	1.6	0.124
	$R^2$	0.99			0.83		
January	$b_1$	17.2	3.7	0.006	8.7	1.8	0.005
	$b_2$	820.4	510.9	0.169	596.4	515.8	0.3
	$b_3$	3.7	1.1	0.019	2.5	1.1	0.075
	$R^2$	0.98			0.96		
February	$b_1$	20.4	2.9	$p < 0.001$	15.2	2.5	$p < 0.001$
	$b_2$	812.4	279.5	0.023	150	75.2	0.093
	$b_3$	3.6	0.6	$p < 0.001$	9.6	3.1	0.022
	$R^2$	0.99			0.98		
March	$b_1$	28.3	3	0	6.6	4.4	0.174
	$b_2$	241.3	89.6	0.027	300.4	713.8	0.686
	$b_3$	15.4	4	0.005	0.9	6	0.882
	$R^2$	0.98			0.71		
April	$b_1$	17.5	14.6	0.245	13.5	4.9	0.013
	$b_2$	192.1	357	0.596	256	254.2	0.326
	$b_3$	9	16.4	0.589	6.7	5.9	0.273
	$R^2$	0.53			0.75		
May	$b_1$	10.9	48.7	0.825	7.3	1.9	0.001
	$b_2$	55.8	320.6	0.863	601.8	1071.7	0.581
	$b_3$	9.2	49.2	0.853	2.5	3.4	0.478
	$R^2$	0.35			0.48		

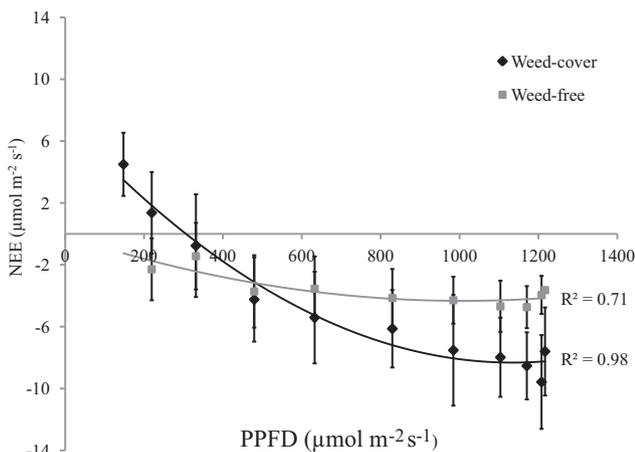
weed-free treatment. Despite frequent data gaps during the study year, uncertainty in the estimation of annual carbon budgets for both treatments was low (14%), making differences between treatments noteworthy.

Regarding productivity, some differences were found between both treatments during the studied year. On average, olive yield (dry weight) was 34.2 kg of olives per tree in the weed-cover treatment and 28.0 kg of olives per tree in the weed-free treatment, thus indicating productivity was 22% higher in the former. According to these results, the C export by olive harvesting was

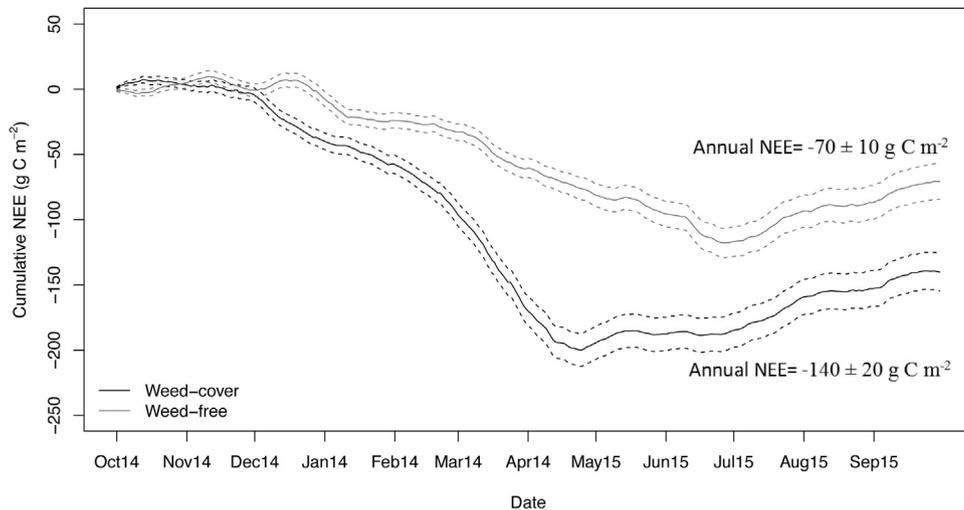
estimated in  $334 \text{ g C m}^{-2}$  in the weed-cover treatment and  $273 \text{ g C m}^{-2}$  in the weed-free treatment.

#### 4. Discussion

Large differences in NEE were observed in the olive orchard under the two treatments. Although plant covers are able to enhance soil respiration by increasing SOC content and microbial activity, alternatively, they can increase C fixation through their photosynthetic activity. Hence, we found that the maintenance of spontaneous weeds from autumn to early spring strongly increased net C fixation compared to the weed-free treatment (Fig. 3). In March, when net C uptake in the olive orchard under both managements was the highest, the treatment with weed cover showed up to 2.7 times higher monthly NEE than the treatment without weed covers, with values of  $-74.43$  and  $-28.09 \text{ g C m}^{-2} \text{ month}^{-1}$ , respectively (Table 1). Assuming that the difference between these values represents the net C uptake by weeds, the resulting value is  $46.34 \text{ g C m}^{-2} \text{ month}^{-1}$ , which is 1.7 times higher than the net C uptake by olive trees in the weed-free treatment ( $-28.09 \text{ g C m}^{-2} \text{ month}^{-1}$ ). Coinciding with this, Palese et al. (2013) reported that spontaneous vegetation (weeds and grasses) was the most important crop component for C fixation in an irrigated olive orchard in southern Italy, contributing to 35% of total  $\text{CO}_2$  fixation (the rest being pruning material and yield). This high C assimilation by weeds can be explained by their short lifetime and the need for higher efficiency in  $\text{CO}_2$  uptake to invest in biomass growth, before the beginning of the senescence dry period. In addition, weed species use different water- and light-use strategies than olive trees, which can explain differences in NEE trends between treatments. Under increasing PPFD, olive trees can limit their photosynthetic activity by closing stomata in response to increased water stress (Testi et al., 2008; Villalobos et al., 2012).



**Fig. 6.** Light-response curves in March for the two treatments, using monthly averages of measured half-hourly daytime NEE. Bars represent standard deviation of monthly averages.



**Fig. 7.** Cumulative NEE (continuous line) and uncertainty (dashed line) of the gap-filled data, as well as annual net C uptake in the two treatments during the study year.

In contrast, weeds are able to maintain their photosynthetic activity under high light intensities, despite relatively high air VPD (Long and Hällgren, 1993; Pérez-Priego et al., 2015). This behavior was reflected in the better relationship found between NEE and PPFD in the weed-cover than the weed-free treatment (Fig. 6).

In our olive orchard, despite irrigation, the effect of light and VPD on diurnal trends of NEE was visible at both treatments (Fig. 4). During the spring growth period, net CO<sub>2</sub> uptake increased with increasing PPFD up to a threshold, coinciding with maximum light intensity, after which net CO<sub>2</sub> assimilation decreased, coinciding with maximum VPD during day (Fig. 4a and c). The relationship between NEE and PPFD during this period was better in the weed-cover than the weed-free treatment, attributed not only to CO<sub>2</sub> uptake by olive trees but also to high CO<sub>2</sub> uptake by weeds and their rapid response to increasing PPFD, as compared to the weed-free treatment, where the response of olive trees to increasing PPFD is subject to stomatal control. During the summer period (August), an increased delay between the CO<sub>2</sub> fixation peak and maximum VPD was observed, and there was also a slight decoupling between the net CO<sub>2</sub> uptake peak and maximum PPFD (Fig. 4b and d). This is indicative of the mechanisms of stomatal control used by olive trees for reducing CO<sub>2</sub> fixation in order to regulate water losses by transpiration under high water stress conditions (high VPD). Indeed, up to 80% of total C uptake occurred before midday during summer months, while only 44% occurred before midday during winter months. Consistent with our analysis, Testi et al. (2008) reported 60% of total C was fixed before midday in summer in an irrigated olive orchard, while this decreased to 40% in the cool season, when VPD exerted a minor effect.

The presence of weeds not only increases C uptake but also significantly increases soil CO<sub>2</sub> efflux (Table 2, Fig. 5). Bertolla et al. (2014) found that soil respiration was higher in an olive orchard with weeds compared to that without weeds and estimated annual emissions due to respiration of 1179 and 784 g C m<sup>-2</sup> in the two treatments, respectively. Nonetheless, the higher CO<sub>2</sub> efflux found in the soil with weeds was more than offset by weed photosynthetic activity during the growth period, thus resulting in higher annual net C assimilation compared to the management for weed suppression (Table 1). Modeled parameters using the light-response equation also showed that both GPP<sub>max</sub> and R<sub>eco</sub> were higher in the treatment with weed than without weed covers (Table 2). These parameters were significant during winter and early spring but not during summer, probably because of the effect of high VPD that could mask the relationship between NEE and PPFD. Aerial and root weed biomass largely contributes to soil C

enrichment (Guzmán et al., 2014). In this study, aboveground weed biomass represented 79.4 g OC m<sup>-2</sup>, which is comparable to the values reported by other authors who have found organic C inputs by spontaneous vegetation between 46.2 and 50.9 g OC m<sup>-2</sup> during years of normal precipitation regime (Repullo-Ruibérriz de Torres et al., 2012). The C fixed by weeds is partly respired back to the atmosphere by decomposition of more labile C compounds, and partly remains in the soil and is incorporated as resistant organic matter in the uppermost layer of the soil, contributing to increasing SOC (Hernández et al., 2005; Castro et al., 2008; Soriano et al., 2014).

The application of glyphosate to control weed emergence was expected to have little effect on CO<sub>2</sub> fluxes of olive trees or bare soil. Previous studies have shown no effect of glyphosate application to weeds on the photosynthetic activity of young olive trees (Cañero et al., 2011) or on the soil microbial community (Weaver et al., 2007). However, some studies have shown that glyphosate increases microbial biomass-C, soil enzymatic activity, and microbial respiration (Zabaloy et al., 2008; Panettieri et al., 2013), as glyphosate could be used by microbes as a C source (Eser et al., 2007). Due to the relatively short degradation time and low persistence of glyphosate in the soil, its effects on the soil can be negligible after about six weeks, depending on soil characteristics (mainly texture), crop type and climatic conditions (Tejada, 2009; Panettieri et al., 2013). The slightly higher nighttime NEE in the weed-free treatment in October might have been caused by an increase in microbial respiration due to glyphosate application, just one month before the beginning of measurements. Once its effect disappeared, similar nighttime NEE was found in the two treatments (November–April). In the long term, weed removal by the herbicide in the weed free treatment could provoke soil compaction, the reduction of SOC and the increase of bulk density (Castro et al., 2008), thereby decreasing infiltration.

After weeds were cut and the hay left on the soil (May and June), opposite to the pattern observed during the growth period, net C uptake was higher in the hay-free treatment than in the hay-cover treatment (Table 1). This lower net C uptake in the hay-cover treatment during late spring can be attributed to the higher respiration promoted by the hay. First, hay decomposition favors the formation of stable microaggregates that are enriched in organic C, enhancing earthworm and soil microfauna activity, which in turn affects respiration and the soil C pool (Pulleman et al., 2005; Plaza-Bonilla et al., 2015). Second, hay also increases the amount of labile C which is readily used for respiration by soil microorganisms. Third, although respiration usually increases

with soil temperature, very high temperatures can constrain respiration by exceeding the optimum temperature for some microorganism activity (O'Connell, 1990). The high temperatures registered in the hay-free soil during the dry season (Fig. 2) could be responsible for lower respiration in these soils relative the hay-covered soil. Fourth, photodegradation of the hay can also contribute to enhancing CO<sub>2</sub> emissions (Brandt et al., 2009; Rutledge et al., 2010). Although soil chamber measurements support this higher CO<sub>2</sub> efflux in the hay-covered soil during May, we found no significant differences in soil CO<sub>2</sub> efflux between soils with or without hay in June, when higher net ecosystem CO<sub>2</sub> uptake was still observed in the site without hay. As soil CO<sub>2</sub> efflux was measured at midday, it is possible that high soil temperatures limited respiratory activity in both soils during this time. Further research on diurnal trends of soil CO<sub>2</sub> efflux under the two soil managements will help to elucidate the role of weed covers in CO<sub>2</sub> emissions and their relative contribution to ecosystem NEE.

Contrary to published studies that have reported net C uptake during summer and throughout the year in irrigated olive orchards under climate conditions similar to ours (Testi et al., 2008; Nardino et al., 2013), we found monthly net CO<sub>2</sub> release in the olive orchard under the two managements during the summer period (from July to September; Table 1). The high evaporative demand recorded during this year (maximum daily air temperature during July and August on average was 36.9 °C and maximum daily VPD on average was 52.8 hPa) could constrain tree photosynthesis, making respiratory processes the main contributors to the ecosystem CO<sub>2</sub> flux. In this regard, although soil respiration in the alleys of our two treatments was low during this period (around 0.9 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), in accordance with other studies (between 1.1 and 1.6 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>, see Testi et al., 2008), due to low soil moisture content, high respiration rates might be expected in the drip-irrigated zones where water availability was not limited. For instance, Testi et al. (2008) reported respiration rates were up to 5.7 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> in irrigated olive groves beneath the tree canopy during summer. Thus, in the irrigated zones, CO<sub>2</sub> efflux from both soil and aboveground respiration from olive trees are expected to significantly contribute to NEE. Leaves and fruits appear to be the main contributors to aboveground respiration in olive trees, while respiration of wood biomass (trunk and branches) represent a very small fraction of CO<sub>2</sub> flux (Pérez-Priego et al., 2014). Thus, the positive values of NEE found in both treatments during summer may be due to leaf, fruit, and soil respiration, the latter originating under the tree canopy.

Nighttime NEE values at our site were higher than those reported by Testi et al. (2008) for winter periods (0.7 versus 1.4 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>, on average, in our site), but lower than those reported by the mentioned authors during non-winter periods (4.8 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> versus 2.2 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>, on average, in our site). Similar to the results of these authors, we found no clear relationship between nighttime NEE and soil or air temperature. This is probably due to a combination of different causes: i) copious missing data during nighttime periods due to battery malfunctioning, and predominance of stable conditions and low friction velocities ( $u_* < 0.15 \text{ m s}^{-1}$ ); ii) influence of soil moisture on ecosystem respiration, since water-limited ecosystems are moisture-pulse dependent (Chen et al., 2009; López-Ballesteros et al., 2016). Thus, while in mid-high latitudes temperature is a key driver for CO<sub>2</sub> fluxes, its relative importance could decrease in semiarid environments where water is the most important driver for vegetation productivity; and iii) strong seasonality of weed and olive tree activity, which could mask the effect of soil temperature on nighttime (as well as daytime) NEE. In support of this, Pérez-Priego et al. (2014) reported a good relationship between aboveground respiration in olive trees and both temperature and phenological stage (i.e. periods of dormancy, flowering and

fruit setting), so that the effects of temperature on CO<sub>2</sub> fluxes could be confounded by plant phenology and/or productivity during flowering and fruit-development periods.

Despite small differences in nighttime NEE between treatments, we can observe that nighttime NEE was slightly higher in the weed-free treatment from May to September (Fig. 3). Possible causes for this higher NEE during nighttime can be: i) frequent dewfall episodes during night have a greater effect on activating soil microbial respiration in the soil without hay, which is directly exposed to dewfall, while dew should be rather deposited on the hay in the hay-cover treatment, making this water input less accessible to soil; or ii) higher nighttime temperature in the soil without hay (on average, 4.9 °C higher than the hay-cover soil during the period from May to August) can greater stimulate microbial respiration.

In general, NEE values recorded in this study were lower than those reported in other irrigated olive orchards under similar soil (clay/clay loam soils) and climate conditions (precipitation regime). While we found NEE values up to  $-0.7 \text{ g C m}^{-2} \text{ day}^{-1}$  during summer, Testi et al. (2008) reported NEE values in a young olive orchard (LAI of the trees was  $1.9 \text{ m}^2 \text{ m}^{-2}$ ) in Southern Spain of  $-2.7 \text{ g C m}^{-2} \text{ day}^{-1}$  during this period. Maximum daytime NEE in our site was  $-4 \text{ g C m}^{-2} \text{ day}^{-1}$ , with an average value of  $-2.5 \text{ g C m}^{-2} \text{ day}^{-1}$  during the period of maximum CO<sub>2</sub> assimilation (March) and  $-0.8 \text{ g C m}^{-2} \text{ day}^{-1}$  during summer. In contrast, López-Bernal et al. (2015) reported an average daytime NEE of  $-4.5 \text{ g C m}^{-2} \text{ day}^{-1}$  in an irrigated olive orchard (LAI =  $1.5 \text{ m}^2 \text{ m}^{-2}$ ) in southern Spain in the period from late June to late September. In an olive orchard (LAI ~  $3 \text{ m}^2 \text{ m}^{-2}$ ) in southern Italy, Nardino et al. (2013) reported maximum monthly NEE values of  $-170 \text{ g C m}^{-2} \text{ month}^{-1}$ , while we found a maximum monthly value of  $-74.4 \text{ g C m}^{-2} \text{ month}^{-1}$  (Table 1). Differences in NEE between our study site and the results reported in other irrigated olive orchards can be attributed to the different age and density of the olive trees, and the inherent inter-annual variability of semiarid ecosystems, among other factors. Contrary to the young age of the olive orchards reported in the previously cited studies, our study was conducted in a mature olive orchard (80 years old trees), where growth of trees is limited and increase of tree biomass is low compared to the rapid growth that can be expected in young olive plantations (5–6 years old). Plant density was also lower in our study (~200 trees ha<sup>-1</sup>) than in the mentioned studies (~400 trees ha<sup>-1</sup>). The annual NEE in our olive orchard was also lower than that reported for young olive orchards with plant cover management. While we found annual C uptake of  $140 \text{ g C m}^{-2} \text{ y}^{-1}$  in the weed-cover treatment (Fig. 7), values from  $1160 \text{ g C m}^{-2} \text{ y}^{-1}$  to  $1345 \text{ g C m}^{-2} \text{ y}^{-1}$  have been reported in 12–16 year old olive orchards (Nardino et al., 2013). In a 50 year old olive orchard on sandy loam soils in Southern Italy and taking into account C inputs by cover crop residues, pruning material, senescent leaves, yield and root biomass of olive trees, Palese et al. (2013) estimated an annual NEE of  $-1550$  and  $1020 \text{ g CO}_2 \text{ m}^{-2} \text{ y}^{-1}$  ( $419$  and  $275 \text{ g C m}^{-2} \text{ y}^{-1}$ ) under sustainable (irrigation with urban wastewater treated and conservation of spontaneous weeds and grasses) and conventional management (rainfed conditions, intensive tillage and pruning), respectively. Our results were similar to those reported by Brilli et al. (2016), who found an annual NEE ranging from  $-137$  to  $-667 \text{ g C m}^{-2} \text{ y}^{-1}$  (average of 3 years,  $364 \text{ g C m}^{-2} \text{ y}^{-1}$ ) in a rain-fed olive orchard in central Italy with surface tillage management. Unfortunately, the lack of literature reporting direct measurements on C uptake in olive orchards under similar conditions to ours (crop characteristic, soil management) makes comparisons difficult.

Some studies have reported a reduction of crop productivity in olive orchards with plant covers due to competition for water and nutrient resources with trees (Gucci et al., 2012; Ferreira et al., 2013). In contrast, other authors have found a positive effect of

weeds on crop productivity. [Palese et al. \(2013\)](#) found that olive yield was, on average, 2.3 times higher in an olive orchard with eight years of sustainable management where no tillage was applied and spontaneous vegetation cover was allowed to grow compared to an olive orchard under conventional management. According to our results, during the first year of differential treatment, the olive yield was 22% higher in the weed-cover than in the weed-free treatment, suggesting that weeds, rather than having a negative effect on crop productivity, appeared to have a positive effect on olive yield. However, this result should be considered with caution due to the few samples considered for olive yield determination in the current study ( $N = 14$  trees per treatment) and the high variability characterizing fruit productivity in olive trees, both spatial and interannual. Thus, a long-term study is necessary to identify trends in olive productivity associated with soil management. The negative effect of weeds on crop productivity reported by the previously mentioned studies might be due to the fact that research was conducted on rain-fed olive orchards or in orchards with very high tree densities, where limiting water and nutrient availabilities likely increased competition for soil resources between plant covers and trees. Nonetheless, all of these studies reported improvements in soil fertility with the presence of herbaceous plants.

Although the weed-cover treatment acted as a C source during a longer period than the weed-free treatment, the higher net C uptake found in the former during the growing period due to the presence of weed cover, resulted in significantly higher C uptake on the annual basis. Weed cover increased the magnitude of NEE by 100% with respect to the treatment without weed cover ([Fig. 7](#)), eventually resulting in an annual value of  $-140 \text{ g C m}^{-2} \text{ y}^{-1}$  (equivalent to 6.9 kg C per tree) in the former versus  $-70 \text{ g C m}^{-2} \text{ y}^{-1}$  (equivalent to 3.5 kg C per tree) in the latter. These findings emphasize the important role of weed covers in increasing C uptake in olive orchards. Although fossil fuel use is the main source of greenhouse gases in fruit tree orchards ([Aguilera et al., 2015](#)), the reduction of  $\text{CO}_2$  emissions by application of conservation practices based on plant covers is not negligible and should be considered when assessing the C footprint in crop systems under sustainable management. [Table 3](#) shows a rough estimation of the annual C budget by considering the Net Ecosystem Productivity ( $\text{NEP} = -\text{NEE}$ ) and anthropogenic emissions derived from management activities for both treatments (for more information, see [Appendix A](#)). While no remarkable differences were found for anthropogenic emissions between treatments, differences in NEP ultimately controlled the annual C budget, which resulted in lower C uptake in the weed-free treatment than in the weed-cover treatment. This assessment does not take into account the lateral C export by harvesting in the estimation of the annual C budget. If we were to consider the Net Biome Productivity ( $\text{NBP} = \text{NEP} - \text{harvest}$ ),

similar values would be found for both treatments (194 and  $203 \text{ g C m}^{-2} \text{ year}^{-1}$  for the weed-cover and weed-free treatments, respectively), which make differences in the annual C budget between treatments smaller (net emissions of 171.4 and  $181 \text{ g C m}^{-2} \text{ year}^{-1}$  by the weed-cover and weed-free treatments, respectively). However, as mentioned above, this estimate of NBP must be considered with caution due to the uncertainty in the olive yield determination and the great inter-annual variation of the olive export by harvesting. Regardless of these sources of uncertainty, our study demonstrates that the management treatment affected annual NBP through its influence on Net Ecosystem Productivity ( $\text{NEP} = -\text{NEE}$ ), which was increased by 100% with the presence of weed cover.

Bearing in mind the limitations previously discussed and assuming a tree density of  $200 \text{ trees ha}^{-1}$ , if we consider the total irrigated olive cultivation surface without spontaneous vegetation or cover crop management in Spain ( $\sim 440 * 10^3 \text{ ha}$ ) ([MAGRAMA, 2012](#)), we can estimate an annual C uptake increase of  $308 * 10^3 \text{ ton C}$  due to implementation of conservation practices based on maintenance of spontaneous vegetation in olive orchards. Nonetheless, this is a rough estimation that needs to be validated. Last, it should be mentioned that, in addition to affecting  $\text{CO}_2$  fluxes, weed cover can affect climate change by modification of the surface albedo. Although effects on albedo have not been addressed in this study, they should be further considered as we found the presence of weeds decreased albedo by 6% (averaged value for the study period) compared to the weed-free treatment.

This study shows for the first time the positive effects of weed cover on the annual C uptake in olive orchards through direct measurements of  $\text{CO}_2$  exchange at ecosystem scale. Nonetheless, these reported effects should be analyzed over the long term, as variables such as precipitation and temperature patterns during the year can strongly condition the C budget and yield in olive orchards. Although plant covers are being increasingly adopted as sustainable management practices in olive orchards and other crops, their implementation in many agricultural lands is still limited and conventional practices such as intensive tillage are widespread in the Mediterranean region. The implementation of conservation practices based on plant cover offers numerous benefits to farmers and land practitioners, not only from the point of view of environmental protection which involves the improvement of physico-chemical soil properties and the increase of  $\text{CO}_2$  fixation and reduction of  $\text{CO}_2$  emissions to the atmosphere, but also from an economic perspective resulting from the reduction of costs for restoration of damaged soils and the possibility of receiving economic subsidies from public bodies for the application of more sustainable agricultural practices.

## 5. Conclusions

Maintenance of alley weed cover in olive orchards increases ecosystem C uptake during periods of weed growth. However, after weeds are cut during late spring, the soil  $\text{CO}_2$  efflux appears to increase due to decomposing weed remnants. This reduces ecosystem C fixation and reverses the behavior of the olive orchard from C sink to C source. Although the presence of weeds increased  $\text{CO}_2$  emissions to the atmosphere during late spring, the maintenance of weed cover increased annual C uptake from the atmosphere by 100% relative to the treatment without weed cover. We measured NEE in the olive orchard under the two treatments, but further research should take into account  $\text{CO}_2$  exchange by the different orchard components in order to elucidate the role that soil, herbaceous plants and olive trees play on  $\text{CO}_2$  uptake and  $\text{CO}_2$  emissions, as well as their seasonal changes throughout the year, and the relative contribution of each component to NEE. On the whole, this study highlights the positive effects of conservation

**Table 3**

Estimation of the annual carbon budget at both treatments, expressed as  $\text{g C m}^{-2} \text{ year}^{-1}$ . Anthropogenic emissions were estimated according to the Carbon Footprint Certification for Castillo de Canena olive oil (see [Appendix A](#) for more information). Annual carbon budget was determined as the difference between NEP and deductions.

Net Effect on C fluxes ( $\text{g C m}^{-2} \text{ year}^{-1}$ )	Weed-cover	Weed-free
Net Ecosystem Productivity ( $\text{NEP} = -\text{NEE}$ )	140	70
Anthropogenic emissions by management activities (deductions)		
Irrigation	15.8	15.8
Foliar treatments (pesticides)	1.2	1.2
Collecting and transport of olives to oil press	5.2	4.3
Mowing	0.4	
Application of glyphosate herbicide		0.7
Total	22.6	22.0
<b>ANNUAL CARBON BUDGET (NEP-deductions)</b>	<b>117.4</b>	<b>48.0</b>

practices based on maintenance of weed cover in net C uptake by olive orchards and the feasibility of using eddy covariance techniques to characterize differences in the C balances of olive orchards under different management practices.

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## Appendix A. Anthropogenic emissions by management activities (deductions) at both treatments according to data provided by Castillo de Canena olive oil S.L.:

C emissions by management activities (g C per liter of oil)	
Irrigation	117.6
Foliar treatments (pesticides)	8.9
*Collecting and transport of olives to oil press	43.6
Mowing	2.6
Application of glyphosate herbicide	5.2

Determination of annual oil production per m <sup>-2</sup>	
Average olive yield (kg olives per tree)	38
Estimated industrial performance (kg of oil per kg of olive)	0.16
Oil density (kg per liter)	0.918
Tree density (trees per ha)	204
Liters of oil per m <sup>-2</sup>	0.13

According to these data, anthropogenic emissions were calculated as  $\text{g C m}^{-2} \text{ year}^{-1}$ .

\*Due to differences in crop productivity between treatments, anthropogenic emissions by collection and transport of olives to oil press was calculated according to olive yield found on each treatment.

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