

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

3

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17

18 **Abstract**

19

20 No-till management and the establishment of plant cover have been implemented in olive crops  
21 in recent years in order to prevent soil erosion and increase soil organic carbon. However, the  
22 effect of these conservation practices on the net CO<sub>2</sub> exchange at the ecosystem scale has not  
23 been explored so far. In this study, we analyze the influence of resident vegetation cover  
24 (hereafter weeds) on the net ecosystem CO<sub>2</sub> exchange (NEE) in an irrigated olive orchard  
25 located in Jaén (SE Spain) by using the eddy covariance technique. NEE was measured in the  
26 olive orchard under two treatments, one with weed cover in the alleys from autumn to spring,  
27 and another where weed growth was avoided by the application of a glyphosate herbicide. Our

28 study demonstrates that the presence of weeds in the alleys increased carbon assimilation in the  
29 weed-cover treatment during the weed growing period (from December to April). However, the  
30 net ecosystem CO<sub>2</sub> uptake decreased in the weed-cover treatment during late spring (May and  
31 June), after weeds were cut and left on the soil, compared to the weed-free treatment, probably  
32 due to an increase in soil respiration. On an annual basis, weed removal decreased carbon  
33 uptake by 50% compared to the weed-cover treatment. The annual NEE was -140 g C m<sup>-2</sup> y<sup>-1</sup> in  
34 the weed-cover treatment and -70 g C m<sup>-2</sup> y<sup>-1</sup> in the weed-free treatment. In summary, our study  
35 demonstrates that, during the first year of differential treatment, maintenance of weed cover in  
36 olive groves has a positive effect on CO<sub>2</sub> uptake and enhances the capacity of the agro-system to  
37 act as a net CO<sub>2</sub> sink.

38

39 **Keywords:** olive tree, carbon uptake, conservation agriculture, eddy covariance, sustainable  
40 management, Mediterranean climate.

41

## 42 **1. Introduction**

43

44 Soil cultivation and anthropogenic climate change have caused a great impact on the global soil  
45 carbon (C) cycle over the last century. Inadequate management of agricultural land has led to  
46 accelerated rates of soil erosion and has exposed trapped C to decomposition, accelerating  
47 mineralization of soil organic carbon (SOC; Lal, 2004). As a consequence, these practices have  
48 modified gains and losses of soil C, altering the natural C balance and increasing greenhouse  
49 gas emissions (Aguilera et al., 2015; Amundson et al., 2015). Some estimates point to global  
50 SOC losses by agricultural erosion of 404 Tg C y<sup>-1</sup> (Doetterl et al., 2012) and to global C  
51 releases to the atmosphere associated with erosion that range from 0.8 to 1.2 Gt C y<sup>-1</sup> (Lal,  
52 2003). These C emissions are equivalent to 12% of global C emissions by fossil fuels and  
53 industry (9.80 Gt C in 2014; Le Quéré et al., 2015). Therefore, the application of sustainable  
54 practices aimed to increase C sequestration in agriculture has become a relevant subject of

55 interest. This can be especially important in Spain, where SOC contents lower than 1% are  
56 frequent, mostly in southern areas and agricultural soils (Rodríguez-Martín et al., 2016).

57

58 Olive trees (*Olea europaea L.*) are one of the most important crops in the Mediterranean basin,  
59 where they cover around 9.5 Mha and account for 98% of the world's olive cultivation area  
60 (Repullo-Ruibérriz de Torres et al., 2012). The largest area dedicated to this crop is found in  
61 Spain, where it occupies 2.6 Mha and represents 72% of world's olive production (data for  
62 2013-2014; IOOC, 2015). Around 60% (1.5 Mha) of the olive cultivation in Spain is located in  
63 Andalusia (southern Iberian Peninsula; MAGRAMA, 2012). Thus, olive groves represent an  
64 important agricultural system in this region due to its environmental, social and economic  
65 benefits. However, olive groves are subject to several environmental problems due to  
66 inadequate conventional soil-management practices such as intensive tillage and overgrazing,  
67 which have caused high runoff and erosion rates, high soil and SOC losses, and the loss of soil  
68 fertility (Álvarez et al., 2007; Francia et al., 2006; Martínez-Mena et al., 2008; Gómez et al.,  
69 2009). In order to mitigate these problems, research has been carried out in recent decades to  
70 improve soil management practices, and prevent the mineralization of organic matter and the  
71 loss of soil structure and fertility (FAO, 2004).

72

73 One of the most widespread conservation practices applied in olive-grove plantations has been  
74 the maintenance of spontaneous resident vegetation cover (hereafter "weeds") in the alleys from  
75 autumn to spring (Marquez-Garcia et al., 2013; Nieto et al., 2013). Weed covers, in addition to  
76 protecting the soil against erosion, offer a number of well-known benefits for soil properties:  
77 improvement of soil physicochemical properties (Ramos et al, 2010); increases in the  
78 interception and storage of rainfall water, as well as in soil water content and water availability  
79 in deep soil (Celano et al., 2011; Palese et al., 2014); increases in atmospheric C fixation and  
80 SOC content, thereby improving soil structure and fertility (Hernández et al., 2005; Castro et al.,  
81 2008; Gómez et al., 2009; Repullo-Ruibérriz de Torres et al., 2012; Marquez-Garcia et al.,  
82 2013; Soriano et al., 2014; Herencia, 2015); and increased biodiversity (Plaza-Bonilla et al.,

83 2015). In this regard, some estimates point to SOC increases between 44% and 85% in topsoil  
84 (0-15 cm) in olive groves after 100 years of cover crop management (Nieto et al., 2013), and  
85 preliminary estimations suggest an increase in soil C sequestration of around 1 ton C ha<sup>-1</sup> y<sup>-1</sup> in  
86 olive orchards under Mediterranean conditions due to the adoption of plant covers (Vicente-  
87 Vicente et al., 2016). Thus, agricultural systems can function as C sinks if adequate  
88 management practices are applied.

89

90 Although numerous studies have examined the effect of weed cover on soil properties and SOC  
91 content, little research has been focused on their effect on soil CO<sub>2</sub> fluxes or how they affect the  
92 ecosystem C balance in olive orchards. Indeed, few studies have reported information on CO<sub>2</sub>  
93 fluxes from olive groves or quantified the ecosystem C uptake accounting for total CO<sub>2</sub> inputs  
94 and outputs (see Testi et al., 2008; Nardino et al., 2013). So far, most CO<sub>2</sub> exchange  
95 measurements have been conducted at the tree (Villalobos et al., 2012; Pérez-Priego et al.,  
96 2010) and soil levels (Bertolla et al., 2014) by using chambers, and soil CO<sub>2</sub> emissions have  
97 been also estimated via modelling approaches (Nieto et al. 2010). In the absence of weed cover,  
98 net ecosystem CO<sub>2</sub> exchange (NEE) from olive groves will result from the balance between CO<sub>2</sub>  
99 inputs by tree photosynthesis and CO<sub>2</sub> outputs by aboveground autotrophic respiration (olive  
100 leaves, trunks and branches), belowground autotrophic respiration (olive roots) and  
101 heterotrophic soil respiration. However, in the presence of weeds, it is necessary to account for  
102 CO<sub>2</sub> uptake via weed photosynthesis and CO<sub>2</sub> emissions via weed and weed-covered soil  
103 respiration for quantification of NEE. Knowledge of how conservation *versus* traditional  
104 practices may affect the net CO<sub>2</sub> uptake in olive groves is lacking and this information is  
105 necessary to elucidate the role that these practices play in C sequestration and thus, their  
106 potential regarding climate change mitigation.

107

108 Non-destructive, ecosystem-scale and long-term measurements of NEE are possible thanks to  
109 the technological development of robust tools such as the eddy covariance (EC) technique  
110 (Dabberdt et al., 1993; Baldocchi, 2003). While this technique has been used to characterize

111 CO<sub>2</sub> and water vapour exchanges in natural (Baldocchi et al., 2001; Reichstein et al., 2007) as  
112 well as agricultural ecosystems under differing management (Baker and Griffis, 2005; Chi et al.,  
113 2016), its application to agricultural systems such as olive orchards is practically non-existent.  
114 Some reasons for the absence of information on these widespread crops in the Mediterranean  
115 region are: i) the steep slopes where these crops are usually located, which complicate the  
116 implementation of these micrometeorological techniques; and ii) the intensive management  
117 including irrigation, fertilization and pruning, which reduces stress for water, nutrients or light,  
118 and strongly modifies CO<sub>2</sub> exchanges compared to other Mediterranean ecosystems or rainfed  
119 crops (Testi et al., 2008; Nardino et al., 2013). Therefore, quantification of CO<sub>2</sub> exchange in  
120 olive groves at the ecosystem scale is necessary to understand how they contribute to the C  
121 balance and how different management practices can amplify or diminish their capacity to act as  
122 sinks of CO<sub>2</sub>. To our knowledge, only a few studies have measured NEE in olive groves at  
123 ecosystem scale using the eddy covariance technique (Testi et al., 2008; Nardino et al., 2013;  
124 López-Bernal et al., 2015). However, these studies were conducted either during short time  
125 periods or in young olive orchards, and none analyzed the influence of no-till practices such as  
126 maintenance of plant cover on the ecosystem C uptake.

127

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

134

## 135 **2. Materials and methods**

136

### 137 *2.1 Study site*

138

139 This research has been conducted in “Cortijo Guadiana” (37°54'39.30"N, 3°13'42.40"W), an  
140 irrigated olive (*Olea europaea* L. ‘Arbequina’) orchard in Úbeda (Jaén, Spain), which belongs to  
141 the oil group “Castillo de Canena, S.L.” (Fig. 1). The site is situated at 370 m above sea level.  
142 The climate is Mediterranean, with a mean annual temperature of 16°C, a mean annual  
143 precipitation of 495 mm, and a mean annual potential evapotranspiration (calculated using the  
144 Penman-Monteith equation) of 1220 mm (from 15-year records at the Agroclimatic Station of  
145 Úbeda, Junta de Andalucía,  
146 <http://www.juntadeandalucia.es/agriculturaypesca/ifapa/ria/servlet/FrontController>).  
147 Predominant winds come from the northwest during the day and from the south and southeast at  
148 night. The farmland has a total extension of 1500 ha, but our experiment was developed in a flat  
149 area, where two homogeneous plots were delimited of 29.3 ha (weed cover) and 20.2 ha (weed  
150 free). Soil organic matter content is 2.9% from 0 to 5 cm and 2.4% from 5 to 15 cm. Soil texture  
151 is clay loam, with 24% sand, 32% silt and 44% clay. Trees are irrigated by drip 3 times a week  
152 from February to October, at a rate of 32 L h<sup>-1</sup> per tree for 8 hours (at night). Within irrigation,  
153 40g of NPK fertilizer per tree is applied together with water (0.156g NPK L<sup>-1</sup> water, every  
154 irrigation night). The olive trees are 80 years old with a 7x7m spacing between them (204 trees  
155 ha<sup>-1</sup>) and tree height is approximately 4 m. The Plant Area Index (PAI) of trees was determined  
156 from the indirect measurement of the gap fraction using upward hemispheric images taken with  
157 a 4.5mm F2.8 EX DC HSM circular fisheye lens (Sigma Corporation of America). Images were  
158 processed with the software CAN-EYE v6.1 (INRA-CSE, Avignon). PAI of the trees (corrected  
159 by clumping effect) was 8.13 ± 0.83 m<sup>2</sup> vegetation/m<sup>2</sup> ground surface.  
160 In the two areas selected in the olive orchard (Fig. 1), two treatments were applied: 1) weed-free  
161 treatment, in which a glyphosate-based herbicide was applied to avoid spontaneous weed  
162 growth (September 2014), and 2) weed-cover treatment, which is the management commonly  
163 applied in the orchard and consists of maintenance of spontaneous weed cover in the alleys from  
164 autumn to spring. In spring (29-30 April), weeds were mechanically whacked and left on the  
165 surface to avoid excessive water consumption and competition for water with trees.

166

## 167 2.2 Eddy covariance and meteorological and soil measurements

168

169 During the hydrological year 2014 (October)-2015 (September), fluxes of CO<sub>2</sub> and latent (LE)  
170 and sensible (H) heat have been determined from fast-response (10 Hz) instruments mounted  
171 atop 10 m-towers, one in each treatment (Fig. 1). The towers were placed in the center of each  
172 treatment and separated by about 500 m to avoid interference from one treatment to another.  
173 Wind vector components and sonic temperature were measured by three-axis sonic  
174 anemometers (CSAT-3, Campbell Scientific, Logan, UT, USA; hereafter CSI), while densities  
175 of CO<sub>2</sub> and H<sub>2</sub>O, together with temperature and pressure, were measured by enclosed path  
176 infrared gas analyzers (IRGA, Li-Cor 7200; Lincoln, NE, USA). The stainless steel intake tubes  
177 are 1 m in length and have outside diameters of 6.35 mm. Flow rates are 15 L min<sup>-1</sup> and pass  
178 through 2 µm filters that reduce dust entering the gas analyzer optical cell. Calibrations of the  
179 IRGAs were done every six months using an ultra-high purity N<sub>2</sub> zero gas, and a 500 ppm  
180 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  
181 from the IRGAs measurements), wind vector components and sonic temperatures were  
182 registered in LI-7550 Analyzer Interface Units.

183

184 At each treatment, additional instrumentation measures environmental and soil states. Air  
185 temperature and humidity were measured at 6 m by a thermo-hygrometer (HC2S3, Rotronic  
186 AG, Bassersdorf, Switzerland), from which vapor pressure deficit (VPDs) was calculated.  
187 Incoming and outgoing short-wave and long-wave radiation components were measured by a 4-  
188 component radiometer (CNR-4, Kipp & Zonen, Delft, Netherlands), installed at 2 m from the  
189 mast at a height of 7 m, allowing the determination of net radiation (R<sub>n</sub>) and albedo. Incident  
190 and reflected photosynthetic photon flux densities (PPFDs) were measured using photodiodes at  
191 7 m (Li-190, Li-Cor, Lincoln, NE, USA). To monitor the temporal evolution of soil moisture,  
192 soil water content (SWC) was measured in an alley of each treatment using two soil moisture  
193 probes installed at 0.10 m depth (CS616, CSI). On each treatment, two thermocouples (TCAV,  
194 CSI) measured soil temperatures at 0.04 m soil depth and two heat flux plates (HFP01,

195 Hukseflux, Delft, the Netherlands) were inserted at 0.08 m. Environmental and soil  
196 measurements were stored as 30 min averages by a datalogger (CR3000, CSI). Finally,  
197 precipitation data were obtained from the Úbeda Agroclimatic Station of the Junta de Andalucía  
198 (<http://www.juntadeandalucia.es/agriculturaypesca/ifapa/ria/servlet/FrontController>) located at 7  
199 km from our study site.

200

### 201 *2.3 Data processing and statistical analysis*

202

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

212

213 Due to high power requirements by the air pump, the system suffered frequent energy losses  
214 that caused data gaps, mainly during nighttime. During May and September, continuous data  
215 losses were found from 4 am to 7 am in the weed-free treatment due to energy loss. Nighttime  
216 fluxes measured during weak turbulence were rejected by filtering with a friction velocity ( $u_*$ )  
217 below 0.15 m s<sup>-1</sup> (Reichstein et al., 2005). In addition, data quality check of the half-hourly  
218 NEE, H<sub>2</sub>O flux and sensible heat flux (H) was applied by filtering according to the following  
219 parameters: 1) For CO<sub>2</sub>: i) quality of data= 0 or 1 (Mauder and Foken, 2004); ii) CO<sub>2</sub>  
220 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)  
221 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)  
222 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°;



223 iv)  $-4 < \text{skewness} < 4$ ; v)  $\text{Kurtosis} < 9$ . Data gaps due to environmental conditions, instrument  
224 malfunction and nighttime low turbulence led to a data coverage of 41% in the weed-cover  
225 treatment (69% during daytime and 22% during nighttime), and 38% in the weed-free treatment  
226 (62% during daytime and 22% during nighttime). Data losses are frequent in eddy covariance  
227 studies (data gap average 35%, see Falge et al., 2001). Despite high data gaps in our site, most  
228 data losses occurred during night when GPP is absent and Reco is generally low, and low  
229 friction velocities ( $u_* < 0.15$ ) lead to not valid  $\text{CO}_2$  fluxes during many nighttime periods. Thus,  
230 frequent data losses during nighttime at our site likely had little influence on monthly and  
231 annual  $\text{CO}_2$  budgets. Data gaps were filled using the marginal distribution sampling technique  
232 described by Reichstein et al. (2005). This technique also calculates uncertainties for actual  
233 measurements by simulating gaps and applying the gap-filling procedure. Twice the standard  
234 deviation of sums of the 30-min uncertainties derived from the gap-filling procedure was  
235 considered as our NEE error for the different time periods we analyzed (monthly and annual  
236 NEE). Positive values of NEE indicate net  $\text{CO}_2$  release to the atmosphere, while negative values  
237 represent net  $\text{CO}_2$  uptake. Half-hour NEE values were integrated to obtain C exchange ( $\text{g C m}^{-2}$ )  
238 at daily, monthly and annual scales.

239

240 An estimation of the flux footprint during daytime and nighttime periods was determined using  
241 the method described by Kljun et al. (2004) to verify that fluxes originated from well within the  
242 fetch (higher than 200 m from the tower). Daytime periods were defined when net radiation was  
243 higher than  $10 \text{ W m}^{-2}$ .

244

245 Flux partitioning into Gross Primary Production (GPP) and Ecosystem Respiration (Reco) was  
246 performed according to the method by Reichstein et al. (2005) and Lasslop et al. (2010).  
247 However, unexpected seasonal behavior and unreliable estimations of annual GPP and Reco  
248 were obtained for both treatments, suggesting these partitioning methods are not suitable for  
249 application at our site. For this reason, the light-response curves were used to model GPP and  
250 Reco. The rectangular hyperbolic light-response function (Falge et al., 2001) was applied to

251 monthly averages of 30-min daytime data, and monthly parameterization coefficients were  
252 obtained according to the equation:

$$253 \quad NEE = \frac{-b_1 * PPF D}{b_2 + PPF D} + b_3 \quad (\text{Equation 1})$$

254  
255 where PPF D is the incident photosynthetic photon flux density, the coefficient  $b_1$  is the  
256 maximum gross primary production ( $GPP_{max}$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ );  $b_2$  represents the level of  
257 PPF D for which GPP is half of  $GPP_{max}$  ( $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ); and the parameter  $b_3$  represents  
258 the ecosystem respiration ( $Reco$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). For determination of the parameterization  
259 coefficients, only measured data (quality = 0 or 1) until noon was considered in order to account  
260 for the effect of PPF D on NEE and avoid the effect of high VPD on NEE. In order to fit the data  
261 to the light-response model described in equation 1, we firstly generate the initial values  
262 (uniform distribution) of the model coefficients randomly by delimiting realistic ranges for  
263 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  
264  $b_3$ . These initial values are necessary to start the fitting procedure, which consists of calculating  
265 the nonlinear (weighted) least-squares estimates of the parameters of the non-linear model (Eq.  
266 1). We used R software version 3.1.3 (R Development Core Team, 2015) to perform this  
267 analysis. Coefficients were considered significant when  $p < 0.05$ .

268

269 To assess the accuracy of the eddy covariance measurements, we analyzed linear regressions  
270 between the sum of latent heat (LE) and sensible heat (H) *versus* net radiation (Rn) minus soil  
271 heat flux (G, calculated as the sum of the soil heat flux at 0.08 m and the heat storage term (Q)  
272 in the 0–0.08 m soil depth):  $Rn - G = LE + H$ . We determined the energy balance closure using  
273 30-min time series of Rn, H, LE and G for the period between April and June. This period was  
274 selected in order to account for the period of maximum weed growth and the later period when  
275 weeds were cut, and also because there were simultaneous measurements of soil heat flux, soil  
276 temperature, and soil water content at both treatments.

277

278 *Soil CO<sub>2</sub> efflux measurements*

279

280 In addition to the eddy covariance measurements, soil CO<sub>2</sub> effluxes were measured in  
281 cylindrical PVC collars (10 cm diameter x 5 cm height) inserted into the soil in the alleys of the  
282 two treatments. Five collars were inserted per treatment and the soil CO<sub>2</sub> efflux was measured at  
283 midday, once a month from March to July, with a manual chamber system model EGM-4/SRC-  
284 1 (PP-Systems, Hitchin, UK). Each collar was measured three times and the average was used  
285 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  
286 fraction (referenced to dry air) measured every 5 seconds during 120 seconds after chamber  
287 closure and was corrected for atmospheric pressure and the chamber air temperature. Significant  
288 differences (P<0.05) in soil CO<sub>2</sub> efflux between the two treatments (weed-covered soil and  
289 weed-free soil) were analyzed using a one-way ANOVA. Analyses were conducted using R  
290 software version 3.1.3 (R Development Core Team, 2015).

291

292 *Weed biomass and weed organic carbon determination*

293

294 Weed sampling was conducted at the beginning of April (before weed whacking) in order to  
295 quantify aboveground weed biomass and the organic C input contributed by weed biomass. Five  
296 square plots of 0.5 m x 0.5 m (0.25 m<sup>2</sup>) were selected and weeds were cut and harvested for  
297 determination of dry weight. Organic C released by weeds was determined using the Walkley  
298 and Black method modified by Mingorance et al. (2007). Samples of 30 mg of plant material  
299 were weighed and 5 mL of potassium dichromate and 7.5 mL of sulfuric acid were added. After  
300 digestion at 155°C for 30 minutes, 10 mL of distilled water was added and absorbance was  
301 measured at 600 nm in a spectrophotometer. The organic C content was determined from the  
302 calibration curve built with increasing concentrations of glucose.

303

304 *2.4 Crop productivity quantification*

305

306 Olive harvesting was carried out in December 2015. Wooden sticks and a trunk-shaker machine  
307 were used to dislodge olives from 14 trees selected randomly at each treatment. Olives were  
308 collected on nets placed on the ground and then weighed. Samples of olives were transported to  
309 the laboratory and dried in an oven at 60°C in order to determine the dry weight. From this  
310 value, we calculated the average crop productivity for each treatment, expressed as kilograms of  
311 olives per tree, as well as the C export by olive yield in  $\text{g C m}^{-2}$  by using the relation: 1 g dry  
312 matter=0.4782 g of C (Palese et al., 2013).

313

### 314 **3. Results**

315

#### 316 *3.1 Meteorological conditions and soil variables*

317

318 Meteorological conditions and evolution of soil variables in the two treatments during the study  
319 year are shown in Fig. 2. Annual rainfall during the study year was 381 mm, mainly  
320 concentrated from November to April, and lower than the climatological average for this site  
321 (495 mm; Fig. 2a). The mean annual temperature was 17°C, and the maximum and minimum  
322 average daily temperatures were 32.4°C (in July) and 0.4°C (in December) (Fig. 2b). The  
323 maximum and minimum averaged daily values of VPD were 42.5 hPa and 0.4 hPa, recorded at  
324 the end of June and in December, respectively. PPFD was the highest during the dry season.  
325 Maximum averaged daily PPFD was 888  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  in June and minimum daily value  
326 was 30  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  in December (Fig. 2c). There were large differences in alley SWC  
327 between the soils with and without weed covers (Fig. 2a) (standard deviation of SWC at each  
328 treatment was very low, with average values for the whole period of 0.03 and maximum and  
329 minimum values of 0.05 and 0.004, respectively). During wet periods, SWC was up to 0.2  $\text{m}^3 \text{m}^{-3}$   
330 higher in the weed-cover than in the weed-free soil. However, during the dry soil period, soil  
331 moisture was similar for both soils. There were also marked differences in soil temperature  
332 between treatments (Fig. 2b). From October to March, soil temperature was similar at both  
333 treatments and strongly coupled with air temperature. However, during spring (April, May and

334 June) and summer (July, August, September) months, the temperature was higher in the soil  
335 with no weeds, reaching daily averages up to 13°C above air temperature and 12°C above the  
336 weed-cover soil temperature.

337

### 338 *3.2 Validity of eddy measurements: flux footprint analysis and energy balance closure*

339

340 The footprint analysis showed that upwind distances contributing to the measured CO<sub>2</sub> flux  
341 were in all cases within the fetch for each treatment. The median of the x<sub>90%</sub> (distance from  
342 anemometer delimiting 90% of the flux) during the studied period was 164 m in the weed-cover  
343 treatment and 172 m in the weed-free treatment at night, and much less during daytime.

344 Regarding the energy balance closure, results were similar at both treatments. The closure  
345 deficit was 27% in the weed-cover treatment and 29% in the weed-free treatment, with R<sup>2</sup> of  
346 0.90 and 0.87, respectively. The energy balance closure improved at both treatments when only  
347 the drier period from May to June was considered, with closure deficits of 26% and 23% and R<sup>2</sup>  
348 of 0.91 and 0.90 at the weed-cover and weed-free treatments, respectively.

349

### 350 *3.3 Temporal variability of NEE between treatments*

351

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

359

360 Thus, despite irrigation, some controlling effects of VPD were found in diurnal trends of NEE.

361 Fig. 4 shows monthly diurnal trends of PPFD, VPD and NEE at both treatments during the

362 growth period in March and the hot dry period in August. During the growth period and under  
363 low water stress (maximum monthly diurnal VPD was 16 hPa), NEE was strongly coupled with  
364 light intensity, and maximum net CO<sub>2</sub> uptake coincided with maximum light intensity  
365 (maximum monthly diurnal PPFD was 1280 μmol photons m<sup>-2</sup> s<sup>-1</sup>; Fig. 4a and Fig. 4c). By  
366 contrast these variables showed lags during periods of high water stress (maximum monthly  
367 diurnal VPD in August was 41 hPa), when net CO<sub>2</sub> uptake peaked several hours before the time  
368 of maximum light intensity (maximum monthly diurnal PPFD was 1630 μmol photons m<sup>-2</sup> s<sup>-1</sup>;  
369 Fig. 4b and Fig. 4d). This net CO<sub>2</sub> uptake peak usually occurred before the time of maximum  
370 VPD in both periods (low and high water stress), but the delay between both was greater during  
371 periods of high water stress (Fig. 4b and Fig. 4d). It can be also seen that during the growth  
372 period, net CO<sub>2</sub> uptake was much higher in the weed-cover (maximum monthly diurnal net CO<sub>2</sub>  
373 uptake was -9.6 μmol m<sup>-2</sup> s<sup>-1</sup>) than in the weed-free treatment (maximum monthly diurnal net  
374 CO<sub>2</sub> uptake was -4.7 μmol m<sup>-2</sup> s<sup>-1</sup>), but both showed similar NEE during August when weeds  
375 had been already cut (maximum monthly diurnal net CO<sub>2</sub> uptake was -3.5 and -4.5 μmol m<sup>-2</sup> s<sup>-1</sup>,  
376 respectively; Fig.4c and Fig. 4d).

377

378 Throughout the year, important differences were observed in monthly NEE between the two  
379 treatments. Results show that NEE was similar in the two treatments in the initial conditions  
380 (October), when there were no weeds in either of the two treatments (Fig. 3). Both treatments  
381 showed positive values during this month, indicating a net CO<sub>2</sub> emission to the atmosphere  
382 (Table 1). For this period, daily NEE values ranged from -0.69 to 1.26 g C m<sup>-2</sup> and from -1.07 to  
383 1.07 g C m<sup>-2</sup> in the weed-cover and weed-free treatment, with daily averages of 0.12 and 0.15 g  
384 C m<sup>-2</sup>, respectively.

385

386 From November to April, negative monthly values of NEE were found at both treatments,  
387 indicating net CO<sub>2</sub> uptake (Table 1). However, as weeds grew, C uptake was much higher in the  
388 weed-cover than the weed-free treatment (Fig. 3), with the maximum difference in March.  
389 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

390 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  
391 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  
392 g C m<sup>-2</sup>, with an average value of -0.91 g C m<sup>-2</sup>.

393

394 In April, weeds reached their maximum size. Average aboveground weed biomass was 220 ± 58  
395 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  
396 CO<sub>2</sub> uptake was still higher in the weed-cover treatment, NEE values became more similar at  
397 both treatments (Fig. 3). Daily NEE ranged from -2.92 to 1.20 g C m<sup>-2</sup> in the weed-cover  
398 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>  
399 in the weed-free treatment, with an average of -0.65 g C m<sup>-2</sup>. This period coincided with the  
400 highest soil CO<sub>2</sub> efflux recorded in the soil covered by weeds at midday (Fig. 5). From March to  
401 May, the soil CO<sub>2</sub> efflux was significantly higher in the weed-covered than weed-free soil.  
402 However, differences were especially marked in April, when the soil CO<sub>2</sub> efflux in the weed-  
403 covered soil was up to 5.6 times higher than in the weed-free soil.

404

405 At the end of April, weeds were cut and left on the soil to allow weed residues (hereafter, “hay”)  
406 to decompose and incorporate into the soil. From May to June, contrary to the pattern observed  
407 during the weed growth period, net CO<sub>2</sub> assimilation in the hay-free treatment surpassed that  
408 observed in the hay-cover treatment (Fig. 3). Negative monthly values of NEE (net CO<sub>2</sub> uptake)  
409 were obtained for the hay-free treatment, whereas the hay-cover showed positive values (net  
410 CO<sub>2</sub> emission to the atmosphere; Table 1). Daily average NEE in the hay-cover and hay-free  
411 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  
412 June.

413

414 During the summer months (July to September), both treatments showed similar and positive  
415 monthly values of NEE (Table 1). Average daily values in July, August and September were  
416 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  
417 hay-free treatment. Soil respiration rates measured during the dry season (June and July) were

418 low (Fig. 5) and soils both with and without hay showed similar respiration rates (average soil  
419 respiration rate was  $0.87 \pm 0.17 \mu\text{mol m}^{-2} \text{s}^{-1}$  in the soil covered by hay, and  $0.78 \pm 0.02 \mu\text{mol}$   
420  $\text{m}^{-2} \text{s}^{-1}$  in the bare soil).

421

#### 422 *3.4 Functional relationships between environmental variables and NEE*

423

424 The light-response curves showed a significant relationship between NEE and PPFD during  
425 winter and early spring (Table 2), whereas no significant relationship was found during the dry  
426 period. Consequently, significant parameterized coefficients of monthly  $\text{GPP}_{\text{max}}$  and  $\text{R}_{\text{eco}}$  were  
427 generally obtained from the light-response equation for both treatments during winter and early  
428 spring (Table 2), but no significant values were obtained for either of them during summer.  
429 Modeled values of  $\text{R}_{\text{eco}}$  were higher in the weed-cover than in the weed-free treatment (with the  
430 exception of February, where modeled  $\text{R}_{\text{eco}}$  was unexpectedly higher in the latter), and modeled  
431  $\text{GPP}_{\text{max}}$  was up to 4.3 times higher in the weed-cover than in the weed-free treatment (maximum  
432 modeled  $\text{GPP}_{\text{max}}$  was  $-28.30$  and  $-15.15 \mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively). Also, a better relationship  
433 between NEE and PPFD was found during the weed growth period (from December to March)  
434 in the weed-cover treatment compared to the weed-free treatment. Concretely, in March when  
435  $\text{GPP}_{\text{max}}$  was highest in the weed-cover treatment, a better fit was found in this treatment  
436 ( $R^2=0.98$ ) compared to the weed-free treatment ( $R^2=0.71$ ). For the same PPFD level ( $1216 \mu\text{mol}$   
437  $\text{photons m}^{-2} \text{s}^{-1}$  at noon), maximum net  $\text{CO}_2$  assimilation was double in the weed-cover than of  
438 the weed-free treatment (Fig. 6).

439 Nevertheless, a worse fit was observed for the weed-cover treatment during April and May,  
440 when weeds were cut and net  $\text{CO}_2$  fixation decreased in the hay-cover treatment. During these  
441 months, significant values of  $\text{GPP}_{\text{max}}$  were obtained in the hay-free treatment, but no significant  
442 values of  $\text{GPP}_{\text{max}}$  or  $\text{R}_{\text{eco}}$  were found in the hay-cover treatment.

443

444 Contrary to expectations, no significant relationship was found between nighttime NEE and  
445 temperature either at daily or monthly scales. Nighttime NEE was low at both treatments during



446 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-  
447 free treatments, respectively. Nonetheless, we could observe some seasonal trends in nighttime  
448 NEE related to temperature for both treatments. Nighttime NEE was low from December to  
449 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-  
450 free treatments, respectively), coinciding with periods of low air temperature (average nighttime  
451 temperature was 5.4°C, and ranged from 2.5°C in January to 9.0°C in March), while higher  
452 values of nighttime NEE were found from April to November (average nighttime NEE was 1.01  
453 and 1.11 g C m<sup>-2</sup> night<sup>-1</sup> in the weed-cover and weed-free treatments, respectively), coinciding  
454 with periods of higher air temperatures (average nighttime temperature was 18.5°C, and ranged  
455 from 11.2°C in November to 24.8 °C in July).

456

### 457 *3.5 Annual NEE and olive productivity between treatments*

458

459 Although higher net CO<sub>2</sub> emissions were found during late spring in the weed-cover treatment  
460 compared to the weed-free treatment, a positive effect of weed cover was found in annual net  
461 ecosystem exchange. The cumulative NEE values during the study year (Fig. 7) resulted in an  
462 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  
463 treatment. Thus, although the weed-free treatment acted as a net C sink during longer period  
464 (from November to June) than the weed-cover treatment (from November to April), the higher  
465 assimilation rate in the former during the weed growth period was able to offset the higher  
466 emissions found in this treatment during late spring. As a result, annual net ecosystem C uptake  
467 was reduced by 50% in the weed-free treatment. Despite frequent data gaps during the study  
468 year, uncertainty in the estimation of annual carbon budgets for both treatments was low (14%),  
469 making differences between treatments noteworthy.

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

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

476

#### 477 **4. Discussion**

478

479 Large differences in NEE were observed in the olive orchard under the two treatments.  
480 Although plant covers are able to enhance soil respiration by increasing SOC content and  
481 microbial activity, alternatively, they can increase C fixation through their photosynthetic  
482 activity. Hence, we found that the maintenance of spontaneous weeds from autumn to early  
483 spring strongly increased net C fixation compared to the weed-free treatment (Fig. 3). In March,  
484 when net C uptake in the olive orchard under both managements was the highest, the treatment  
485 with weed cover showed up to 2.7 times higher monthly NEE than the treatment without weed  
486 covers, with values of  $-74.43$  and  $-28.09 \text{ g C m}^{-2} \text{ month}^{-1}$ , respectively (Table 1). Assuming that  
487 the difference between these values represents the net C uptake by weeds, the resulting value is  
488  $46.34 \text{ g C m}^{-2} \text{ month}^{-1}$ , which is 1.7 times higher than the net C uptake by olive trees in the  
489 weed-free treatment ( $-28.09 \text{ g C m}^{-2} \text{ month}^{-1}$ ). Coinciding with this, Palese et al. (2013) reported  
490 that spontaneous vegetation (weeds and grasses) was the most important crop component for C  
491 fixation in an irrigated olive orchard in southern Italy, contributing to 35% of total  $\text{CO}_2$  fixation  
492 (the rest being pruning material and yield). This high C assimilation by weeds can be explained  
493 by their short lifetime and the need for higher efficiency in  $\text{CO}_2$  uptake to invest in biomass  
494 growth, before the beginning of the senescence dry period. In addition, weed species use  
495 different water- and light-use strategies than olive trees, which can explain differences in NEE  
496 trends between treatments. Under increasing PPFD, olive trees can limit their photosynthetic  
497 activity by closing stomata in response to increased water stress (Testi et al., 2008; Villalobos et  
498 al., 2012). In contrast, weeds are able to maintain their photosynthetic activity under high light  
499 intensities, despite relatively high air VPD (Long and Hällgren, 1993; Pérez-Priego et al., 2015).  
500 This behaviour was reflected in the better relationship found between NEE and PPFD in the  
501 weed-cover than the weed-free treatment (Fig. 6).

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

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

530 enrichment (Guzmán et al., 2014). In this study, aboveground weed biomass represented 79.4 g  
531 OC m<sup>-2</sup>, which is comparable to the values reported by other authors who have found organic C  
532 inputs by spontaneous vegetation between 46.2 and 50.9 g OC m<sup>-2</sup> during years of normal  
533 precipitation regime (Repullo-Ruibérriz de Torres et al., 2012). The C fixed by weeds is partly  
534 respired back to the atmosphere by decomposition of more labile C compounds, and partly  
535 remains in the soil and is incorporated as resistant organic matter in the uppermost layer of the  
536 soil, contributing to increasing SOC (Hernández et al., 2005; Castro et al., 2008; Soriano et al.,  
537 2014).

538

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

555

556 After weeds were cut and the hay left on the soil (May and June), opposite to the pattern  
557 observed during the growth period, net C uptake was higher in the hay-free treatment than in the

558 hay-cover treatment (Table 1). This lower net C uptake in the hay-cover treatment during late  
559 spring can be attributed to the higher respiration promoted by the hay. First, hay decomposition  
560 favors the formation of stable microaggregates that are enriched in organic C, enhancing  
561 earthworm and soil microfauna activity, which in turn affects respiration and the soil C pool  
562 (Pulleman et al., 2005; Plaza-Bonilla et al., 2005). Second, hay also increases the amount of  
563 labile C which is readily used for respiration by soil microorganisms. Third, although  
564 respiration usually increases with soil temperature, very high temperatures can constrain  
565 respiration by exceeding the optimum temperature for some microorganism activity (O'Connell,  
566 1990). The high temperatures registered in the hay-free soil during the dry season (Fig. 2) could  
567 be responsible for lower respiration in these soils relative the hay-covered soil. Fourth,  
568 photodegradation of the hay can also contribute to enhancing CO<sub>2</sub> emissions (Brandt et al.,  
569 2009; Rutledge et al., 2010). Although soil chamber measurements support this higher CO<sub>2</sub>  
570 efflux in the hay-covered soil during May, we found no significant differences in soil CO<sub>2</sub>  
571 efflux between soils with or without hay in June, when higher net ecosystem CO<sub>2</sub> uptake was  
572 still observed in the site without hay. As soil CO<sub>2</sub> efflux was measured at midday, it is possible  
573 that high soil temperatures limited respiratory activity in both soils during this time. Further  
574 research on diurnal trends of soil CO<sub>2</sub> efflux under the two soil managements will help to  
575 elucidate the role of weed covers in CO<sub>2</sub> emissions and their relative contribution to ecosystem  
576 NEE.

577

578 Contrary to published studies that have reported net C uptake during summer and throughout  
579 the year in irrigated olive orchards under climate conditions similar to ours (Testi et al., 2008;  
580 Nardino et al., 2013), we found monthly net CO<sub>2</sub> release in the olive orchard under the two  
581 managements during the summer period (from July to September; Table 1). The high  
582 evaporative demand recorded during this year (maximum daily air temperature during July and  
583 August on average was 36.9°C and maximum daily VPD on average was 52.8 hPa) could  
584 constrain tree photosynthesis, making respiratory processes the main contributors to the  
585 ecosystem CO<sub>2</sub> flux. In this regard, although soil respiration in the alleys of our two treatments

586 was low during this period (around  $0.9 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), in accordance with other studies  
587 (between  $1.1$  and  $1.6 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ , see Testi et al., 2008), due to low soil moisture content,  
588 high respiration rates might be expected in the drip-irrigated zones where water availability was  
589 not limited. For instance, Testi et al. (2008) reported respiration rates were up to  $5.7 \mu\text{mol CO}_2$   
590  $\text{m}^{-2} \text{ s}^{-1}$  in irrigated olive groves beneath the tree canopy during summer. Thus, in the irrigated  
591 zones,  $\text{CO}_2$  efflux from both soil and aboveground respiration from olive trees are expected to  
592 significantly contribute to NEE. Leaves and fruits appear to be the main contributors to  
593 aboveground respiration in olive trees, while respiration of wood biomass (trunk and branches)  
594 represent a very small fraction of  $\text{CO}_2$  flux (Pérez-Priego et al., 2014). Thus, the positive values  
595 of NEE found in both treatments during summer may be due to leaf, fruit, and soil respiration,  
596 the latter originating under the tree canopy.

597

598 Nighttime NEE values at our site were higher than those reported by Testi et al. (2008) for  
599 winter periods ( $0.7$  versus  $1.4 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ , on average, in our site), but lower than those  
600 reported by the mentioned authors during non-winter periods ( $4.8 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  versus  $2.2$   
601  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ , on average, in our site). Similar to the results of these authors, we found no  
602 clear relationship between nighttime NEE and soil or air temperature. This is probably due to a  
603 combination of different causes: i) copious missing data during nighttime periods due to battery  
604 malfunctioning, and predominance of stable conditions and low friction velocities ( $u_* < 0.15 \text{ m s}^{-1}$ );  
605 ii) influence of soil moisture on ecosystem respiration, since water-limited ecosystems are  
606 moisture-pulse dependent (Chen et al., 2009; López-Ballesteros et al., 2016). Thus, while in  
607 mid-high latitudes temperature is a key driver for  $\text{CO}_2$  fluxes, its relative importance could  
608 decrease in semiarid environments where water is the most important driver for vegetation  
609 productivity; and iii) strong seasonality of weed and olive tree activity, which could mask the  
610 effect of soil temperature on nighttime (as well as daytime) NEE. In support of this, Pérez-  
611 Priego et al. (2014) reported a good relationship between aboveground respiration in olive trees  
612 and both temperature and phenological stage (i.e. periods of dormancy, flowering and fruit

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

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

623

624 In general, NEE values recorded in this study were lower than those reported in other irrigated  
625 olive orchards under similar soil (clay/clay loam soils) and climate conditions (precipitation  
626 regime). While we found NEE values up to -0.7 g C m<sup>-2</sup> day<sup>-1</sup> during summer, Testi et al.  
627 (2008) reported NEE values in a young olive orchard (LAI of the trees was 1.9 m<sup>2</sup> m<sup>-2</sup>) in  
628 Southern Spain of -2.7 g C m<sup>-2</sup> day<sup>-1</sup> during this period. Maximum daytime NEE in our site was  
629 -4 g C m<sup>-2</sup> day<sup>-1</sup>, with an average value of -2.5 g C m<sup>-2</sup> day<sup>-1</sup> during the period of maximum CO<sub>2</sub>  
630 assimilation (March) and -0.8 g C m<sup>-2</sup> day<sup>-1</sup> during summer. In contrast, López-Bernal et al.  
631 (2015) reported an average daytime NEE of -4.5 g C m<sup>-2</sup> day<sup>-1</sup> in an irrigated olive orchard  
632 (LAI=1.5 m<sup>2</sup> m<sup>-2</sup>) in southern Spain in the period from late June to late September. In an olive  
633 orchard (LAI~3 m<sup>2</sup> m<sup>-2</sup>) in southern Italy, Nardino et al. (2013) reported maximum monthly  
634 NEE values of -170 g C m<sup>-2</sup> month<sup>-1</sup>, while we found a maximum monthly value of -74.4 g C m<sup>-2</sup>  
635 month<sup>-1</sup> (Table 1). Differences in NEE between our study site and the results reported in other  
636 irrigated olive orchards can be attributed to the different age and density of the olive trees, and  
637 the inherent inter-annual variability of semiarid ecosystems, among other factors. Contrary to  
638 the young age of the olive orchards reported in the previously cited studies, our study was  
639 conducted in a mature olive orchard (80 years old trees), where growth of trees is limited and  
640 increase of tree biomass is low compared to the rapid growth that can be expected in young

641 olive plantations (5-6 years old). Plant density was also lower in our study (~200 trees ha<sup>-1</sup>) than  
642 in the mentioned studies (~400 trees ha<sup>-1</sup>). The annual NEE in our olive orchard was also lower  
643 than that reported for young olive orchards with plant cover management. While we found  
644 annual C uptake of 140 g C m<sup>-2</sup> y<sup>-1</sup> in the weed-cover treatment (Fig. 7), values from 1160 g C  
645 m<sup>-2</sup> y<sup>-1</sup> to 1345 g C m<sup>-2</sup> y<sup>-1</sup> have been reported in 12–16 year old olive orchards (Nardino et al.,  
646 2013). In a 50 year old olive orchard on sandy loam soils in Southern Italy and taking into  
647 account C inputs by cover crop residues, pruning material, senescent leaves, yield and root  
648 biomass of olive trees, Palese et al. (2013) estimated an annual NEE of -1550 and 1020 g CO<sub>2</sub>  
649 m<sup>-2</sup> y<sup>-1</sup> (4200 and 2800 g C m<sup>-2</sup> y<sup>-1</sup>) under sustainable (irrigation with urban wastewater treated  
650 and conservation of spontaneous weeds and grasses) and conventional management (rainfed  
651 conditions, intensive tillage and pruning), respectively. Our results were similar to those  
652 reported by Brillì et al. (2016), who found an annual NEE ranging from -137 to -667 g C m<sup>-2</sup> y<sup>-1</sup>  
653 (average of 3 years, 364 g C m<sup>-2</sup> y<sup>-1</sup>) in a rain-fed olive orchard in central Italy with surface  
654 tillage management. Unfortunately, the lack of literature reporting direct measurements on C  
655 uptake in olive orchards under similar conditions to ours (crop characteristic, soil management)  
656 makes comparisons difficult.

657

658 Some studies have reported a reduction of crop productivity in olive orchards with plant covers  
659 due to competition for water and nutrient resources with trees (Gucci et al., 2012; Ferreira et al.,  
660 2013). In contrast, other authors have found a positive effect of weeds on crop productivity.  
661 Palese et al. (2013) found that olive yield was, on average, 2.3 times higher in an olive orchard  
662 with eight years of sustainable management where no tillage was applied and spontaneous  
663 vegetation cover was allowed to grow compared to an olive orchard under conventional  
664 management. According to our results, during the first year of differential treatment, the olive  
665 yield was 22% higher in the weed-cover than in the weed-free treatment, suggesting that weeds,  
666 rather than having a negative effect on crop productivity, appeared to have a positive effect on  
667 olive yield. However, this result should be considered with caution due to the few samples  
668 considered for olive yield determination in the current study (N=14 trees per treatment) and the



669 high variability characterizing fruit productivity in olive trees, both spatial and interannual.  
670 Thus, a long-term study is necessary to identify trends in olive productivity associated with soil  
671 management. The negative effect of weeds on crop productivity reported by the previously  
672 mentioned studies might be due to the fact that research was conducted on rain-fed olive  
673 orchards or in orchards with very high tree densities, where limiting water and nutrient  
674 availabilities likely increased competition for soil resources between plant covers and trees.  
675 Nonetheless, all of these studies reported improvements in soil fertility with the presence of  
676 herbaceous plants.

677

678 Although the weed-cover treatment acted as a C source during a longer period than the weed-  
679 free treatment, the higher net C uptake found in the former during the growing period due to the  
680 presence of weed cover, resulted in significantly higher C uptake on the annual basis. Weed  
681 cover increased the magnitude of NEE by 100% with respect to the treatment without weed  
682 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  
683 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  
684 findings emphasize the important role of weed covers in increasing C uptake in olive orchards.  
685 Although fossil fuel use is the main source of greenhouse gases in fruit tree orchards (Aguilera  
686 et al., 2015), the reduction of  $\text{CO}_2$  emissions by application of conservation practices based on  
687 plant covers is not negligible and should be considered when assessing the C footprint in crop  
688 systems under sustainable management. Table 3 shows a rough estimation of the annual C  
689 budget by considering the Net Ecosystem Productivity ( $\text{NEP} = -\text{NEE}$ ) and anthropogenic  
690 emissions derived from management activities for both treatments (for more information, see  
691 Appendix 1). While no remarkable differences were found for anthropogenic emissions between  
692 treatments, differences in NEP ultimately controlled the annual C budget, which resulted in  
693 lower C uptake in the weed-free treatment than in the weed-cover treatment. This assessment  
694 does not into account the lateral C export by harvesting in the estimation of the annual C  
695 budget. If we were to consider the Net Biome Productivity ( $\text{NBP} = \text{NEP} - \text{harvest}$ ), similar values  
696 would be found for both treatments ( $194$  and  $203 \text{ g C m}^{-2} \text{ year}^{-1}$  for the weed-cover and weed-

697 free treatments, respectively), which make differences in the annual C budget between  
698 treatments smaller (net emissions of 171.4 and 181 g C m<sup>-2</sup> year<sup>-1</sup> by the weed-cover and weed-  
699 free treatments, respectively). However, as mentioned above, this estimate of NBP must be  
700 considered with caution due to the uncertainty in the olive yield determination and the great  
701 inter-annual variation of the olive export by harvesting. Regardless of these sources of  
702 uncertainty, our study demonstrates that the management treatment affected annual NBP  
703 through its influence on Net Ecosystem Productivity (NEP=-NEE), which was increased by  
704 100% with the presence of weed cover.

705 Bearing in mind the limitations previously discussed and assuming a tree density of 200 trees  
706 ha<sup>-1</sup>, if we consider the total irrigated olive cultivation surface without spontaneous vegetation  
707 or cover crop management in Spain (~440 \* 10<sup>3</sup> ha) (MAGRAMA, 2012), we can estimate an  
708 annual C uptake increase of 308 \* 10<sup>3</sup> ton C due to implementation of conservation practices  
709 based on maintenance of spontaneous vegetation in olive orchards. Nonetheless, this is a rough  
710 estimation that needs to be validated. Last, it should be mentioned that, in addition to affecting  
711 CO<sub>2</sub> fluxes, weed cover can affect climate change by modification of the surface albedo.  
712 Although effects on albedo have not been addressed in this study, they should be further  
713 considered as we found the presence of weeds decreased albedo by 6% (averaged value for the  
714 study period) compared to the weed-free treatment.

715 This study shows for the first time the positive effects of weed cover on the annual C uptake in  
716 olive orchards through direct measurements of CO<sub>2</sub> exchange at ecosystem scale. Nonetheless,  
717 these reported effects should be analyzed over the long term, as variables such as precipitation  
718 and temperature patterns during the year can strongly condition the C budget and yield in olive  
719 orchards. Although plant covers are being increasingly adopted as sustainable management  
720 practices in olive orchards and other crops, their implementation in many agricultural lands is  
721 still limited and conventional practices such as intensive tillage are widespread in the  
722 Mediterranean region. The implementation of conservation practices based on plant cover offers  
723 numerous benefits to farmers and land practitioners, not only from the point of view of  
724 environmental protection which involves the improvement of physico-chemical soil properties

725 and the increase of CO<sub>2</sub> fixation and reduction of CO<sub>2</sub> emissions to the atmosphere, but also  
726 from an economic perspective resulting from the reduction of costs for restoration of damaged  
727 soils and the possibility of receiving economic subsidies from public bodies for the application  
728 of more sustainable agricultural practices.

729

## 730 **5. Conclusions**

731

732 Maintenance of alley weed cover in olive orchards increases ecosystem C uptake during periods  
733 of weed growth. However, after weeds are cut during late spring, the soil CO<sub>2</sub> efflux appears to  
734 increase due to decomposing weed remnants. This reduces ecosystem C fixation and reverses  
735 the behavior of the olive orchard from C sink to C source. Although the presence of weeds  
736 increased CO<sub>2</sub> emissions to the atmosphere during late spring, the maintenance of weed cover  
737 increased annual C uptake from the atmosphere by 100% relative to the treatment without weed  
738 cover. We measured NEE in the olive orchard under the two treatments, but further research  
739 should take into account CO<sub>2</sub> exchange by the different orchard components in order to  
740 elucidate the role that soil, herbaceous plants and olive trees play on CO<sub>2</sub> uptake and CO<sub>2</sub>  
741 emissions, as well as their seasonal changes throughout the year, and the relative contribution of  
742 each component to NEE. On the whole, this study highlights the positive effects of conservation  
743 practices based on maintenance of weed cover in net C uptake by olive orchards and the  
744 feasibility of using eddy covariance techniques to characterize differences in the C balances of  
745 olive orchards under different management practices.

746

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748

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758

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951 Table 1. Monthly NEE (gap-filled data) (and error) for each month at the two treatments.  
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Monthly NEE (g C m <sup>-2</sup> )		
	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)

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982 Table 2. Coefficients for  $GPP_{max}$  ( $b_1$ ),  $R_{eco}$  ( $b_3$ ) and PPFD when  $GPP_{max}$  was half ( $b_2$ ) obtained by  
 983 applying the Falge et al. (2001) equation using monthly averages of daytime 30-min data until noon for  
 984 each month. The coefficient of determination of the relationship between NEE and PPFD is also shown.  
 985 Only months with at least one significant parameterization coefficient are shown.  
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		Weed-cover			Weed-free		
		Estimate	Standard error	<i>p</i> value	Estimate	Standard error	<i>p</i> 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	0.300
	$b_3$	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	150.0	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	0.000	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	0.005	0.9	6.0	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	0.596	256.0	254.2	0.326
	$b_3$	9.0	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		

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996 Table 3. Estimation of the annual carbon budget at both treatments, expressed as g C m<sup>-2</sup> year<sup>-1</sup>.  
 997 Anthropogenic emissions were estimated according to the Carbon Footprint Certification for Castillo de  
 998 Canena olive oil (see Appendix 1 for more information). Annual carbon budget was determined as the  
 999 difference between NEP and deductions.

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

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## Figure captions

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1021 Fig. 1. Location of the olive orchard and picture of the eddy tower installed at each treatment  
1022 considered at this site: maintenance of spontaneous weeds (weed cover) and weed removal by  
1023 application of an herbicide (weed free). Points indicate the location of the eddy covariance  
1024 towers. The colored area indicates the fetch for each treatment.

1025 Fig. 2. Daily averages of environmental and soil variables during the hydrological year: a) Soil  
1026 water content (SWC,  $\text{m}^3 \text{m}^{-3}$ ; average of the two moisture probes) measured in an alley at 0.10  
1027 m in the weed-covered and weed-free soil, and rain (mm) during the studied period. b) Air  
1028 temperature (data average of the two treatments) and soil temperature ( $^{\circ}\text{C}$ ) in the weed-covered  
1029 and weed-free soil (average of the two thermocouples). c) Photosynthetic photon flux density  
1030 (PPFD,  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and vapor pressure deficit (VPD, hPa) (data average of the two  
1031 treatments).

1032 Fig. 3. Average monthly diurnal trends in net ecosystem  $\text{CO}_2$  exchange (NEE) in the two  
1033 treatments. Monthly averages were calculated from measured hourly data of NEE.

1034 Fig. 4. Monthly diurnal trend of PPFD and VPD during March (a) and August (b) and monthly  
1035 diurnal trend of NEE in both treatments during March (c) and August (d). Monthly averages  
1036 were calculated from measured half-hourly data of NEE.

1037 Fig. 5. Soil  $\text{CO}_2$  efflux (mean  $\pm$  sd,  $n=5$ ) measured at midday in the alleys of the two  
1038 treatments.

1039 Fig. 6. Light-response curves in March for the two treatments, using monthly averages of  
1040 measured half-hourly daytime NEE. Bars represent standard deviation of monthly averages.

1041 Fig. 7. Cumulative NEE and uncertainty of the gap-filled data, as well as annual net C uptake in  
1042 the two treatments during the study year.

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1048 **Appendix 1**

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1050 Anthropogenic emissions by management activities (deductions) at both treatments were

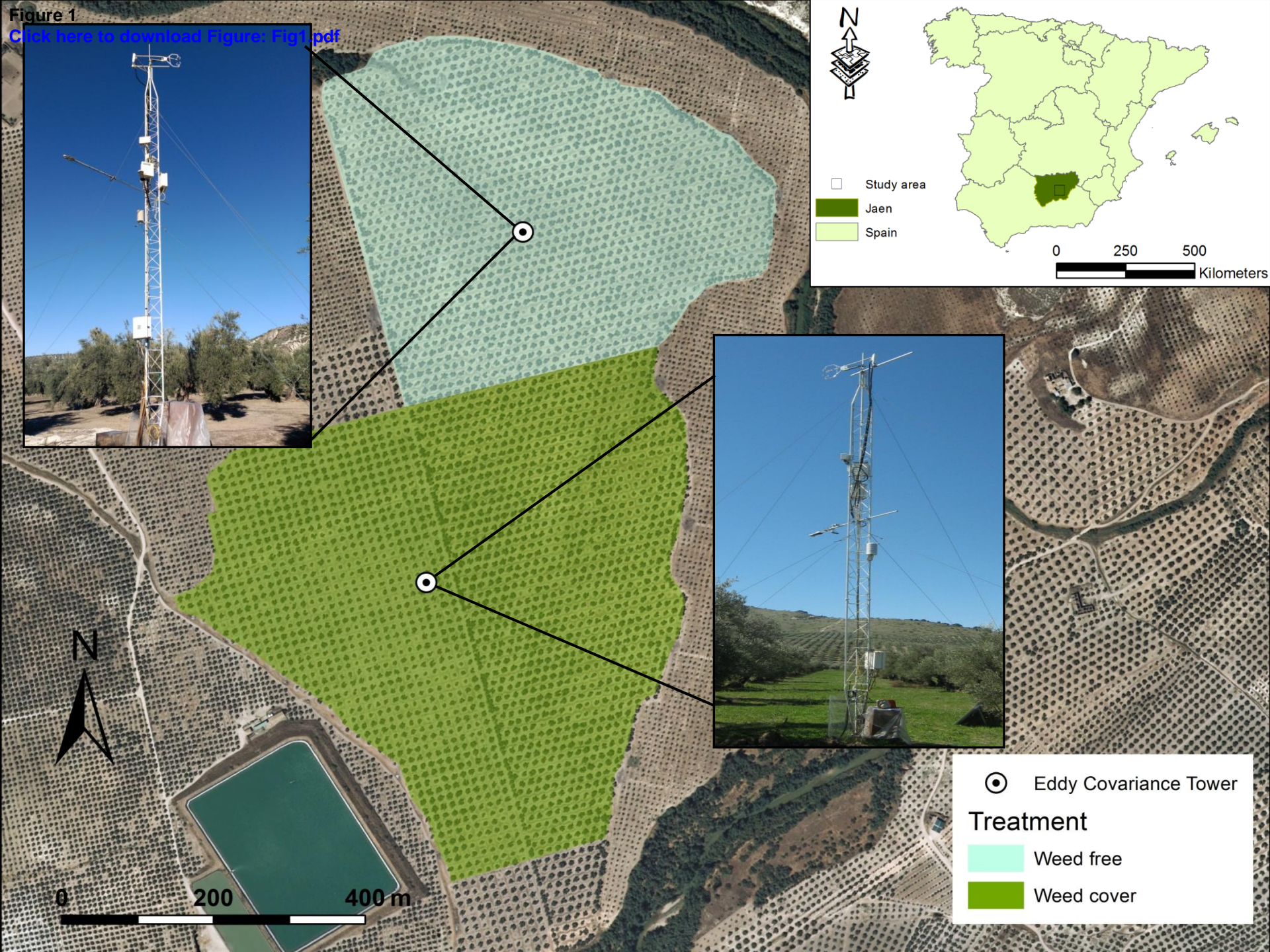
1051 estimated according to the following data provided by Castillo de Canena olive oil S.L.:

1052	
C emissions by management activities ( g C per liter of oil)	
<i>Irrigation</i>	10546
<i>Foliar treatments (pesticides)</i>	10559
<i>**Collecting and transport of olives to oil press</i>	10566
<i>Mowing</i>	10576
<i>Application of glyphosate herbicide</i>	10582
Determination of annual oil production per m <sup>-2</sup>	
<i>Average olive yield (kg olives per tree)</i>	106038
<i>Estimated industrial performance (kg of oil per kg of olive)</i>	0.16
<i>Oil density (kg per liter)</i>	0.918
<i>Tree density (trees per ha)</i>	204
<i>Liters of oil per m<sup>-2</sup></i>	10613
*According to these data, anthropogenic emissions were calculated as g C m <sup>-2</sup> year <sup>-1</sup> .	
**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.	

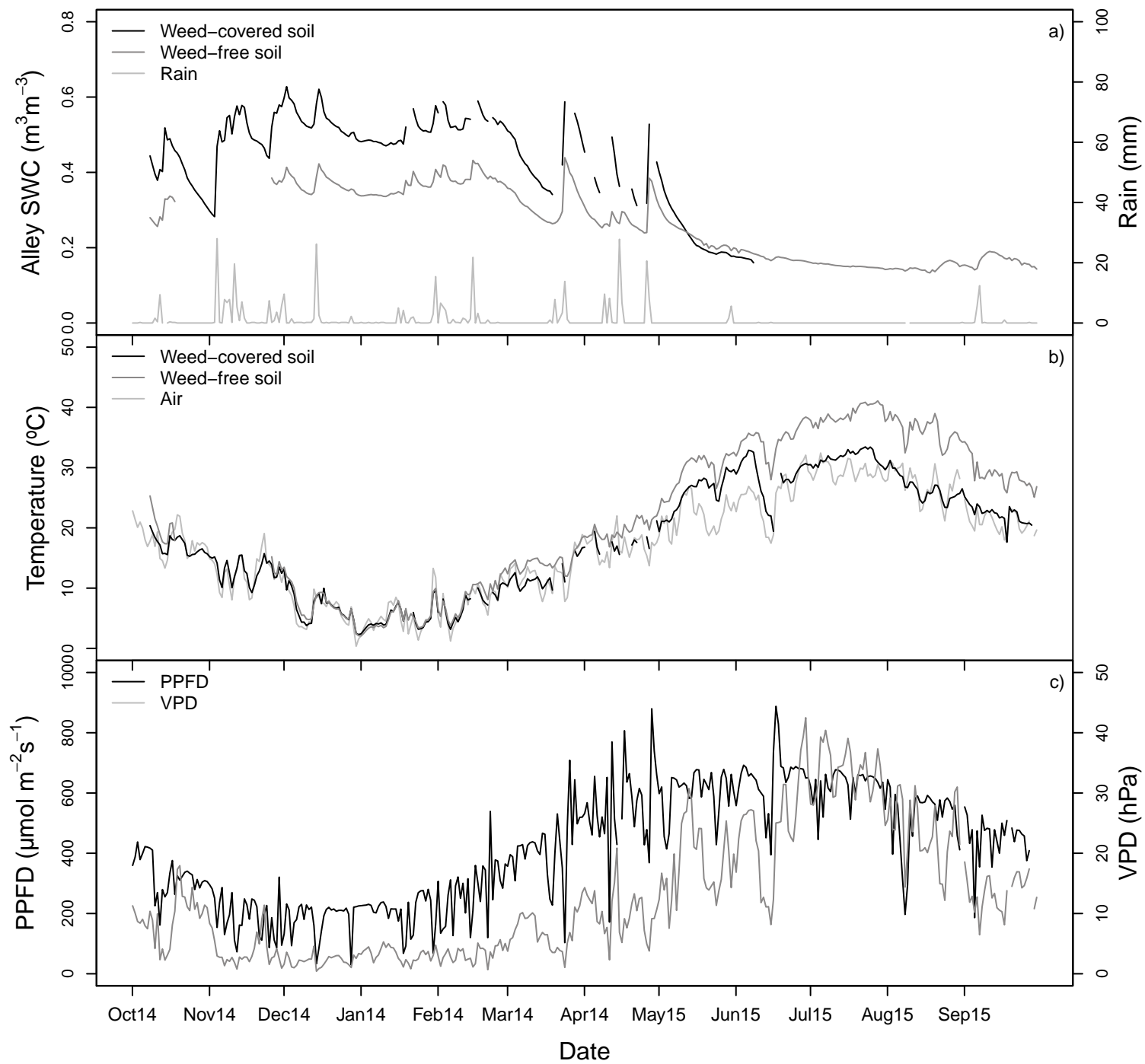


Figure 1

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**Figure 2**[Click here to download Figure: Fig2.pdf](#)

**Figure 3**

[Click here to download Figure: Fig3.pdf](#)

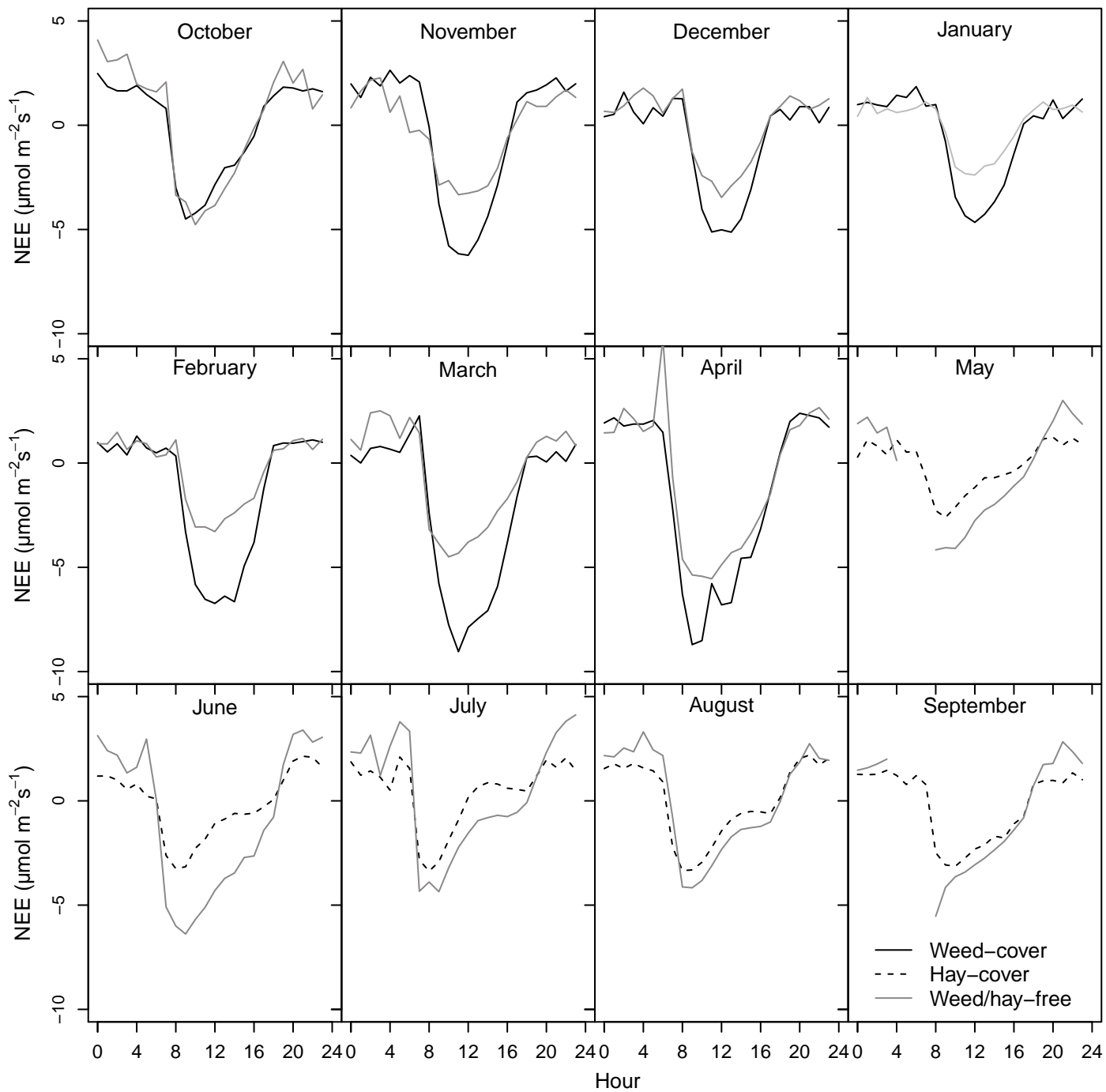


Figure 4

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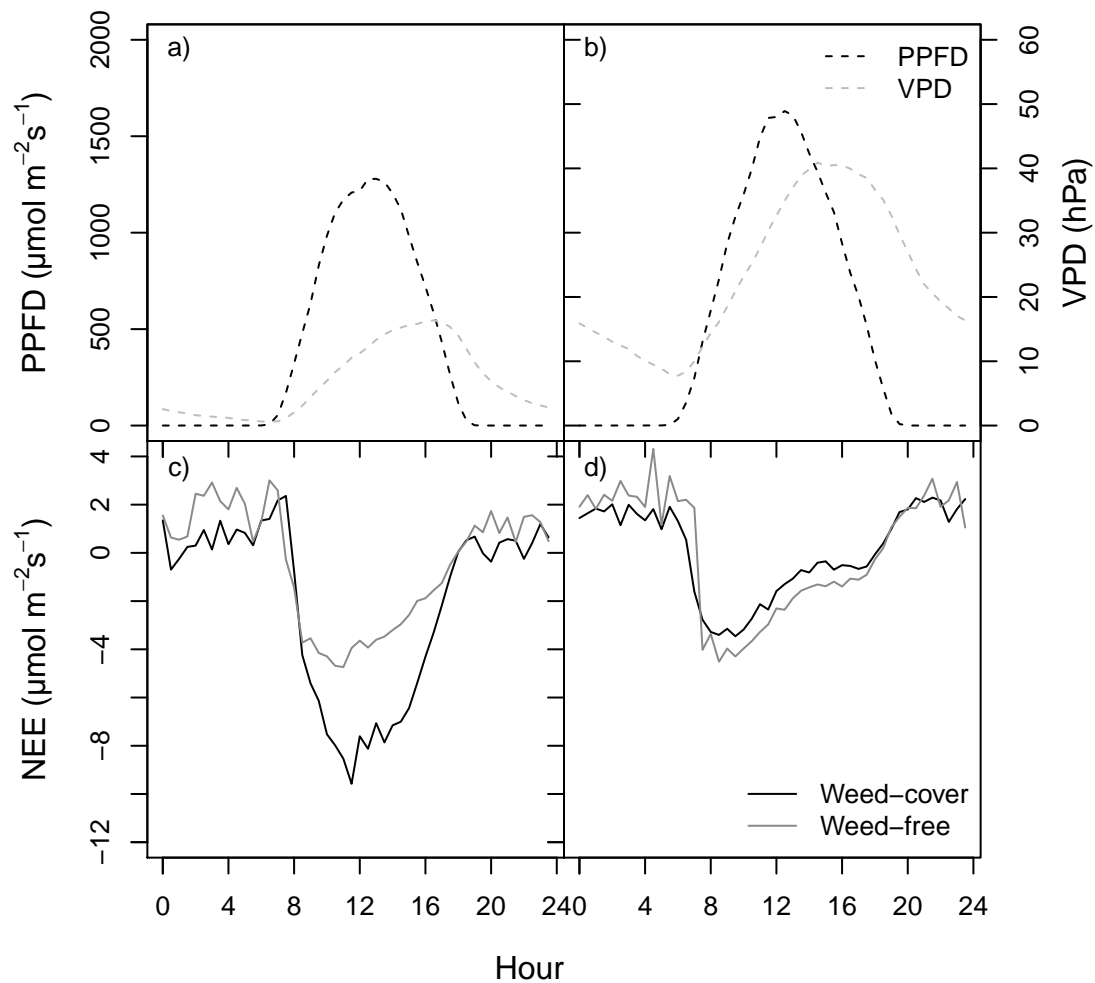


Figure 5  
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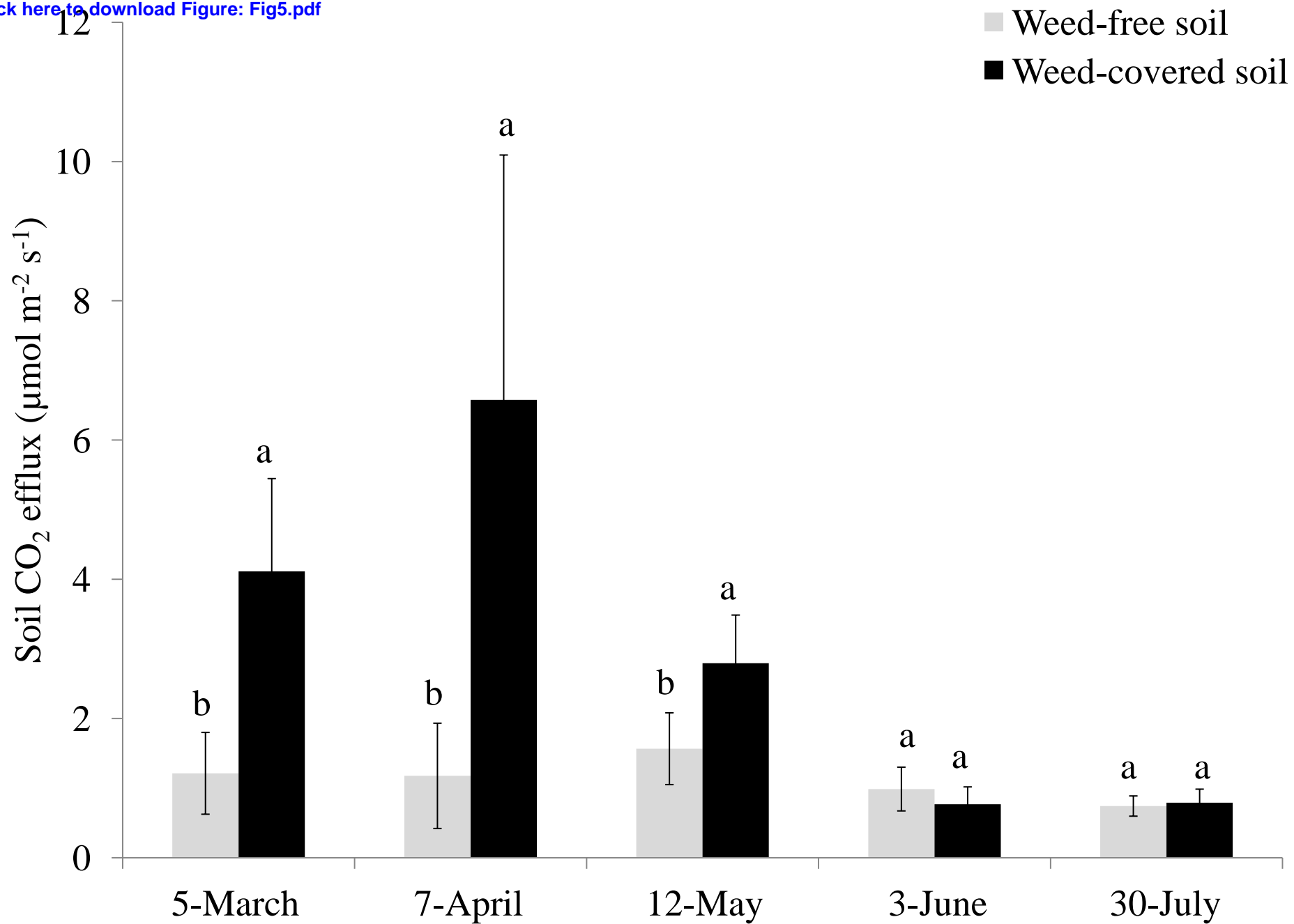


Figure 6  
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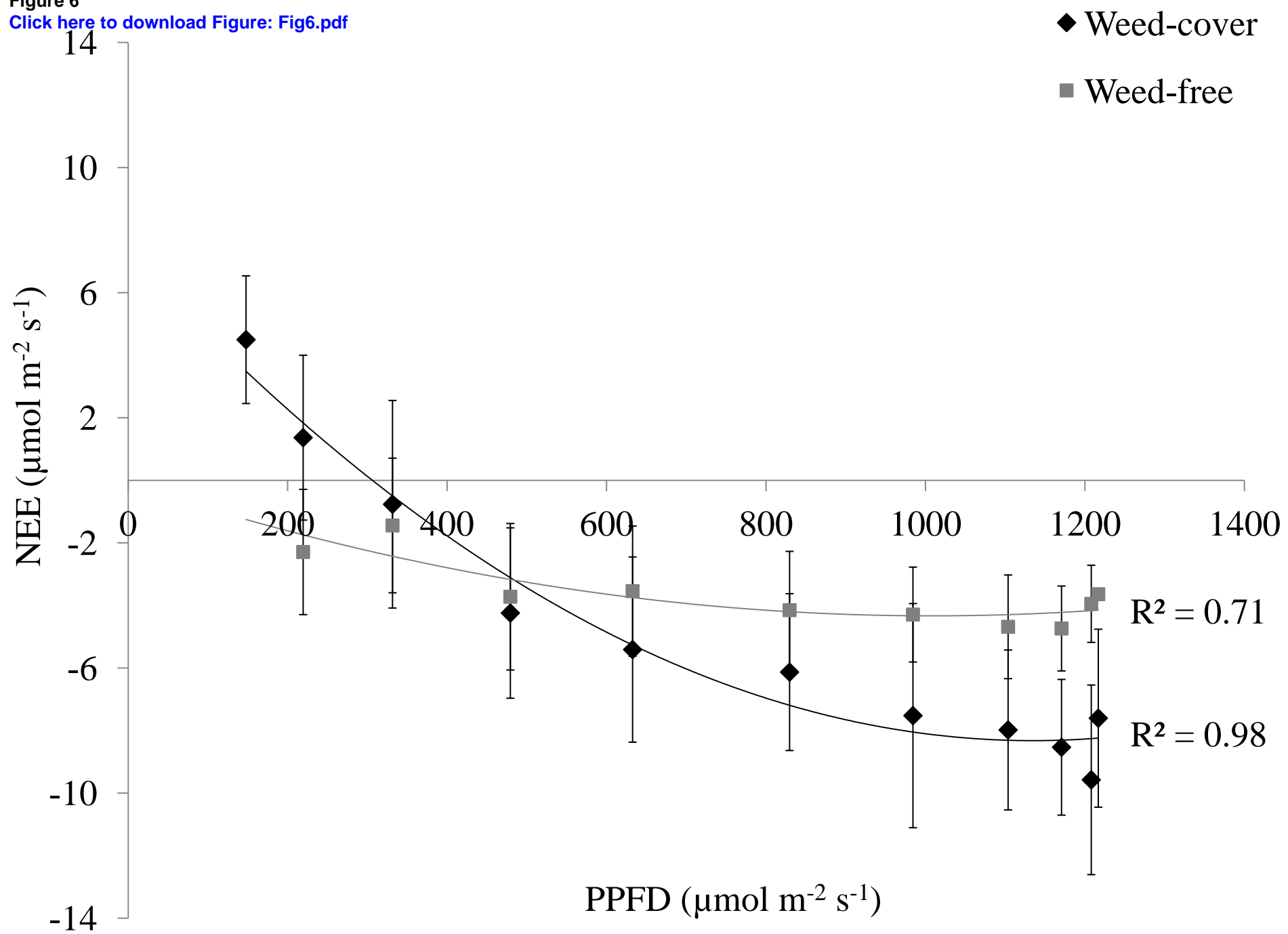


Figure 7

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