Autotrophic and heterotrophic contributions to short-term soil CO_2 efflux following simulated summer precipitation pulses in a Mediterranean dehesa

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[1] Autotrophic and heterotrophic components of soil CO_2 efflux may have differential responses to environmental factors, so estimating the relative contribution of each component during summer precipitation pulses is essential to predict C balance in soils experiencing regular drought conditions. As even small summer rains induced high instantaneous soil respiration rates in Mediterranean wooded grasslands, we hypothesized that standing dead mass, surface litter, and topsoil layer could play a dominant role in the initial flush of CO_2 produced immediately after soil rewetting; in contrast, soil CO_2 effluxes during drought periods should be mostly derived from tree root activity. In a grazed dehesa, we simulated four summer rain events and measured soil CO_2 efflux discontinuously, estimating its $\delta^{13}C$ through a Keeling plot nonsteady state static chamber approach. In addition, we estimated litter contribution to soil CO_2 efflux and extracted soil available C fractions (K_2SO_4-extracted C and chloroform-fumigated extracted C). The $\delta^{13}C$-CO_2 from in-tube incubated excised tree roots and rewetted root-free soil was −25.0‰ (±0.2) and −28.4‰ (±0.2), respectively. Assuming those values as end-members' sources, the autotrophic component of soil CO_2 efflux was dominant during the severe drought, whereas the heterotrophic contribution dominated from the very beginning of precipitation pulses. As standing dead mass and fresh litter contribution was low (<25%) in the first day and negligible after, we concluded that CO_2 efflux after rewetting was mostly derived from microbial mineralization of available soil organic C fractions.

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1. Introduction

[2] Ecosystem respiration is one of the largest gross fluxes in the global C budget and integrates quite different processes of plant and microbial function [*Trumbore*, 2006]. Soil CO_2 efflux results from a combination of (1) root respiration and associated rhizosphere activities that mineralize relatively recent photosynthetic assimilates; and (2) the activity of heterotrophic microorganisms that decompose necromass and organic matter in soils. The controls and mechanisms underlying these two components of soil respiration are different and may therefore respond differently to changes in environmental conditions [*Huxman et al.*, 2004b]. The fraction of soil CO_2 efflux derived from the heterotrophic component is largely independent from the autotrophic one, except for the increasing evidences of priming effect [*Kuzyakov*, 2002; *Paterson*, 2003], and contributes to decrease the amount of C sequestered in soils. Thus, estimating the relative contribution of both components under changing environmental conditions is relevant to understand the processes regulating soil organic matter turnover and to accurately infer the effects of global change on long-term soil C balance [*Kuzyakov*, 2006; *Ryan and Law*, 2005; *Yan et al.*, 2010]. Although forecasts on a regional scale remain difficult [*Weltzin et al.*, 2003], global climate models predict future changing precipitation regimes, with longer drought periods for tropical and subtropical regions and more frequent extreme rainfall events [*Allan and Soden*, 2008; *Alley et al.*, 2007; *Millán et al.*, 2005]. Given the sensitivity of arid and Mediterranean ecosystems to water availability, it is critical to characterize the origin and magnitude of soil C fluxes and their response to precipitation pulses in order to predict accurately the effects of soil rewetting on ecosystem C balance.

[3] In Mediterranean and arid ecosystems, organic matter and microbial biomass mostly accumulate in the uppermost soil layers, with an abrupt decrease below the first centimeters

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Table 1. Soil Main Properties in the Uppermost Layers of the Grazed Dehesa[a]

	0–5 cm		5–15 cm	
Bulk density (g cm^{-3})	1.51	±0.10	1.81	±0.04
SWC (%)	0.35	±0.09	1.16	±0.37
SOC (mg g^{-1})	13.6	±2.5	4.80	±0.5
C-to-N ratio	12.03	±1.63	9.45	±0.24
CFE (μg g^{-1})	272.33	±35.14	55.34	±5.02
EOC (μg g^{-1})	53.02	±15.02	12.70	±0.53

[a]Mean ± SE (n = 3). Soil samples were obtained 1 day before the first irrigation. SWC, gravimetric soil water content (60° for 48 h); SOC, soil organic carbon; CFE, $CHCl_3$-fumigated K_2SO_4-extracted soil carbon; EOC, K_2SO_4-extracted soil carbon.

of soil. After a long drought period in summer, heterotrophic activity in surface layers is strongly limited by soil water content [*Borken and Matzner*, 2009], whereas it may be limited by labile C or N availabilities in depth. In contrast, soil moisture decreases much slowly in deep soil, which enables deep rooting plants, as trees, to maintain water uptake and their root activity over an extended period. In consequence, during dry periods, soil CO_2 efflux could mostly derive from respiration associated with tree root maintenance and associate rhizosphere activity. Thus, high respiration rates can occur during summer drought period, as it was observed in a semiarid Mediterranean forest soil, coinciding with the lowest contribution of C mineralization from organic and uppermost mineral layers [*Casals et al.*, 2000]. However, in the short-term flush of soil CO_2 following the rewetting of extreme dry soil, both autotrophic and heterotrophic components are susceptible to contribute, even though only the CO_2 derived from soil organic matter decomposition may significantly affect soil C balance.

[4] In arid or Mediterranean climates, occasional summer rainfalls, even those of low magnitude, normally induce a disproportionate increase in soil CO_2 efflux [*Casals et al.*, 2009; *Cisneros-Dozal et al.*, 2007; *Huxman et al.*, 2004b; *Aires et al.*, 2008]. The relevance of this CO_2 release on the soil C balance will depend on the relative importance of the autotrophic and heterotrophic components involved. The spatial variability in the released C-CO_2 was largely related to the soil available C pool (estimated as chloroform-fumigated K_2SO_4-extracted C) present just before the rewetting [*Casals et al.*, 2009]. Other works argue that the physical displacement of the CO_2-enriched soil atmosphere out of the pore spaces due to percolating water may also contribute to the CO_2 flush immediately after rewetting [*Frank and Dugas*, 2001; *Huxman et al.*, 2004b; *Thomas and Hoon*, 2010]. Presumably, this CO_2, accumulated in the pore spaces during dry periods, originate from activity of roots and their closely associated microorganisms. As even small summer rain events (less than 3 mm) induced high instantaneous soil respiration rates [e.g., *Casals et al.*, 2009], we alternatively hypothesized that standing dead mass, surface litter and topsoil layer could have a dominant role in the initial flush of CO_2 produced immediately after rewetting.

[5] In most C-cycle models, the soil respiration algorithms do not account for differential contributions from litter, root and soil organic C pools due to drying and wetting cycles [*Hanson et al.*, 2004]. In this field experiment, we aimed to estimate the relative contribution of those possible sources to the short-term CO_2 efflux response to summer precipitation pulses by simulating rain events in a Mediterranean dehesa. A range of methods can be used to partition the soil CO_2 efflux into autotrophic and heterotrophic components [*Hanson et al.*, 2000; *Subke et al.*, 2006; *Trumbore*, 2006]. Isotope approaches have the important advantage over other methods to limit soil and root disturbance. However, identifying the origin of CO_2 requires that the sourced materials are sufficiently different in isotopic composition [*Kuzyakov*, 2006; *Hanson et al.*, 2000]. In the present experiment, the relative contribution of soil components to the response of CO_2 efflux to simulated precipitation is estimated by comparing the δ^{13}C of soil CO_2 efflux, calculated by a Keeling plot nonsteady state static chamber, before and after rewetting, with the isotopic compositions of the CO_2 respired by excised tree root or root-free soil as end-member sources.

2. Material and Methods

2.1. Study Site

[6] The site is located in an open holm oak woodland (dehesa) in Majadas del Tietar, Caceres, Spain (39°56′ N; 5°46′ W, 260 m a.s.l.). The soil is classified as Cambisol (Dystric) [*IUSS Working Group WRB*, 2006] and originated from Pliocene-Miocene alluvial deposits. The soil profile is about 90 cm deep with a clay horizon upper limit at a depth between 30 and 60 cm; in contrast, the texture in the upper horizons is sandy (9.3% clay, 10.7% silt, 80.0% sand). The pH (H_2O) is about 5.6 in the first 15 cm. Soil organic matter and associated extractable C fractions decrease sharply in the first 15 cm of soil (Table 1). The climate is continental Mediterranean with temperate winters. Mean annual temperature is 16.7°C and mean annual precipitation is 528 mm, with less than 6% of precipitation occurring during summer. Occasional rainfalls occur during five to ten summer days per year with usually less than 20 mm per day. The open tree stratum (24.8 trees ha^{-1}) is composed of evergreen holm oak (*Quercus ilex* ssp *ballota* Lam.) with occasional presence of *Q. suber* L. or *Q. faginea* Lam. (<5%). Tree canopy fraction is 19.8% on average. The herbaceous stratum is mainly composed of annual species (*Vulpia bromoides* (L.) SF Grey; *V. geniculata* (L.) Link; *Trifolium subterraneum* L., *Ornithopus compressus* L.), that senesce by the end of May. The site is subjected to traditional grazing management (see *Lopez-Sangil et al.* [2011] for more details).

2.2. Experimental Design

[7] During the summer of 2006, three irrigations (2006-R1, -R2; -R3) were performed at 2 week intervals (5 and 19 July; 2 August) during nighttime (from about 20:00 to 06:00 GMT) to avoid excessive evaporation. An additional irrigation was performed in summer 2008 (2008-R1). During each irrigation experiment, 20 L m^{-2} of water (about 200 m^3) were homogeneously distributed over 1 hectare area by 18 sprinklers (1.5 m in height).

[8] We established six permanent plots (1 m^2), in 2006, and five in 2008. In 2006, three plots were established under tree canopy and three in open grassland. Data from both situations were merged in the following analysis. The five plots in 2008 were in open grassland and with a different location than those of 2006. As the amount of irrigated

Table 2. Concentration (ppm) and Isotope Signature (δ^{13}C ‰) of CO_2 Sampled Within the PP-System Chamber Just Before Closing (Background) and After Leaving CO_2 to Accumulate in the Four Irrigation Events[a]

Irrigation Events		Sampling Time Related to Irrigation Event Starts							
		Before, −2 h		After 2 h		After 16 h		After 40 h	
		Background[b]							
	CO_2	378.0	±0.5	385.8[b]	±0.5	380.9[b]	±0.7	379.4	±0.6
	δ^{13}C	−12.06	±0.07	−12.35	±0.03	−12.05	±0.11	−12.40	±0.08
		Accumulate							
2006-R1[c]	CO_2	519.1	±27.8	1419.0	±42.7	1356.7	±190.4	1135.8	±151.8
	δ^{13}C	−17.97	±0.88	−24.99	±0.49	−24.12	±1.39	−23.79	±0.90
2006-R2	CO_2	762.2	±62.4	974.7	±150.3	1677.1	±242.1	1109.2	±55.1
	δ^{13}C	−18.53	±0.51	−20.79	±1.03	−23.44	±0.65	−21.18	±0.21
2006-R3	CO_2	646.2	±42.4	1072.2	±74.5	909.9	±95.2	936.5	±32.2
	δ^{13}C	−17.56	±0.40	−22.00	±0.58	−21.81	±0.74	−21.82	±0.48
2008-R1	CO_2	658.0	±17.5	1763.1	±360.2	1179.0	±53.0	775.3	±13.1
	δ^{13}C	−16.37	±0.79	−22.22	±1.61	−22.77	±0.31	−19.74	±0.34

[a]Mean ± SE (n = 6, except in 2008-R1, n = 5).
[b]As no significant differences existed in the background data between irrigation events, mean values using the three irrigation periods (2006-R2, 2006-R3, 2008) are shown. Abnormal values higher than 410 ppm after closing the chamber, 2 h and 16 h after rewetting, were not used to calculated the background mean but included in the Keeling plot (Figure 3).
[c]Concentrations and signatures measured in 2006-R1 were obtained after leaving soil CO_2 efflux to accumulate in a previously purged chamber.

water showed a good linear relationship with the distance to the closest sprinkler (R^2 = 0.78, data not shown), the effect of spatial heterogeneity in the simulated irrigation was limited by locating the plots at a constant distance (7 m) from the sprinklers. In each plot and irrigation event, before and after irrigation starts, we measured the soil CO_2 efflux rate and concentration by a closed dynamic system (EGM-4, SRC-1; PP-Systems, United Kingdom) and sampled the air in the chamber headspace for δ^{13}C-CO_2 analysis (nonsteady state static chamber method). In addition, during the 2008 irrigation, litter contribution to soil total efflux was estimated by the close dynamic system comparing CO_2 efflux from intact cores with efflux from the litter collected and disposed in a separate chamber.

2.3. Soil CO_2 Efflux

[9] In 2006, soil CO_2 efflux was measured continuously on irrigated and nonirrigated open grassland sites from June to October by means of automated open dynamic systems with five chambers each (as described by *Casals et al.* [2009]). In 2006 and 2008 irrigations, discontinuous CO_2 efflux measurements were carried out with a closed dynamic system (PP-Systems) with dark chambers that fitted on PVC collars (10 cm height, 15.5 cm diameter). Collars were inserted 6 cm into the ground in early spring. The volume of the entire chamber was 1975 cm^3. CO_2 efflux measurements were performed 2 h before and 2, 5, 12, 16, 24, 40 and 48 h after starting the irrigation event.

2.4. Litter CO_2 Efflux

[10] In 2008, a set of extra PVC collars was installed for litter respiration measurements. CO_2 efflux measurements were performed 2 h before and 0.5, 1.5, 8 and 24 h after starting the irrigation event in five collars per time. Just after each measurement of the intact collar, standing mass and surface litter were carefully removed and were placed in similar PVC collars with the bottom sealed in order to measure the litter CO_2 efflux contribution. Afterward, litter was stored, oven-dried and weighted in the laboratory.

2.5. δ^{13}C-CO_2 Sampling and Analysis

[11] CO_2 samples for isotopic analysis were taken 2 h before and 2, 16 and 40 h after starting each irrigation event from a nonsteady state static chamber with slightly variation between the first irrigation (2006-R1) and the following irrigations (2006-R2, -R3; 2008-R1). Thus, for the 2006-R1 and in each plot and time, we sampled the air accumulated for 20 min in the headspace of the PP-chamber, previously purged by recirculating the chamber air for 20 min in a circuit with soda-lime (purged nonsteady state static chamber). At end of the purged period, CO_2 concentration in the chamber was below 260 ppm before irrigation while it reached ~675 to 990 ppm after rewetting. During the following irrigations (2006-R2, -R3; 2008-R1) and in each plot and time, the chamber-headspace atmosphere was sampled two times: just after chamber closure and after accumulating soil CO_2 efflux for 20 min. CO_2 concentration in the chamber was measured by the PP-system just before sampling the air for isotopic analysis. In this approach, the chamber was not initially purged and the isotope signature of soil CO_2 efflux was estimated by the keeling plot as described in section 2.6, nonsteady state static chamber-keeling plot method [*Ohlsson et al.*, 2005; *Pataki et al.*, 2003]. The CO_2 range between both samples was higher than 250 ppm (Table 2) which reduces standard error in ^{13}C estimation with Keeling plots below 0.5‰ [*Joos et al.*, 2008; *Ohlsson et al.*, 2005]. Lateral diffusion may also introduce an additional error in our estimates although this was presumably minimized using collars inserted to 6 cm depth [*Hutchinson and Livingston*, 2001; *Ohlsson*, 2009].

[12] Air samples were collected using 50 mL syringes (SGE International PTY LTD, Ringwood, Australia), kept in previously N_2-purged 10 mL vacutainers (BD Vacutainers, Plymouth, United Kingdom), and then analyzed by Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry (GC-C-IRMS), as described by *Nogués et al.* [2008]. Carbon isotope values (δ^{13}C) of CO_2 are expressed in parts per thousand differences from the international

standard Vienna–Pee Dee belemnite using the classical equation for natural abundance:

$$\delta^{13}C(‰) = \left[\left(R_{sample}/R_{standard}\right) - 1\right] \times 10^3$$

2.6. Isotopic Signature of Soil CO_2 Efflux

[13] In 2006-R1, the isotopic signature of soil CO_2 efflux was assumed the same that the air accumulated in the headspace of the PP-chamber (previously purged, so the accumulated CO_2 should derive mostly from soil efflux). As this method has assumptions that may introduce important sources of error (see *Nickerson and Risk* [2009b] and *Ohlsson et al.* [2005] for more details), in the following irrigations the isotopic signature of the soil CO_2 efflux was calculated by a Keeling mixing model [*Ohlsson et al.*, 2005; *Pataki et al.*, 2003, and references herein]. The results obtained by both methods are not comparable [*Nickerson and Risk*, 2009b].

[14] The Keeling-mixing model assumes that the isotopic signature of the headspace air is a mixture of two sources of ^{13}C-CO_2: the background atmosphere and the source of respiration. The Keeling linear mixing model equation resulting to solve the two-component mixing model

$$C_F = C_I + C_R \quad (1)$$

$$\delta_F C_F = \delta_I\ C_I + \delta_R\ C_R \quad (2)$$

relates the concentrations and signatures of CO_2 sampled before and after chamber accumulation:

$$\delta_F = C_I(\delta_I - \delta_R)(1/C_F) + \delta_R \quad (3)$$

where subscripts F and I denote final and initial chamber headspace CO_2, respectively, and R the respired CO_2. From equation (3) the δ_R is estimated as the intercept of the linear regression of δ_F (y variable) against $1/C_F$ data (x variable) [*Keeling*, 1958]. In each time and irrigation event, the linear regression was calculated using data from the six (five in 2008) plots sampled. Following *Ohlsson et al.* [2005], we used least squares regressions, which presuppose that errors are present in the y variable only. This method assumes a negligible effect on the soil CO_2 efflux and isotope composition from the presence of chamber, but the systematic error could be of a significant magnitude [*Nickerson and Risk*, 2009a; *Ohlsson*, 2009] as summarized in section 4.

2.7. End-Member Isotope Estimations

[15] To estimate the δ^{13}C signature of CO_2 respired by roots, small oak roots (2–6 mm diameter) were collected from four different oak trees by digging below their canopies until 10–20 cm depth. Once localized, they were extracted and carefully cleaned with distilled water to remove adjacent soil. The 1–2 g of oak roots (dry-weight equivalent) were placed into 10 mL vacutainers, which were thoroughly cleaned by placing two needles on the lid (septum) and applying a CO_2-free N_2 stream at a constant flow of 2 mL s^{-1} for 90 s. Roots were then kept in the dark at ambient temperature (~28°C) for 30 min and finally the accumulated CO_2 transferred into equivalent clean vacutainers (previously N_2 purged and 8 mL emptied) by using a 50 mL glass syringe.

[16] To assess for the δ^{13}C signature of the heterotrophic source, soil samples were collected also at four points from the uppermost horizon (0–5 cm depth) and 2 mm sieved. 3.5 g of equivalent dry-weight soil were placed into 10 mL vacutainers, rewetted with 0.85 mL distilled water, left to respire in dark conditions for 30 min, and the accumulated CO_2 finally transferred to clean vials following the same procedure than with oak roots.

[17] Soil and root samplings were performed about 1 h before 2008 irrigation started (19:00–20:00 GMT). Additional isotope root and soil respiration measurements were performed (1) with samples collected at 07:00–8:00 GMT, to assess daily differences in isotope signature (data not shown); and (2) leaving 120 min for CO_2 accumulation, to check the influence of incubation period. *Midwood et al.* [2006] observed slight shifts in the isotopic signal of root-respired CO_2 between 20 and 120 min. As a quality control of the process, a δ^{13}C -depleted CO_2 standard was injected in situ in four vacutainers and transferred also using the same procedure. No differences were found between the standard (−39.42‰ VPDB) and the CO_2 signature measured in these vials (−39.44 ± 0.06‰ VPDB). Air samples were analyzed by gas chromatograph-combustion-mass spectrometry (GC-C-IRMS) as mentioned above.

[18] In addition, the ^{13}C and ^{15}N natural abundance of oak roots, soil organic matter (0–5 cm) and fumigated-extracted soil organic C fraction (CFE; see section 2.9) were measured. The tree roots used to estimate the signature of respired CO_2, and a subsample of dry soil were dried and grounded with a ball mill (MM200 Retsch®) for isotopic analysis. For the signature of CFE fraction, an aliquot of extracts containing about 20–40 μg of C was transferred into a capsule containing quartz sand (previously heated at 550°C for 48 h to remove organic C) and dried (30°, 48 h). Total C and N concentrations and ^{13}C and ^{15}N isotopic ratios were analyzed using an ANCA interfaced to a 20–20 Europa isotope ratio mass spectrometer (Sercon Ltd. Cheshire, United Kingdom).

2.8. Estimation of Autotrophic and Heterotrophic Components

[19] Assuming that only two different sources contribute to soil CO_2 efflux, an isotope-mixing model was used to estimate the fraction of soil surface CO_2 efflux due to the autotrophic component (*Af*):

$$Af = \left[\left(\delta^{13}C_{SE} - \delta^{13}C_{SoilR}\right)/\left(\delta^{13}C_{rootR} - \delta^{13}C_{SoilR}\right)\right] \quad (4)$$

$$Hf = 1 - Af \quad (5)$$

where the subscripts *SE*, *SoilR* and *rootR* refer to the surface soil CO_2 efflux and the CO_2 derived from both soil (*SoilR*) and root (*RootR*) incubations. Heterotrophic component (*Hf*) is the complementary of *Af*.

2.9. Soil Sampling and Extractable Organic C Fractions

[20] In the 2008 event, soil undisturbed cores (5 × 5 cm and 15 cm depth) were extracted close to each collar (<0.5 m) and divided into two layers: 0–5 cm and 5–15 cm. Soil samples were taken out 4 h before and 2, 12 and 48 h after the start of

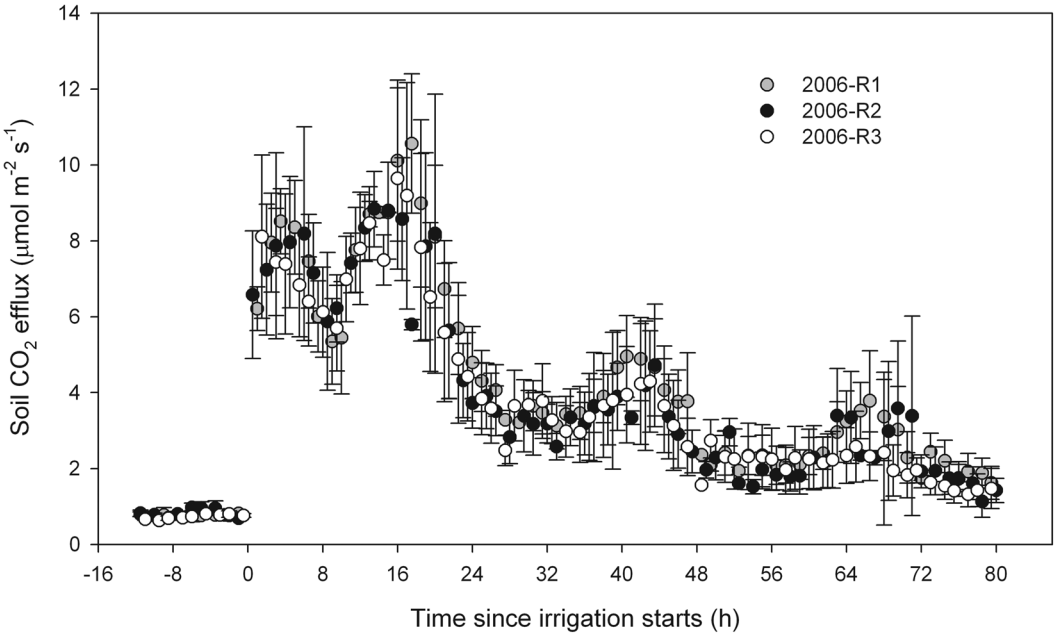

Figure 1. Continuous soil CO_2 efflux (μmol m^{-2} s^{-1}) measured by an open dynamic system in the grazed dehesa site during the 2006 irrigation events. Data are mean ± SE (n = 5). Time 0:00 corresponds to irrigation start.

irrigation. Samples were kept undisturbed and refrigerated until laboratory analysis (within the next 4 days). After sieving (2 mm) and homogenizing, field-moist subsamples were used for determination of soil water content (gravimetrically, 48 h at 60°C) and soil extractable organic C fractions (*Vance et al.* [1987], following the recommendations of *Jones and Willet* [2006]): the amount of nonbiomass soil available C, measured as K_2SO_4-extracted soil organic C (EOC); and a combination of both the microbial C and EOC fractions, measured as the chloroform-fumigated K_2SO_4-extracted soil organic C (CFE).

2.10. Statistical Analysis

[21] The differences in soil characteristics (soil water and C fractions) before and after irrigation were evaluated through a Repeated Measures Analysis of Variance, including the sampling time (before and after) as a within factor. Variables were log-transformed prior statistical analysis. Differences in δ^{13}C keeling plot regressions between irrigation events in each time were assessed by an Analysis of Covariance. Normality of distribution was verified for all variables prior to statistical analysis. Unless otherwise indicated, all data are presented as mean ± standard error. All the statistical analyses were performed using the SPSS v. 15.0.

3. Results

3.1. Dynamics of Soil and Litter CO_2 Efflux

[22] Continuous soil CO_2 efflux measured by the open dynamic systems was lower than 1.16 μmol m^{-2} s^{-1} (1.2 g C m^{-2} d^{-1}) in nonirrigated plots, whereas it sharply increased about seven to ten times in the first hours after starting the irrigations (Figure 1). Accumulated CO_2 effluxes in the first day after rewetting ranged from 6.34 ± 2.37 g C m^{-2} to 7.74 ± 0.52 g C m^{-2}. The increase in soil CO_2 efflux due to irrigation was important during 5 days and the accumulated C loss was about 19.7 ± 1.5 g C m^{-2} over this period (mean ± SE of the 4 irrigation events).

[23] Instantaneous soil CO_2 efflux 2 h before precipitation pulses was 1.00 ± 0.11 μmol m^{-2} s^{-1}, while it increased to 9.91 ± 0.36, 7.75 ± 0.52 and 4.32 ± 0.10 μmol m^{-2} s^{-1} 2, 16 and 40 h after (mean ± SE of the 4 irrigation events). Before simulated precipitation pulses, litter respiration was negligible. Half an hour after starting the rewetting, litter CO_2 efflux rates ranged from 1.39 to 1.90 μmol m^{-2} s^{-1} and kept on similar values 1 h after (2.07 ± 0.23 μmol m^{-2} s^{-1}). These flushes account for about 12–16% of total soil CO_2 efflux (Figure 2). Ten hours after irrigation started, at 06:00 GMT, when all the 20 mm of water were applied, litter respiration rate only decreased slightly (1.56 ± 0.18 μmol m^{-2} s^{-1}) but as total soil CO_2 efflux decreases sharply, the contribution of litter to total soil CO_2 efflux increases till around 24% (Figure 2). Litter water content versus litter dry matter was about 127.34 ± 7.53% (n = 12) in the first 2 h after irrigation started and, slightly lower after 8 h (84.32 ± 6.49%, n = 6). As expected, litter dries out quicker than soil during the day and, 24 h after starting the irrigation, litter respiration rates go back to undetectable initial values (Figure 2).

[24] Standing dead mass and litter collected in the closed system-collars ranged from 171.3 to 445.9 g dry-matter m^{-2} (n = 18). Stepwise regression selected both litter dry-matter (regression coefficient: 0.004 ± 0.001 g m^{-2}) and litter water content (regression coefficient: 1.436 ± 0.551 g g^{-1}) to explain observed variations in litter CO_2 efflux in the first 8 h (adj R^2 = 0.48, p = 0.008, n = 18). Consequently, CO_2

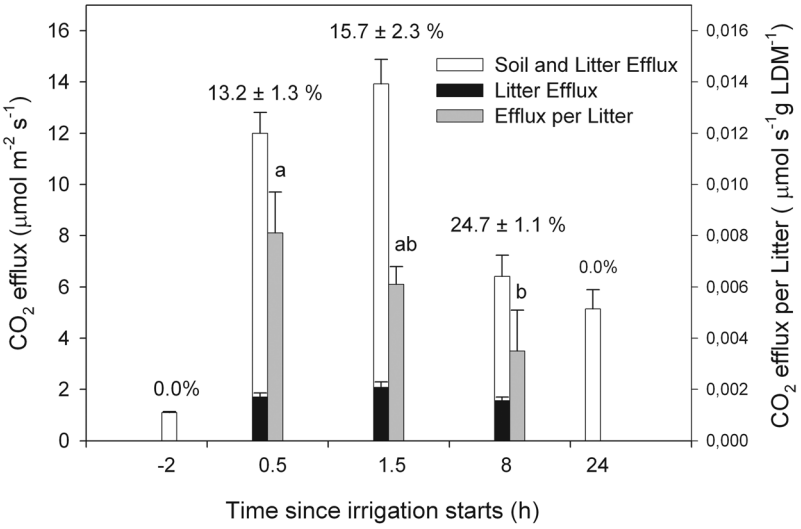

Figure 2. CO_2 efflux of soil and litter (left axis, μmol m^{-2} s^{-1}) and litter-CO_2 efflux per dry-matter litter (right axis, μmol m^{-2} g LDM^{-1}) measured discontinuously by a closed system (PP-systems), 2 h before and 0.5, 1.5, 8, and 24 h after starting the 2008 irrigation event. The proportion (%) of litter CO_2 efflux compared to the litter and soil efflux is indicated (2 h before irrigation and 24 h after the litter CO_2 efflux was negligible). Different lowercase letters denote significant differences between times in litter-CO_2 efflux per dry-matter litter. Data are mean ± SE (n = 4).

efflux per litter dry-matter in 8 h after irrigation starts was lower than in the first half an hour (Figure 2).

3.2. The δ^{13}C Signature of Soil CO_2 Efflux

[25] Background CO_2 concentration in the headspace just before closing the chamber ranged from 370 ppm to 386 ppm and δ^{13}C signature, from −11.61‰ to −12.37‰ (Table 2). In the 2006-R2, -R3 and 2008 irrigations, the concentration and signature of accumulated soil CO_2 efflux ranged from 658 ppm and −17.63‰ 2 h before irrigation to 2271 ppm and −24.99‰ after (Table 2). In the 2006-R1, the chamber was previously purged and both concentration and signature were not directly comparable with data from the following irrigation events. However, the raw data showed that both concentration and signature followed a similar pattern than those of the subsequent irrigations (Table 2).

[26] Overall, the δ^{13}C signature of CO_2 efflux, estimated as the raw data in 2006-R1 (Table 2) and the y intercept of the least squares linear regressions of Keeling plots in the other experiments (Table 3), was higher before than after rewetting in all the four irrigation experiments. The δ^{13}C of soil CO_2 efflux 2 h after starting the irrigation was similar in magnitude to those of 16 or 40 h after (Table 3). Either 2 h before starting the irrigations or 2 h after, no differences appeared in the y intercept between events (Figure 3). In contrast, some slight differences between events were observed 16 and 40 h after measurements.

3.3. Partitioning Soil CO_2 Efflux

[27] The δ^{13}C of CO_2 respired by oak roots was about 3.5‰ units higher compared to that respired by the rewetted soil (n = 4; Table 4). Length of the root-soil incubation periods (0.5, 2 and 30 h) did entail neither clear trends nor significant differences in the δ^{13}C of respired CO_2 ($p > 0.05$; Table 4). The δ^{13}C-CO_2 of root respiration had also been estimated earlier (in July 2007, data not shown) with

Table 3. Keeling Intercept Values (B ± SE; ‰) and Coefficient of Model Regression (r) in Each Irrigation Event, 2 h Before and 2, 16, and 40 h After Irrigation Starts[a]

	Time Since Irrigation Starts and Irrigation Event											
				After								
	Before, −2 h			2 h			16 h			40 h		
	2006-R2	2006-R3	2008-R1	2006-R2	2006-R3	2008-R1	2006-R2	2006-R3	2008-R1	2006-R2	2006-R3	2008-R1
	Intercept Values (‰)											
B	−25.32	−25.44	−23.09	−27.21	−27.49	−27.30	−27.50	−29.07	−27.72	−25.74	−28.32	−27.00
SE	±0.50	±0.78	±1.40	±0.84	±0.82	±0.34	±0.32	±0.93	±0.25	±0.12	±0.52	±0.51
	Coefficient of Regression											
r	0.989	0.975	0.920	0.975	0.975	0.995	0.996	0.970	0.998	0.999	0.991	0.993

[a]The intercept value and the regression model were always significant ($p < 0.001$, n = 12 for 2006-R2 and -R3 and n = 10 for 2008-R1).

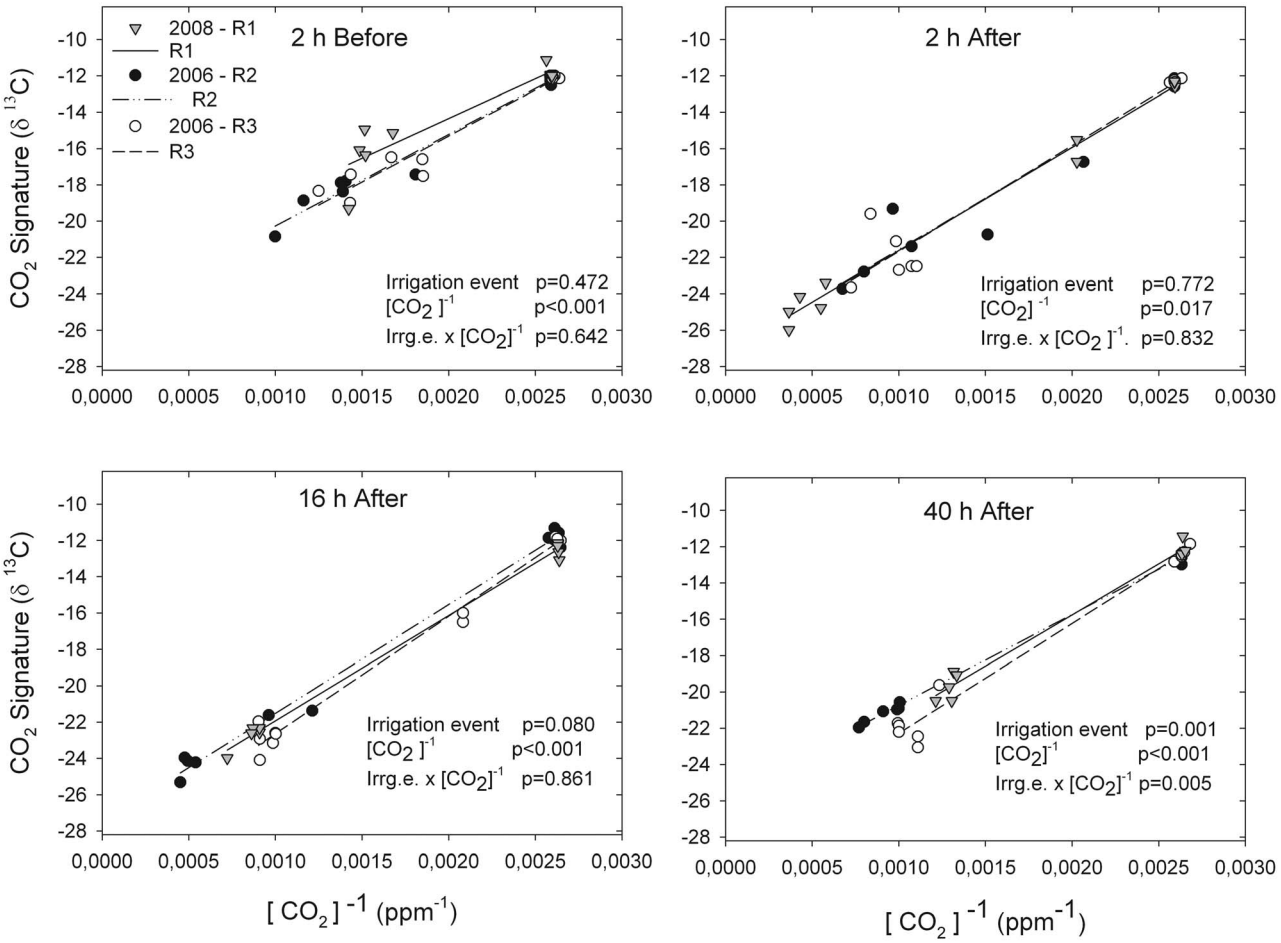

Figure 3. Inverse of CO_2 concentration against isotope signature (Keeling plot) of the three irrigation events (2006-R2 and -R3; and 2008-R1) 2 h before and 2, 16, and 40 h after starting irrigation events. Six plots in the 2006-R2 and -R3 and five plots in 2008-R1 irrigations were sampled twice, before and after accumulate air in a non-static-state chamber. Linear regression was performed for each irrigation event.

equivalent results to those presented here. The δ^{13}C of bulk soil organic matter, CFE fraction extracted after irrigation, and small roots were similar (Table 5) and no different from that of the CO_2 respired by incubated soil (Table 4). However, the δ^{13}C of CFE extracted before irrigation was slightly depleted ($p < 0.05$).

[28] The δ^{13}C of soil CO_2 efflux at each time, before or after irrigation, lay between these to end-members' values. Assuming that the measured signature of CO_2 respired by roots incubated for 0.5 h (−24.99 ± 0.21‰) was representative of the autotrophic source and that respired by incubated soil (−28.44 ± 0.18‰) was representative of the heterotrophic source, the autotrophic component (Af) is the responsible of the total soil CO_2 efflux before irrigation (Figure 4). Af decreased to 31 and 10%, 2 and 16 h after irrigation started, respectively. After 40 h, it represents about 43% of soil efflux, and after 15 days (before 2006-R2 and 2006-R3), it increased again to about 100%.

3.4. Soil Water and C Fractions

[29] The amount of EOC at the surface soil horizon (0–5 cm) decreased throughout the first 2 days after rewetting (2008-R1; Figure 5). Thus, EOC dropped progressively from 0.31 and 0.22 mg C g^{-1} soil (under canopy and open grassland, respectively) to 0.23 and 0.09 mg C g^{-1}, comparing the levels before and 48 h after the start of irrigation (Figure 5). In contrast, the rewetting process entailed an extremely rapid evolution of the CFE fraction. CFE doubled 2 h after the start of irrigation in both under-canopy and open grassland soils, increasing from 0.56 and

Table 4. Signature (δ^{13}C) of Respired CO_2 From Root and Soil Samples Taken up in the Morning or at Night and Incubated During Either 0.5, 2 or 30 h[a]

Incubation Times	Root δ^{13}C-CO_2		Soil δ^{13}C-CO_2	
	Night (19:00–20:00 GMT)			
0.5 h	−24.99	±0.21	−28.44	±0.19
2 h	−25.47	±0.28	−28.62	±0.08
30 h	−25.43	±0.23	−27.74	±0.06
	Morning (07:00–8:00 GMT)			
0.5 h	−24.16	±0.40	−27.41	

[a]Mean ± SE (n = 4, except soil in 07:00–08:00 incubation, n = 1).

Table 5. Signature (δ^{13}C) and Content of the Fumigated-Extracted Soil C Fraction (CFE) Before and After Starting Irrigation (2006-R1) and C and N Signatures and Contents of Soil Organic Matter and Small Roots[a]

	δ^{13}C (‰)			C (mg g^{-1})			δ^{15}N (‰)			N (mg g^{-1})		
CFE before irrigation	−31.86	±1.02	a	0.32	±0.03	a	n.a.[b]			n.a.[b]		
CFE after irrigation	−28.21	±0.63	b	0.40	±0.05	a	n.a.[b]			n.a.[b]		
Soil organic matter	−27.90	±0.38	b	26.93	±7.56	b	2.68	±0.24	a	1.84	±0.57	a
Small roots	−29.18	±0.20	b	356.2	±6.66	c	−0.82	±0.36	a	6.26	±0.38	b

[a]Mean ± SE (n = 4); in each variable, different letters denote statistical differences between fractions.
[b]N.a., not available because of the low amount of N in the extracts.

0.34 mg C g^{-1} to 1.13 and 0.60 mg C g^{-1}, respectively, and gradually turned back to similar values to those before rewetting 48 h after the start of irrigation (Figure 5).

4. Discussion

4.1. Autotrophic and Heterotrophic Contributions to Soil CO_2 Flush

[30] CO_2 efflux during drought period was ^{13}C-enriched compared to CO_2 evolved after simulated precipitation pulses, about 2 to 5‰ higher. This pattern was consistent throughout the four irrigation experiments and points to different sources of CO_2 efflux before and just after soil rewetting. Although the source of CO_2 efflux is uncertain, its δ^{13}C prior to rewetting was equivalent to the signature of CO_2 respired by the excised oak roots, whereas the isotopic signature of soil CO_2 efflux after rewetting was much closer to that of CO_2 from incubated rewetted soil respiration.

[31] Assuming the measured signatures of CO_2 respired by excised root or rewetted soil as end-members, the heterotrophic component of CO_2 efflux from dry soil was negligible, whereas it increased up to 70% just after the beginning of soil rewetting and even to 90% the day after. However, the cumulated uncertainties associated to the method used to estimate the Keeling plot intercepts impede inferring the absolute contributions of autotrophic and heterotrophic respirations. Thus, there are evidences in the literature indicating a deviation from the linearity assumed by the Keeling plot method in nonsteady state diffusive environments [*Midwood and Millard*, 2011; *Nickerson and Risk*, 2009a; *Ohlsson*, 2009], which compromise the absolute comparison of the δ^{13}C keeling intercepts between dry and wet soil conditions in our experiment. According to these authors, this deviation depends on several factors, such as the parameter settings for each scenario (e.g., biological production rate, soil porosity and diffusivity) and the chamber design [*Ohlsson*, 2009], that affect the diffusive fluxes for $^{12}CO_2$ and $^{13}CO_2$ (the retardation effect) and a partial redirection of the flux to the atmosphere surrounding the chamber (the bypass effect). Thus, in their summer-dry scenario, *Nickerson and Risk* [2009b] found that simulations of the keeling plot method using a static chamber had an error of 0.92‰ higher relative to the source used as a reference. In their simulations, the chamber was 0.05 m height and inserted 2.5 cm into the soil. Comparatively, in our experiment lateral diffusion effect was intended to be minimized using collars inserted to 6 cm depth [*Hutchinson and Livingston*, 2001; *Ohlsson*, 2009]. Moreover, the time deployed to accumulate CO_2 before rewetting was 20 min. The time for CO_2 accumulation was a compromise between obtaining a range in concentration higher than 250 ppm, which reduces standard error in ^{13}C estimation [*Joos et al.*

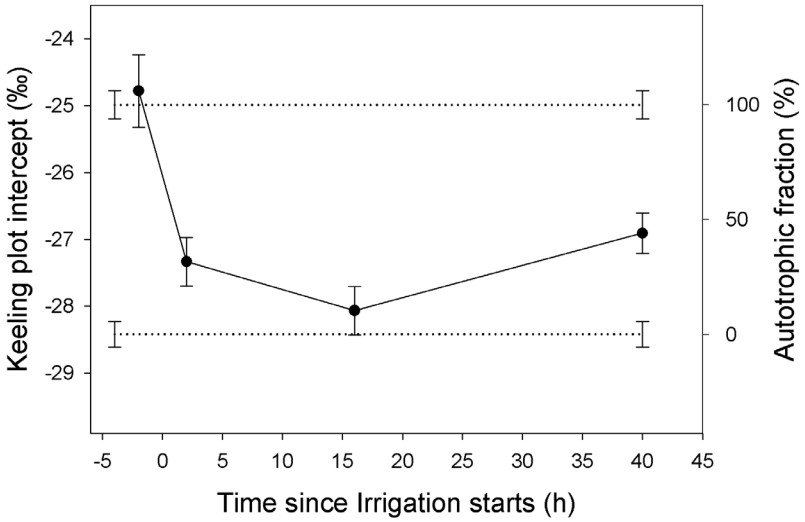

Figure 4. Change in the signature of the soil CO_2 efflux estimated as the Keeling plot intercept in each sampling time before and after irrigation starts. In each time, the Keeling plot intercept corresponds to the mean (±SE) of the intercept values of the three irrigations (Table 3). The resulting autotrophic fraction (%) estimated comparing the signature of the soil CO_2 efflux against the signature of CO_2 respired by both small roots and rewetted soil (dotted lines; Table 4) was included as an additional y axis.

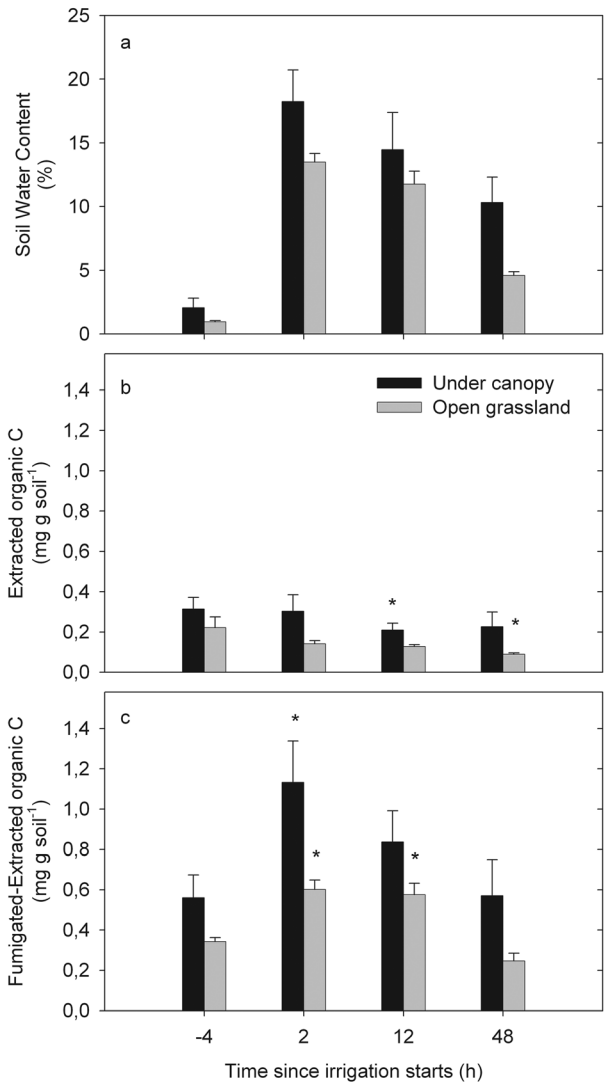

Figure 5. (a) Soil water content (% by weight, 105°C); (b) nonfumigated K_2SO_4-extracted organic C (EOC); and (c) chloroform-fumigated K_2SO_4-extracted organic C (CFE) in the first 5 cm of soil under tree canopy and in open grassland, during the 2008 irrigation event (4 h before and 2, 12, and 48 h after the start of irrigation). Data are mean ± SE (n = 4). Asterisks in EOC and CFE denote statistical difference compared with the level prior to irrigation (−4 h; repeated measurements test).

2008; *Ohlsson et al.*, 2005], and to reduce the overpressurized effect on the differential isotope diffusion between soil and chamber headspace or lateral diffusion [*Ohlsson*, 2009]. Finally, the soil production rate before rewetting was clearly lower than that used in their simulation. Thus, before rewetting, the retardation effect may produce a positive bias in our Keeling plot intercept estimations with respect to that of the source but it is expected to be lower than 1‰ in absolute values. After rewetting, in a high productivity and low diffusion scenario the bias is expected to be lower than that of dry soils. In their summer-wet scenario, *Nickerson and Risk* [2009b] found that the bias was reduced to 0.18‰.

[32] The rapid flush of CO_2 following a precipitation pulse was commonly attributed in part to the physical displacement of higher concentrations of CO_2 accumulated in pore spaces during dry periods and, thus, avoided to take into account in several drying-rewetting studies [*Huxman et al.*, 2004b; *Millard et al.*, 2008; *Thomas and Hoon*, 2010]. As discussed in the literature [e.g., *Lee et al.*, 2004], theoretical calculations show that the increase in CO_2 efflux could not be interpreted as the consequence of the air displacement by percolating water within the soil pores. Since the cumulated amount of irrigation 2 h after starting rewetting was about 4 mm and assuming a CO_2 concentration in the uppermost dry soil layer pores of about 1000 ppm, the rate of CO_2 displacement out of the soil caused by 4 mm of water will be less than 0.2 μmol CO_2 m^{-2} s^{-1} during the first 2 h. A magnitude clearly lower than the measured fluxes in the field following rewetting (9.9 μmol CO_2 m^{-2} s^{-1}). Moreover, this amount of water did not penetrate into the soil more than a few mm, and it is highly unlikely that the respiration rate of living tree roots had increased. Therefore, the increase in CO_2 efflux in the short-term flush after rewetting could not be interpreted as the consequence of air displacement by percolating water or enhancement of root activity in this Mediterranean ecosystem, where most of the herbs were senescent in summer. As, at this moment, the CO_2 efflux signature was similar to that of the in-tube soil respiration, heterotrophic component of respiration is expected to be dominant. However, the presumably heterogeneity, in terms of diffusivity, pore space or CO_2 production, of the first cm of soil shortly after starting the irrigation prevent from going in depth discussing small differences between 2 h or 16 h after rewetting.

4.2. Origin of Enriched Soil CO_2 During Drought Period

[33] Before rewetting, soil water content in the first 5 cm was lower than 3% (Figure 5 and *Casals et al.* [2009]). In these conditions, soil CO_2 efflux was enriched compared to the organic matter of soil or roots. *Rochette et al.* [1999] suggested that soil CO_2 signature in drought soils might be enriched with respect to that of the source due to a convective transfer downward from the atmosphere into the soil, when the temperature is colder at the soil surface than below. An alternative explanation may actually be related with root respiration. δ^{13}C of the CO_2 respired by oak roots was about −24.99‰, similar to that of soil CO_2 efflux before rewetting, but four units more enriched than the signatures of the root material or soil organic matter. This result was quite surprising and contrasts with the general assumption that root respiration is depleted in ^{13}C compared to root organic matter [*Bowling et al.*, 2008, and references herein]. It is worth noting that our result refers to excised root of oak trees and collected after ~2 months of sever drought. Oak is a slow growing perennial tree, with structural and functional strategies to cope with long drought periods [*Chiatante et al.*, 2005; *Ogaya and Peñuelas*, 2003], and clearly different from the fast growing or herbaceous plants commonly used in most other studies to estimated root respiration, generally growing in non-water-limited conditions [*Badeck et al.*, 2005; *Bathellier et al.*, 2008; *Klumpp et al.*, 2005; *Schnyder and Lattanzi*, 2005]. Our in-tube root CO_2 measurements agree with an enrichment of

CO_2 signatures in the ecosystem respiration during drought events detected by different authors [*Lai et al.*, 2005; *McDowell et al.*, 2004; *Pataki et al.*, 2003]. This enrichment may be related to changes in the respiration pathways from a low metabolic demand for respiratory products to a secondary compound metabolism for storage or maintenance [*Nogués et al.*, 2004; *Priault et al.*, 2009; *Tcherkez et al.*, 2003].

[34] CO_2 signature respired by tree leaves show rapid dynamics: from minutes to hours [*Unger et al.*, 2010; *Werner et al.*, 2007]. Interestingly, the diurnal amplitude decreased with drought [*Sun et al.*, 2009; *Unger et al.*, 2010]. Similarly, our results did not detect any differences in the CO_2 signature respired by root excised in the morning with respect to those collected in the evening. Furthermore, no clear differences were found between roots incubated 0.5, 2 or 30 h. Both results might suggest a dominant and constant pathway of *Q. ilex* root respiration during drought period.

[35] Little is known about root metabolism and C discrimination [*Bowling et al.*, 2008]. In summary, there are several possible mechanisms that may contribute to the enriched ^{13}C signature of the CO_2 respired by roots compared with that of root organic matter during long drought periods: (1) an enriched signature of the recent photosynthate substrate as a consequence of the adjustment of stomata closure to avoid dehydratation; (2) a fractionation during phloem loading or transport leading to ^{13}C-enriched metabolites for root respiration; and (3) a change of respiration substrates from growth to maintenance as drought increases. In addition, both root growth and the flow of C from the root system to the soil are reduced during drought [*Gorissen et al.*, 2004] and, consequently, rhizosphere activity decreases [*Borken and Matzner*, 2009]. Despite the signature of CO_2 derived from rhizosphere activity is seldom known, as bacteria and fungi are expected to be ^{13}C-enriched with respect their substrate [*Ehleringer et al.*, 2000, and references herein], CO_2 efflux should be depleted [*Bowling et al.*, 2008]. Thus, in addition to tree physiology, a dominance of root respiration with respect to rhizosphere activity may contribute to explain the apparent disparity of enriched root respiration compared to root organic matter, under severe drought conditions.

4.3. Origin of Depleted Soil CO_2 After Rewetting

[36] After rewetting, the δ^{13}C signature of soil CO_2 efflux was similar to that of the respired CO_2 by incubated rewetted soil as well as to the signature of the bulk soil organic matter and extractable C fraction (CFE). Our results suggest that the heterotrophic component of soil respiration becomes essentially dominant just after starting a summer rainfall. In temperate forest, *Cisneros-Dozal et al.* [2007] found that leaf litter decomposition represents a substantial source of soil respiration after rewetting (37 ± 8%). By contrast, our results suggested that standing dead mass and litter has only a small contribution to total soil efflux (12–16% in the first 2 h) and decrease sharply as it becomes dry. One possible explanation for this discrepancy may lie in the material that was considered as litter. *Cisneros-Dozal et al.* [2007] experiment included the respiration of semidecomposed leaf litter, whereas in our study the litter CO_2 efflux was estimated from standing dead mass and nonfragmented leaf litter. The rapid CO_2 flush observed 30 min after starting the irrigation experiment (irrigation amount of 1 mm), but also as a consequence of small natural rainfalls (<3 mm, [e.g., *Casals et al.*, 2009]), may be due to the mineralization of solutes released from dead microorganisms by exoenzymes and survival microorganisms [*Schimel and Weintraub*, 2003]. The decrease of CO_2 efflux per unit of litter within the first hours after wetting may reflect the exhaustion of easily mineralizable substrate in the standing dead plant mass and recent litter.

[37] The immediate recovery of soil microbial biomass after rewetting actually suggests that microbial decomposition of soil organic matter may be responsible for the rapid flush of CO_2 observed in the first hours of rewetting. The observed decrease of the EOC fraction evidences a gradual, although very rapid consumption of the available organic compounds within the soil solution, which is attributed to microbial respiration over the course of the rewetting event. Thus, microbial mineralization prompted a significant reduction of the soil respiration substrate, similarly to what was observed by *Nguyen and Guckert* [2001]. These authors added ^{14}C-glucose to soil, resulting in an extremely fast microbial mineralization (peaking during the first 10 min) associated to the consumption of that C source. In contrast to EOC fractions, the CFE (which includes EOC fraction and a given percentage of the soil microbial C [*Vance et al.*, 1987]) followed a very different evolution after rewetting. The twofold increase observed after 2 h from the beginning of rewetting is likely related to a rapid increase in microbial biomass. At the same time, rewetting the dry soil could have activated a great proportion of the soil microbes that were dormant or inactive and less affected by the chloroform fumigation [*Foster*, 1988; *Martin and Foster*, 1985].

[38] CO_2 signature of incubated soil was similar to that of CFE extracted 24 h after rewetting, made up to a great extent of microbial biomass. In contrast, depleted ^{13}C of the CFE fraction extracted before irrigation may relate to the low proportion of microbial biomass in the dry soil and the accumulation of residues from lignin decomposition. Accordingly, *Coyle et al.* [2009] found that extractable C was about 4‰–6‰ depleted compared to soil organic matter or microbial biomass. Unfortunately, the enrichment of the EOC could not be determined in our study due to the low C content of soil extracts.

4.4. Implications for a Net C Balance

[39] A sudden soil CO_2 release following rewetting of dry soils has been observed in many ecosystems [*Jarvis et al.*, 2007, and references herein]. In our rainfall simulation experiments, rewetting the dry soil led to an immediately and large pulse of CO_2 that extended over the following days. The extra amount of C-CO_2 released in each event compared to the nonirrigated plots was 14.98 ± 2.13 g C m^{-2} (mean ± SE of the four events) during the first 5 days (120 h) after irrigation. This flux represents about 15% of the net ecosystem exchange measured by Eddy Covariance in a nearby nonirrigated plot in the same dehesa site (an estimated annual NEE of −80.2 and −99.0 g m^{-2} in 2006 and 2008, respectively). The dominance of the heterotrophic component in these rapid CO_2 flushes actually denotes that summer precipitation pulses may liberate into the atmosphere important quantities of C from soil organic matter

decomposition. However, the long-term effect on ecosystem C balance remains an issue, as changes in the frequency of rainfall are expected to affect plant physiology as well, an so net primary productivity of the ecosystem [*Huxman et al.* 2004a; *Borken and Matzner*, 2009].

5. Conclusions

[40] Our study provides evidence that, in a Mediterranean dehesa, a woodland ecosystem with scattered oak trees over an annual pasture mostly dead in summer, the autotrophic component of soil CO_2 efflux was largely dominant during the long periods of drought. In contrast, the heterotrophic component became dominant from just the rewetting moment to several days after, as evidenced by ^{13}C-depleted soil CO_2 efflux. As standing dead mass and recent litter contribution was low during the first hours and negligible after the first day as it dried, we concluded that CO_2 efflux after rewetting was mostly derived from microbial mineralization of available soil organic C fractions.

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