Seasonal soil and leaf CO$_2$ exchange rates in a Mediterranean holm oak forest and their responses to drought conditions

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Abstract

We measured the soil and leaf CO$_2$ exchange in Quercus ilex and Phillyrea latifolia seasonally throughout the year in a representative site of the Mediterranean region, a natural holm oak forest growing in the Prades Mountains in southeastern Catalonia. In the wet seasons (spring and autumn), we experimentally decreased soil moisture by 30%, by excluding rainfall and water runoff in 12 plots, 1$\times$10 m, and left 12 further plots as controls. Our aim was to predict the response of these gas exchanges to the drought forecasted for the next decades for this region by GCM and ecophysiological models.

Annual average soil CO$_2$ exchange rate was 2.27 ± 0.27 μmol CO$_2$ m$^{-2}$ s$^{-1}$. Annual average leaf CO$_2$ exchange rates were 8 ± 1 and 5 ± 1 μmol m$^{-2}$ s$^{-1}$ in Q. ilex and P. latifolia, respectively. Soil respiration rates in control treatments followed a seasonal pattern similar to photosynthetic activity. They reached maximum values in spring and autumn (2.5–3.8 μmol m$^{-2}$ s$^{-1}$ soil CO$_2$ emission rates and 7–15 μmol m$^{-2}$ s$^{-1}$ net photosynthetic rates) and minimum values (almost 0 for both variables) in summer, showing that soil moisture was the most important factor driving the soil microbial activity and the photosynthetic activity of plants. In autumn, drought treatment strongly decreased net photosynthesis rates and stomatal conductance of Q. ilex by 44% and 53%, respectively. Soil respiration was also reduced by 43% under drought treatment in the wet seasons. In summer there were larger soil CO$_2$ emissions in drought plots than in control plots, probably driven by autotrophic (roots) metabolism. The results indicate that leaf and soil CO$_2$ exchange may be strongly reduced (by ca. 44%) by the predicted decreases of soil water availability in the next decades. Long-term studies are needed to confirm these predictions or to find out possible acclimation of those processes.

Keywords: Soil CO$_2$ exchange; Foliar net photosynthetic rates; Mediterranean holm oak forest; Climate change; Drought; Roots; Microorganisms

1. Introduction

Soil respiration and foliar photosynthesis represent large natural fluxes in the dynamics of carbon exchange. While net primary production estimates are 50 × 10$^{15}$ g C yr$^{-1}$ (Field et al., 1998) carbon losses by soils are estimated at approximately 75 × 10$^{15}$ g C yr$^{-1}$ (Schlesinger and Andrews, 2000). Therefore, the study of soil respiration is important to understand the balance between biospheric and atmospheric carbon.

Soil respiration and above-ground processes are linked because photosynthesis supplies carbon substrate for root metabolism and nutrition. Root
metabolism produces the release of exudates to the rhizosphere and these carbon-rich substances supply organic residues to decomposers (Schlesinger and Andrews, 2000; Ryan and Law, 2005). Concurrent study of soil respiration and plant activity (photosynthesis and conductance) can provide more insight in the understanding of terrestrial carbon cycling and fluxes between the atmosphere and the terrestrial biosphere.

Soil respiration includes two principal below-ground processes: autotrophic and heterotrophic respiration (Hanson et al., 2000). The autotrophic respiration results from the growth and maintenance of roots and associated rhizosphere microorganisms (Pendall et al., 2004). The heterotrophic respiration is the sum of heterotrophic bacteria and fungi activity and soil faunal activity (Hanson et al., 2000). The proportion of soil respiration from autotrophic and heterotrophic contributions may vary seasonally and among ecosystems (Hanson et al., 2000) and may respond differently to environmental factors (Ryan and Law, 2005).

We conducted a study in a typical Mediterranean holm oak forest. The dominant species, *Quercus ilex* L. and *Phyllirea latifolia* L. are widely distributed in the Mediterranean basin. Both species are well adapted to drought, although *P. latifolia* has been described as more drought resistant than *Q. ilex* (Tretiach, 1993; Peñuelas et al., 2001; Ogaya and Peñuelas, 2003a, b).

The goals of our study were (i) to assess the seasonal CO2 gas exchange from soils and plants, (ii) to test their dependence on abiotic factors such as soil moisture and temperature, (iii) to study the linkage between above- and below-ground exchange processes and (iv) to study the response of soil and leaf CO2 exchange to the lower soil water availability predicted for the next decades for Mediterranean ecosystems by IPCC and ecophysiological models (IPCC, 2001; Sabate et al., 2002; Peñuelas et al., 2005).

2. Material and methods

2.1. Sampling site

This study was conducted between Spring 2003 and Spring 2004. Measurements were carried out in a natural holm oak forest growing in the Prades Mountain region, in southern Catalonia (41°13’N, 0°55’E), on a south-facing slope (25% slope) at 930 m above sea level. The soil is a stony xerochrept on a bedrock of metamorphic sandstone, and its depth ranges between 35 and 90 cm. The average annual temperature is 12°C and the annual rainfall 658 mm. Summer drought occurs approximately from mid-June to mid-September. The vegetation of the area is short holm oak forest characterised by 3- or 4-m tall trees and shrubs. This forest is dominated by *Q. ilex* L. *P. latifolia* L. is also very abundant. *Arbutus unedo* L., some shrubs of *Erica arborea* L., *Juniperus oxycedrus* L. and *Cistus albidus* L. and occasional individuals of deciduous species (*Sorbus torminalis* L. Crantz and *Acer monspessulanum* L.) occur occasionally (Ogaya and Peñuelas, 2003a, b).

2.2. Experimental design

Twenty-four 1 × 10 m plots were randomly distributed at the same altitude along the slope in the study area. Half of the plots were subjected to a drought treatment and the remainder plots were control plots. The drought treatment consisted of rainfall exclusion by suspending transparent PVC strips at a height of 0.5–0.8 m above the soil. In addition a 0.8–1 m deep ditch was excavated along the entire 1 m top edge of the upper part of the treatment plots to intercept runoff water. Water intercepted by strips and ditches was drained to an area outside and downhill of the plots. Rainfall exclusion by plastic strips does not affect the light interception by the trees because the whole tree canopies are located above the plastic strips.

Litterfall on the plastic strips was moved underneath them each month to sustain the humic composition of the soil. Therefore any nutrient differences below and outside the strips were due only to the change in water available for decomposition of this litterfall.

Drought treatment started in March 1999 and continues to the present.

2.3. Measurements of soil CO2 flux, temperature and moisture

Soil respiration was measured in situ using a flow-through chamber method and an infrared gas analyser system (EGM-4, PP Systems, Hitchin, Hertfordshire, England). A vented soil chamber system was performed with PVC collars (12.5 cm in diameter and 8 cm in height) installed permanently 3–4 cm into the soil. The collars were covered by a PVC lid with two outlets. One outlet was connected...
to the IRGA analyser by a Teflon tube. The other outlet was open to exterior air entry. Air inside the chamber was flowed (constant flux 0.4 L min\(^{-1}\)) to the CO\(_2\) analyser by the EGM-4 integral DC pump. The flow was measured with a bubbler flowmeter. Equilibration of CO\(_2\) concentration in the effluent stream occurred after 20 min. Before the collar was covered, we measured exterior air CO\(_2\) concentrations. Net soil CO\(_2\) fluxes were calculated by considering the stable difference in CO\(_2\) concentration between the outlet and the inlet air. Measurements were automatically corrected for temperature and pressure by the EGM-4 analyser. The accuracy of CO\(_2\) measurements was estimated as 1%. Stability of the measurements were assured with the periodic “Auto-Zero” resulting in automatic correction for sample cell contamination, source aging, detector sensitivity variations and pre-amplifier gain changes.

Twelve collars in both control and drought plots (one collar per plot, \(n = 12\)) were distributed randomly. The collars were installed in Winter 2002 and they were permanently placed into soil, in order to minimise possible effects of the mechanical disturbance during measurements. Before sampling litter recently fallen inside the PVC collars was removed to obtain CO\(_2\) emissions only from soil roots and soil microorganisms. We measured one soil respiration value per collar.

Soil temperature and moisture were measured at 10 cm depth, just beside each PVC collar to avoid mechanical disturbances to the enclosed soil. Soil temperature above the soil surface (air temperature) was also measured. A soil digital thermometer was used to measure temperature (TO 15, Jules Richard instruments, Argenteuil, France) and a HH2 soil moisture meter connected to a ML2x soil moisture sensor (Delta-T Devices Ltd., Cambridge, England) was used to measure soil moisture.

2.4. Leaf CO\(_2\) exchange rates and leaf water status measurements

Leaf net CO\(_2\) exchange rates (\(A\)) and stomatal conductances (\(g_s\)) were measured in situ with a portable gas exchange system CIRAS2 (PP Systems, Hitchin, Hertfordshire, UK) at a 1500 \(\mu\)mol m\(^{-2}\) h\(^{-1}\) PPFD. Intact leaves were clamped in a Parkinson leaf cuvette (Std Broad 2.5, PP Systems, Hitchin, UK) connected to the CIRAS2.

Conductance for water vapour was calculated as:

\[
g_s = 1/r_s \quad \text{where} \quad r_s \text{ is the stomatal resistance to water vapour}.
\]

vapour: \(r_s = (W_{\text{leaf}} - W_{\text{an}})/\Delta W \times u_s - r_b\), and where:

\[
W_{\text{leaf}} = e_s/p, \quad e_s \text{ the saturated vapour pressure at leaf surface, } p \text{ the atmospheric pressure, } \Delta W \text{ the water vapour differential across leaf chamber, } W_{\text{an}} \text{ the water vapour concentration out of leaf chamber, } r_b \text{ the boundary layer resistance to water vapour and } u_s \text{ the mass flow of air per m}^2 \text{ of leaf area}.
\]

Net photosynthetic rate and stomatal conductance were measured in one sunlit leaf of \(Q. \text{ ilex}\) and one sunlit leaf of \(P. \text{ latifolia}\) per plot. We conducted these measurements in six control and six drought plots, which had accessible leaves to manual sampling. Sampled leaves had always the same age and similar position within the canopy.

Water potential was measured in one terminal twig of two different plants per species, in control and drought plots, using a Scholander pressure chamber (PMS, Corvallis, OR, USA). Relative water content (RWC) was measured early in the morning for five sunlit leaves of \(Q. \text{ ilex}\) and \(P. \text{ latifolia}\) in each plot. RWC was calculated as:

\[
\text{RWC} = (M_F - M_D)/(M_F - M_D), \quad \text{where } M_F \text{ is leaf fresh mass, } M_D \text{ is leaf dry mass and } M_T \text{ is leaf turgor mass, measured as water saturated leaf weight after 10–12 h in water saturating conditions (petiole in water).}
\]

2.5. Sampling strategy

Measurement campaigns were carried out during three consecutive sunny days in each season: spring 2003 (April 22, 23 and 24), summer 2003 (August 12, 13 and 14), autumn 2003 (November 3, 4 and 5), winter 2004 (February 17 and 18) and spring 2004 (April 21, 22 and 23). Soil and leaf CO\(_2\) exchange rates were measured during the mornings (from 7 to 11 a.m.). Soil respiration measurements in each plot took 20 min. The interval sample from plot to plot was 15 min. Net photosynthetic rates measurements took 15 min per plot. The interval sample between plots was 20 min. RWC was measured early in the morning, and leaf water potential at midday.

2.6. Statistical analyses

Repeated measures analyses of variance (ANOVA) were conducted with soil CO\(_2\) fluxes, soil moisture and temperature, leaf CO\(_2\) exchange rates, RWC and leaf water potential as dependent variables and with treatment and season as independent factors. Data were log transformed when necessary to meet the ANOVA assumptions. All
analyses were performed with STATVIEW 5.01 software package (Abacus Concepts Inc., 1998).

3. Results

Annual average soil temperature and moisture during the sampling period were 13 ± 3 °C and 17 ± 5%, respectively. We did not find significant differences in the soil surface temperature between control and drought plots (data not shown). Soil temperature at 10 cm depth was significantly higher in drought plots than in control plots only in November 2003 (Fig. 1), a season with no significant differences in soil respiration between treatments (Fig. 2). The lowest values of soil moisture were found in summer (1.6 ± 0.3%), coinciding with maximum temperatures (25 ± 1 °C). The drought treatment decreased soil moisture by 30% in spring and autumn (p<0.0001) when soil moisture was at maximum values for the year (Fig. 1a).

Mean values of soil CO2 efflux ranged from 2.00 to 2.53 μmol CO2 m⁻² s⁻¹ for control treatment and from 1.64 to 1.92 μmol CO2 m⁻² s⁻¹ for drought treatment (Fig. 2). There were seasonal variations in soil respiration during the year with the highest values in the springs (3.22 ± 0.49 and 3.76 ± 0.85 μmol CO2 m⁻² s⁻¹) and the lowest values in summer (0.13 ± 0.01 μmol CO2 m⁻² s⁻¹). Significant differences between drought and control plots in soil CO2 fluxes were found only in the spring seasons when they were higher in control plots (p<0.05 and < 0.1, respectively), and in summer when CO2 fluxes were higher in drought plots (control 0.13 ± 0.01 μmol m⁻² s⁻¹, drought 0.75 ± 0.18 μmol m⁻² s⁻¹; p<0.01) (Fig. 2).

![Fig. 1. Seasonal course of soil moisture and soil temperature.](image1)

![Fig. 2. Seasonal course of soil CO2 emission rates during the studied period.](image2)
*P. latifolia* exhibited lower annual average of leaf water potential values than *Q. ilex* (−2.9 ± 0.2 and −2.1 ± 0.1 MPa, respectively). Both species had maximum values in spring and autumn, and minimum values in summer (Fig. 3a and b). The summer drought response was greater in *P. latifolia*, with values that reached −6.7 ± 0.3 MPa, while values for *Q. ilex* only reached −3.1 ± 0.1 MPa. Slightly significant differences between treatments were found in spring 2004 in *Q. ilex* and *P. latifolia* (*p* = 0.056 and 0.059, respectively) (Fig. 3a,b).

Leaf water potentials were strongly correlated with soil moisture (logarithmic regression: *R* = 0.92, *n* = 10, *p* = 0.0001 in *Q. ilex* and *R* = 0.94, *n* = 10, *p* < 0.0001 in *P. latifolia*).

Annual mean values of RWC were similar in both species (0.83 ± 0.01 for *Q. ilex* and 0.80 ± 0.02 for *P. latifolia*). They showed a variation pattern similar to the LWP (Fig. 3c,d), especially in control plants, although the range of variation in each species was different. *P. latifolia* minimum in summer (0.63 ± 0.07), and maximum in both spring and autumn, with slightly lower values in drought than in control plots treatments in the autumn season (*p* < 0.1). For *Q. ilex* RWC values were constant throughout the year. No overall significant effects of drought treatment were detected (Fig. 3c,d).

Annual leaf CO₂ exchange rates in *Q. ilex* were higher than in *P. latifolia* (8 ± 1 and 5 ± 1 μmol m⁻² s⁻¹ respectively, Fig. 4a and b). Both species’ leaf CO₂ exchange was minimum in summer with values near to 0 or even slightly negative in *P. latifolia*. The maximum values in both species were found in spring and autumn, when the water availability was high (Fig. 1a,b).

Leaf CO₂ exchange rates were well correlated to leaf water potentials (logarithmic regression: *r*² = 0.62, *n* = 8, *p* < 0.05 in *Q. ilex* and *r*² = 0.83, *n* = 8, *p* < 0.005 in *P. latifolia*). There were significant decreases (45%) of leaf CO₂ exchange in drought plots in spring (*P. latifolia, p* < 0.05) and autumn (*Q. ilex, p* < 0.1), when there was rain to be excluded, i.e., in the raining seasons, spring and autumn.

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**Fig. 3.** Seasonal course of leaf water potential in the two dominant plant species of the studied holm oak forest, *Q. ilex* (a) and *P. latifolia* (b), seasonal course of relative water content in *Q. ilex* (c) and *P. latifolia* (d). Vertical bars indicate standard errors of the mean (*n* = 8 samples per season, treatment and species). One asterisk indicates significant differences between the two treatments (*p* < 0.1) in each season. No overall significant drought effect was found for any of the two species (repeated measurements ANOVA).
autumn, and therefore there were significant effects of treatment on soil moisture (Fig. 1a).

Stomatal conductances were usually higher in *Q. ilex* than in *P. latifolia* in all seasons (annual mean values 75 ± 9 and 45 ± 9 mmol m⁻² s⁻¹, respectively) and varied with net photosynthetic rates (Fig. 4c and d). Significant decreases in *gₛ* in drought treatments for *Q. ilex* were found in summer and autumn (*p < 0.1* and *p < 0.05*, respectively), whereas *P. latifolia* showed similar *gₛ* values in control and drought plots during the year, except for a slightly significant decrease in drought plots in spring (*p < 0.1*) (Fig. 4c and d).

### 4. Discussion

#### 4.1. Seasonal course of soil CO₂ exchange rates in control and drought treatments

The 30% soil moisture decrease in the wet seasons and the lower soil CO₂ fluxes and photosynthetic rates measured in drought plots show the effectiveness of the drought treatment. Root growth towards water sources beyond the influence of plastic strips in drought plots could be possible. However, the reduction of growth rates reported in this experimental system (Ogaya et al., 2003), in addition to our results, show that water availability is lower in drought plots and the whole plant is affected by this water reduction.

Soil respiration values coincided with values reported for other Mediterranean forests (Joffre et al., 2003) but they were higher than values reported for the same study area in previous reports (Piñol et al., 1995). The increase of the annual soil temperature in the 2003–04 period compared with 1991–92 may account for the differences.

In both spring and autumn, the wet seasons, the plant and microbial activities were high. This was also reflected in the seasonal patterns of leaf CO₂ uptake (Fig. 5). These results contrast with those...
reported for a southern boreal aspen forest (Griffis et al., 2004), where seasonal variability in soil respiration was mainly controlled by temperature with maximum rates in summer. In Mediterranean ecosystems, water is the principal factor controlling most of the above- and below-ground processes resulting in a soil-moisture-dependent seasonal pattern for soil CO₂ emissions and leaf CO₂ uptake.

Soil respiration is well correlated with microbial activity (Orchand and Cook, 1983). During summer drought, the physiological activity of microorganisms in response to the increase in temperature appears to be constrained by low soil moisture (Conant et al., 2004, Martin and Bolstad, 2005). Similarly, drought treatment reduced soil respiration by 43% \( (p < 0.05) \) during the rainy season in spring (Fig. 2). The reduction of soil respiration by excluding rainfall was found also by Borken et al. (1999, 2006) in similar studies on the soil respiration responses to experimental drought. To what extent is this reduction caused by lower microbial activity is unknown. Borken et al. (2006) suggest a stronger effect of drought in heterotrophs than in autotrophs because they observed under drought smaller decreases in photosynthesis than in soil respiration. In our study, photosynthetic rates decreased by 40% in drought plots in spring only in \( P. \) latifolia (Fig. 4). Moreover, it is known that the root contribution to total soil respiration is higher during the growing season (Tang et al., 2005); thus, the reduction in the CO₂ efflux measured in drought plots in spring might be attributed to lower microbial activity.

Nevertheless results showed a different response of soil CO₂ emissions to drought treatment in wet spring compared with the dry summer. In summer, CO₂ emissions in drought plots were higher than in control plots which were practically zero (Fig. 2). Soil moisture in summer was very low in both treatments (Fig 1a), but plants and microorganisms under drought treatment had been subjected to a lower water availability for a longer time than those in control plots. Prolonged low water availability in the drought treatment plots might have favoured root growth in those plots, resulting in enhanced root respiration. There are several reasons for high root respiration rate under severe drought stress (Li et al., 2004) in addition to the physical larger root surface. A change in the plant source–sink relationship may lead to a greater proportion of assimilates transferring to roots, providing more substrate supply for root respiration. This could be a strategy to improve the nutrient availability and water uptake for the drought-stressed plant. Thus, in summer, CO₂ emissions in drought plots could be mostly driven by autotrophic metabolism as a result of changes in the importance of root versus soil microbial activity. Although this hypothesis needs further investigation, the significant correlation \( (r^2 = 0.56, p < 0.05) \) between leaf and soil CO₂ exchange in summer in drought plots, that was not found in control plots \( (r^2 = 0.0001, p = 0.76) \), provides support for the hypothesis that prolonged low water availability favour root growth.

4.2. Coupling between soil respiration and leaf CO₂ exchange

Numerous studies of leaf gas exchange have demonstrated similar leaf responses to those described here of net photosynthetic rates decreasing
from spring to summer (Fig. 4) with increasing drought (Oechel et al., 1981; Tenhunen et al., 1990; Peñuelas et al., 1998; Ogaya and Peñuelas, 2003a, b). For instance, in a Mediterranean maquis characterised by tall shrubs in Castelporziano (Italy), the seasonal variation of leaf CO2 exchange rates and stomatal conductances (Gratani and Varone, 2004) were very similar to those found at Prades. The study of soil and leaf CO2 exchange throughout the year showed that soil moisture and temperature were the main factors driving CO2 exchange.

Root respiration comprises a significant fraction of soil respiration (Irvine et al., 2005) and it strongly reflects plant metabolism (Ekblad and Högb erg, 2001). We have found a significant correlation between photosynthetic rates and soil respiration rates (Fig. 6). However, this could be an indirect relationship resulting from seasonal variations of temperature, moisture and phenology. Moreover, there could be time lags between the assimilation of carbon in leaves and the carbon transport to the roots (McDowell et al., 2004). Further studies considering autotrophic versus heterotrophic respiration separately in a high frequency of measurements over a number of days are needed to further confirm this correlation and gain knowledge on the relationships between above- and below-ground plant processes. Although our experimental methods did not allow separation of the two components of the total soil respiration, seasonal data correlation between carbon fixation and soil respiration in control plots suggests a link between both the variables.

The different soil respiration response to drought in spring and summer highlights the need of a better understanding of the contribution of autotrophic respiration to total soil CO2 exchange. The results suggested changes in drought conditions towards a decrease in the microorganisms/roots ratio of activities in the rhizosphere, especially in summer.

Finally, coupled GCM and ecophysiology models predict a 20–30% decrease in water availability over the next three or four decades (IPCC, 2001, Peñuelas et al., 2005). Our results suggest a 44% reduction in soil and foliar CO2 exchange rates in wet seasons in response to this decrease in water availability, demonstrating the importance of considering climate change effects on soil CO2 flux and foliar CO2 uptake in the budgeting of carbon in the atmosphere and the biosphere. However, acclimation of soil respiration and photosynthesis to prolonged drought could occur and therefore long-term studies of soil and leaf CO2 exchange are needed to discern the climate change effects on soil CO2 fluxes.

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