Components, drivers, and temporal dynamics of ecosystem respiration in a Mediterranean pine forest

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Abstract

To investigate the climate impacts on the different components of ecosystem respiration, we combined soil efflux data from a tree-girdling experiment with eddy covariance CO2 fluxes in a Mediterranean maritime pine (Pinus pinaster) forest in Central Italy. 73 trees were stem girdled to stop the flow of photosynthates from the canopy to the roots, and weekly soil respiration surveys were carried out for one year. Heterotrophic respiration (R_h) was estimated from the soil CO2 flux measured in girdled plots, and rhizosphere respiration (R_ab) was calculated as the difference between respiration from controls (R_s) and girdled plots (R_g).

Results show that the R_s dynamics were clearly driven by R_d (average R_d/R_s ratio 0.74). R_d predictably responded to environmental variables, being predominantly controlled by soil water availability during the hot and dry growing season (May–October) and by soil temperature during the wetter and colder months (November–March). High R_d and R_h peaks were recorded after rain pulses greater than 10 mm on dry soil, indicating that large soil carbon emissions were driven by the rapid microbial oxidation of labile carbon compounds. We also observed a time-lag of one week between water pulses and R_d peaks, which might be due to the delay in the translocation of recently assimilated photosynthates from the canopy to the root system. At the ecosystem scale, total autotrophic respiration (R_at, i.e. the sum of carbon respired by the rhizosphere and aboveground biomass) amounted to 60% of ecosystem respiration. R_at was predominantly controlled by photosynthesis, and showed high temperature sensitivity (Q10) only during the wet periods. Despite the fact that the study coincided with an anomalous dry year and results might therefore not represent a general pattern, these data highlight the complex climatic control of the respiratory processes responsible for ecosystem CO2 emissions.

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1. Introduction

The amount of carbon released by ecosystem respiration represents the second largest CO2 flux after photosynthesis (IPCC, 2013). In order to predict likely changes of the terrestrial carbon balance under varying environmental conditions, it is fundamental to quantify the different sources of the ecosystem CO2 efflux and their dependence on biotic and environmental drivers. The total flux of CO2 released from the ecosystem (R_co2) can be partitioned into soil respiration (R_s) and aboveground autotrophic respiration (R_at). R_s can be further partitioned into that originating from roots and closely associated microorganisms, such as mycorrhizas (i.e. belowground autotrophic or rhizosphere respiration, R_ab) and the flux produced from the decomposition of dead organic matter (i.e. microbial or heterotrophic respiration, R_h) (Eq. (1), Hanson et al., 2000; Kuzyakov, 2006; Heinemeyer et al., 2007):

\[ R_{co2} = R_s + R_{ab}, \quad R_s = R_{ab} + R_h \] (1)
The monitoring of the total soil CO\textsubscript{2} efflux can be performed with robust and consolidated methods, such as static or dynamic chamber systems equipped with infrared gas analysers (Davidson et al., 2002). However, the partitioning of \( R_{\text{eco}} \) and \( R_S \) into individual sources remains a challenging task, for which several techniques have been developed and tested over the past few decades. Most of these methods cannot be applied to forests since they are often impractical and/or limited in their spatial and temporal analysis of the phenomena (for a review, see Hanson et al. (2000) and Kuzyakov (2006)).

In the past decade, the tree girdling approach has been successfully applied to forest ecosystems (Hogberg et al., 2001; Bhupinderpal-Singh et al., 2003; Subke et al., 2011). The method is based on the removal of the bark and outer vascular tissues (phloem) to stop the flow of photosynthates to the roots, which causes a rapid and strong suppression of root and rhizomicrobial respiration (Hogberg et al., 2001). Soil respiration measurements performed on girdled plots after an initial settling-down period can be used to directly derive \( R_H \) (Hogberg et al., 2001). Tree-girdling can lead to an overestimation of the proportion of \( R_H \) due to roots’ residual respiration and decomposition (Bhupinderpal-Singh et al., 2003). However, compared to other partition techniques (i.e. root smoothing), girdling has the advantage of preserving the soil–root structural integrity and the xylematic water transport, so that soil moisture and temperature are generally unaltered for several months after the treatment (Kuzyakov, 2006; Ekberg et al., 2007)

Published results of tree girdling experiments report that the \( R_{\text{At}} \) contribution to \( R_S \) ranges from 24 to 65% in forest soils across different biomes and ecosystem types (Hogberg et al., 2001; Bhupinderpal-Singh et al., 2003; Andersen et al., 2005; Olsson et al., 2005; Binkley et al., 2006; Frey et al., 2006; Scott-Denton et al., 2006; Johnsen et al., 2007; Hogberg et al., 2009; Chen et al., 2010; Subke et al., 2011; Levy-Varon et al., 2012; Bloemen et al., 2014). However, most of these girdling experiments have been performed in boreal, temperate or subtropical forests, and there is a general lack of information on water-limited environments such as Mediterranean ecosystems.

Mediterranean forests and shrublands cover an area of about 2.75 million km\textsuperscript{2} (Rambal, 2001), and are characterised by high inter-annual climate variability (Luterbacher et al., 2006). Several studies highlight the fact that soil and ecosystem carbon fluxes in these ecosystems are strongly affected by the erratic seasonal and inter-annual distribution of rain events (Valentini et al., 2000; Almagro et al., 2009; Jongen et al., 2011; Ross et al., 2012; Poulter et al., 2014). While \( R_S \) is generally constrained by low soil water content during summer months, abrupt and large soil CO\textsubscript{2} pulses have been observed after rewetting the dry soil (Birch, 1958; Jarvis et al., 2007; Inglima et al., 2009; Unger et al., 2012; Matteucci et al., 2014). These intense emissions can substantially affect the annual carbon balance and highlight the vulnerability of soil carbon stocks in these ecosystems (Borken and Matzner, 2009; Moyano et al., 2013). The Mediterranean climate is expected to be affected by a reduction in the intensity and distribution of rainfall (Trenberth et al., 2003; Tebaldi et al., 2006; Christensen et al., 2007; Trenberth, 2011; IPCC, 2013). The prediction of the impacts of these climate changes on soil carbon dynamics requires new insights into and observations of the processes and drivers that affect the different components of respiration.

For this purpose we present the results of a tree girdling experiment carried out in a Mediterranean maritime pine forest (\textit{Pinus pinaster}) in Central Italy, which is equipped with an eddy covariance micrometeorological tower. Ecosystem fluxes were analysed together with soil CO\textsubscript{2} fluxes throughout the first year following the girdling treatment. The novel combination of these two methodologies allowed us to quantify all the different components of ecosystem respiration and to: (1) partition soil fluxes into their autotrophic and heterotrophic components; (2) partition total autotrophic respiration (\( R_{\text{At}} \)) into above- and belowground sources; (3) follow the seasonal evolution of the partitioned components; (4) analyse in detail the environmental and biological drivers that affect the response of the different components of \( R_{\text{eco}} \).

The study period coincided with a severe drought interspersed with sporadic rain events. Particular attention was given to the temporal response of \( R_S \) to sudden rain pulses.

2. Materials and methods

2.1. Site description

The study site is located in a maritime pine forest in Central Italy (Tuscany). The experimental area lies within the boundaries of the Regional Park of San Rossore–Migliarino–Massaciuccoli (43°43’44” N, 10°17’13” E, 6 m a.s.l.), in an almost flat area (slope < 3%) characterised by the presence of sandy dunes and located between the Arno and Serchio rivers, 800 m inland from the sea coast.

The area is characterised by a typical Mediterranean climate, with humid and mild winters and dry and hot summers. The long-term (1980–2012) mean annual air temperature is 15.35 °C, with the highest value measured in August (24.16 °C) and the lowest in January (7.4 °C). Mean annual precipitation (1980–2012) amounts to 883 mm, 50% of this being concentrated in the autumn months. The driest month is July with a mean rainfall of 18 mm, while October and November are the wettest, with 138 mm each. Long-term climate data were obtained from a meteorological station (Regional Hydrologic Service of Tuscany) located 10 km from the study site. Meteorological data have been collected at the flux tower since the year 2000. The wind regime is characterised by a sea–land breeze circulation, i.e. the air flows quite predictably from the West (sea) during the day and from the East (land) during the night (Fig. 1, inset).

The dominant species is \textit{P. pinaster} Ait., with sparse \textit{Pinus pinea} L. and \textit{Quercus ilex} L. trees. The average stem density is 565 trees ha\textsuperscript{-1}, the average diameter at breast height is 29 cm, and the average canopy height is 18 m. Ground vegetation is represented by sparse \textit{Erica arborea}, \textit{Phyllirea angustifolia}, \textit{Rhamnus alaternus} and \textit{Myrtus communis}.

The vegetation was naturally renovated following a wildfire in 1944 and therefore, at the moment of this study, was 67 years old. Root biomass is concentrated at between 0 and 40 cm. Total root biomass in the first 25 cm of the soil profile is 2.2 kg fresh weight m\textsuperscript{-2}, and the root/shoot ratio is 0.18. The water table depth ranged from 69 cm in April 2011 to 174 cm in November 2011, and can be reached by the taproot of maritime pine (Zenone et al., 2008).

The soil is a sandy calcareous regosol with a content of 93% sand, 4% clay and 3% silt in the first 10 cm of the soil profile. The organic layer has a thickness of 2.7 ± 0.4 cm, 43.8% soil organic carbon content, a C/N ratio of 32.5 and a pH of 4.4. The carbon content and C/N ratio are, respectively, 13.9% and 13.5 in 10-cm deep mineral soil. The annual N deposition is about 12 kg N ha\textsuperscript{-1} (Rosenkranz et al., 2006).

2.2. Soil respiration measurements

In March 2011, six circular plots (20-m diameter) were delimited at the long-term eddy covariance experimental site of San Rossore. In order to minimise the impact of the girdling experiment (see below) on eddy covariance measurements, plots were located at
about 40 m south of the tower, in a west–east direction parallel to the prevailing wind directions (Fig. 1). Each plot was populated by an average of 25 maritime pine trees, while ground vegetation was absent or insignificant.

The soil CO2 efflux was measured on a weekly basis starting from 4 April 2011 within a radius of 2 m from the centre of each plot by means of a portable closed dynamic chamber system (EGM4 analyser with SRC-1 chamber, PP System, Amesbury MA, USA). In order to optimise the sampling of the spatial variability, four sampling points were selected in each of the six plots, according to a stratified sampling strategy in 12 randomly selected locations (Rodeghiero and Cescatti, 2008) (Fig. 1). In order to guarantee repeatability over time, soil respiration measurements were taken over pre-set fixed-positioned metal collars (10-cm diameter, 5-cm height) inserted 2 cm into the soil. The portable chamber was modified to guarantee an optimal seal with the metal collars, and the final volume (chamber volume plus collar head-space) was adjusted in the analyser settings. Superficial (0–10 cm) soil water content (SWC, Trime-HD handheld device with Trime-EZ time domain reflectometry probe, IMKO, Ettingen, DE) and superficial (5 cm) soil temperature (STP-1 soil temperature probe, PP System, Amesbury MA, USA) were recorded for each sampling point at the time of the soil respiration surveys. Measurements were taken between 11 a.m. and 3 p.m. (local time). From 4 April 2011 to 31 March 2012, 48 surveys were carried out, for a total of 1152 soil respiration measurements. A litter trap (0.5-m² area) was positioned in the centre of each plot to estimate litter input to the soil. Litter was collected at the end of each month, and transported to the laboratory where its dry-weight was calculated. The cumulated amount of litter in control and girdled plots were 1.1 and 1.3 kg dry weight m⁻² yr⁻¹, respectively (p > 0.05).

2.3. Partitioning soil respiration by tree girdling

On 4 May 2011, three of the original six soil respiration plots were randomly selected for a tree-girdling experiment (Fig. 1). All of the trees (73 in total) within the selected plots were stem girdled: a strip of bark (30 cm width) was removed from the trunk of the trees at about 1.5-m height, taking special care not to damage the underlying xylematic tissue. In order to detect short-term effects, soil respiration was measured every second day on both girdled and control plots for the first week after the girdling treatment, while the succeeding soil respiration surveys followed the weekly routine outlined above.

Using the tree-girdling approach, \( R_{HI} \) was estimated from the soil CO2 efflux measured in girdled plots, while \( R_{Ab} \) was calculated as the difference between the respiration from controls (\( R_a \)) and girdled plots (\( R_{Hi} \)), according to Eq. (1) (Hogberg et al., 2001; Bhupinderpal-Singh et al., 2003; Kuzyakov, 2006).

\[
\Delta R = R_{HI} = R_a - R_{Hi} = \frac{R_{Hi}}{R_{Hi} + R_{Hi}} \cdot \frac{R_{Hi} + R_{Hi}}{R_{Hi} + R_{Hi}} = \frac{R_{Hi}}{R_{Hi} + R_{Hi}} \cdot \frac{R_{Hi} + R_{Hi}}{R_{Hi} + R_{Hi}}
\]

2.4. Ecosystem CO2 flux measurement and partitioning

At the San Rossore experimental site, fluxes of CO2, H2O and sensible heat were measured continuously on a half-hourly basis starting from 1999 by means of the eddy covariance technique (Baldocchi et al., 1988). Net ecosystem CO2 exchange (NEE) was determined as the covariance between fluctuations in vertical wind speed (\( w' \)) and CO2 concentration (\( c' \)):

\[
F_c = \overline{w'c'},
\]

where the overbar denotes the time average. Measurements were performed using an open-path infrared gas analyser (IRGA; LI-7500, Li-Cor, Lincoln NE, USA) and a three-dimensional sonic anemometer (R3, Gill instruments, Lymington, UK) installed on top of a retractable mast at a height of 23 m (5 m above the forest canopy). Raw data were logged at 10 Hz on a local laptop connected to the sonic anemometer by means of EddyMeas software (Olaf Kolle, www.bgc-jena.mpg.de). Eddy fluxes were calculated as 30-min averages according to EUROFLUX methodology (Aubinet et al., 2000). The EdiRe software package (R. Clement,
University of Edinburgh, http://www.geos.ed.ac.uk/abs/research/micromet/EdiRe) was used for data processing, including frequency and Webb—Pearman—Leuning corrections. Half-hourly data were classified in three quality classes (QF = 0, 1, 2) according to the steady state test and the integral turbulence characteristics, as proposed by Foken and Wichura (1996). Data measured when the theoretical requirements of the eddy covariance technique were not met (QF 2) were discarded. Data were additionally filtered for friction velocity ($u^*$), and only fluxes observed during periods with $0.196 < u^* < 0.948$ were further considered. During the observation period, poor quality and missing data amounted to 10 and 11%, respectively, while data discarded after $u^*$-filtering amounted to 21%. On the whole, coverage with good data amounted to 58%.

Gaps in the dataset were replaced with the online eddy covariance data gap-filling and flux-partitioning tool (Reichstein et al., 2005; see also www.bgc-jena.mpg.de/~MDIwork/eddyproc/method.php). Using this online tool, the partitioning of the measured NEE into GPP and $R_{eco}$ is based on a stepwise procedure and algorithms (Owen et al., 2007). This method derives short-term temperature-dependent ecosystem respiration from night-time NEE in order to estimate the parameters of the Lloyd and Taylor (1994) equation:

$$R_{eco} = R_{ref} \times e^{(Eo \times [(1/T_{ref} - T_0) - (1/T_{ref} - T_0))]}$$

where $T_{ref}$ is 10 °C, $T_0$ is constant (−46.02 °C), $T_{air}$ is air temperature, $E_0$ is the activation energy, and $R_{ref}$ is the reference ecosystem respiration at 10 °C. Estimates for $R_{ref}$ are based on time windows of 10 days and a step of four days, assuming a constant value for $E_0$ during the year (Reichstein et al., 2005). $R_{eco}$ for each half-hour is finally calculated using the values of $R_{ref}$ and the constant $E_0$. GPP is then obtained as:

$$GPP = R_{eco} - NEE$$

Within this study we followed the convention of adopting positive values for both GPP and $R_{eco}$. Aboveground autotrophic respiration ($R_{Ab}$) was calculated by subtracting $R_{f}$ from $R_{eco}$ fluxes measured at the same time as $R_{f}$ measurements. Subsequently, $R_{At}$ was calculated as the sum of $R_{Ab}$ and $R_{Ab}$ derived from the tree-girdling partitioning experiment.

2.5. Meteorological data and environmental parameters

The soil microclimatology was sampled with a combination of sensors and loggers in the proximity of the girdling plots to maintain continuous recording of the SWC vertical profile at 10 cm, 50 cm and 120 cm (Trime-Mux 6, time domain reflectometry probes, IMKO, Ettingen, DE), soil temperature vertical profile at 3 cm, 15 cm and 50 cm (PT-1000 temperature sensors), and water table depth (Micro-Diver D60xxx, Schlumberger Water Services, Houston TX, USA). Rainfall was measured above the canopy with an ARG100 rain gauge (Environmental Measurements, North Shields, UK). Air temperature and relative humidity were measured using a thermistor and capacitive relative humidity sensor probe (RTF2, UMS, Munich, DE). The incoming short wavelength solar radiation and photosynthetic active radiation were measured with a Li-200 Pyranometer (Li-Cor, Lincoln NE, USA) and a sunshine sensor (BF3, Delta-T, Cambridge, UK).

Half-hourly averages of meteorological and environmental parameters were stored locally on a datalogging system (DL2e datalogger, Delta-T, Cambridge, UK).

2.6. Data analysis

The differences in soil respiration fluxes, soil water content (SWC) and soil temperature between girdled and control plots were statistically tested by two-way repeated measures ANOVA using girdling treatment and time as independent variables.

The relationship between temperature and respiration fluxes was fitted using the simple empirical exponential model proposed by Vant-Hoff:

$$R_x = R_0 \times e^{\beta T},$$

where $R_x$ is the measured respiration rate, $R_0$ is the respiration rate at 0 °C, $\beta$ is a temperature response coefficient, and T is either air or soil temperature. The temperature sensitivity coefficient describing the change in respiration caused by a change in temperature of 10 °C is defined as:

$$Q_{10} = \frac{R_{T_{10} \rightarrow \text{At}}}{R_{T_{0}}},$$

where $R_{T_{0}}$ and $R_{T_{10}}$ are the respiration rates at temperatures $T_0$ and $T_{10}$, respectively. $Q_{10}$ can be estimated from the coefficient $\beta$ of regression Equation (5), as follows:

$$Q_{10} = e^{10\beta}.$$

The temporal evolution of the apparent temperature—sensitivity coefficient ($Q_{10}$) was calculated using a two-months moving time-window shifted forward one week at a time until the end of the observation period. We found that the best fitting of Equation (5) was achieved with the superficial soil temperature (3-cm depth) for belowground fluxes ($R_{f}$, $R_{f1}$, and $R_{f2}$), and with air temperature for aboveground respiration ($R_{Ab}$). Therefore, we used these variables to estimate the $Q_{10}$ values of the different partitioned components. The standard error for $Q_{10}$ values was calculated according to Boone et al. (1998).

3. Results

3.1. Interannual and seasonal climate

The climate at the San Rossore site is historically contextualised in Fig. 2. With a total annual precipitation of only 584 mm, 2011 was the driest year since the establishment of the experimental site (Fig. 2a). The drought was particularly severe from May to October 2011 (approximately the length of the growing season) with a cumulated rainfall of 109 mm, 73% lower than the corresponding long-term average (Fig. 2b). Substantially lower precipitation also persisted from November 2011 to March 2012, with 258 mm cumulative rainfall, 36% lower than the long-term average. The period November 2011 – March 2012 was also the second coldest since the establishment of the experimental site (Fig. 2c).

The temporal evolution of the main environmental variables from 1 April 2011 to 31 March 2012 is illustrated in Fig. 3a and b. Consistent with the typical Mediterranean climate of the region, air and soil temperatures reached their highest values in August 2011 and their lowest values in February 2012, when an anomalous cold spell led air temperature to drop by 9 °C in a few days. Superficial volumetric soil water content (SWC at 10-cm depth) was strongly dependent on rainfall. Extended periods of drought (SWC < 5%) were recorded from May to October 2011, with scattered precipitation events of intensity > 10 mm day$^{-1}$ transiently raising the superficial SWC.
The carbon assimilated by photosynthesis (GPP) reached maximum values in June 2011, following the trend in the photosynthetic photon flux density (PPFD), and gradually declined with the onset of the cold season until the February 2012 minimum.

3.2. Partitioning soil respiration into autotrophic and heterotrophic components

$R_S$ in control plots ranged from 0.26 to 0.71 g CO$_2$ m$^{-2}$ h$^{-1}$ during May–October 2011, and from 0.14 to 0.60 g CO$_2$ m$^{-2}$ h$^{-1}$ from November 2011 to March 2012 (Fig. 3c). Large soil CO$_2$ emissions were recorded when rain levels greater than 10 mm day$^{-1}$ fell on the dry soil. A strong suppression of $R_S$ was measured during the cold period of February 2012.

Tree girdling significantly reduced the soil carbon efflux. The first statistically significant difference was recorded on 16 May 2011, 12 days after the girdling treatment, when soil respiration measured on girdled plots was 24% lower than on the control plots. The difference between the control and the girdled plots reached 30% after 20 days and 36% after two months, and remained statistically significant throughout the whole observation period (two-way repeated measures ANOVA: whole period, $F = 8.2$, $p < 0.01$; May–October, $F = 10$, $p < 0.01$; November–March, $F = 4.8$, $p < 0.05$).

Overall, the total amount of CO$_2$ released from the control ($R_S$) and girdled ($R_H$) plots over the whole observation period approximated to 872 and 649 g C m$^{-2}$, respectively; this resulted in an average estimate of $R_H/R_S = 0.74$ and $R_Ab/R_S = 0.26$ (Table 1).

No significant difference in SWC and soil temperature was observed between the control and the girdled plots throughout the experiment. The canopy of girdled trees started to show the first signs of yellowing in January 2012, and had dried up almost completely by the end of June 2012. Nonetheless, during the observation period, litter input and light and rain interception within the experimental plots did not show any significant difference between treatments (data not shown).

3.3. Partitioning ecosystem respiration into above- and belowground respiration

Fig. 4 shows the relative contribution of the different respiratory components of $R_{eco}$. $R_S$ was the component with the largest temporal variability, ranging from 30 to 40% of $R_{eco}$ during the driest periods, and up to 60–80% of $R_{eco}$ during the wettest periods. Most of the variability in the $R_S/R_{eco}$ ratio was clearly due to $R_H$, whose dynamics were in turn principally related to the superficial SWC. $R_{Ab}/R_{eco}$ was less variable, while total autotrophic respiration ($R_{At} = R_{Aa} + R_{Ab}$) was generally higher during the growing season.
than during the cold months (November 2011–March 2012) (62% and 53% of \( R_{\text{eco}} \), respectively). On the whole, \( R_{\text{At}} \) was dominated by \( R_{\text{Aa}} \), which accounted for 76% of \( R_{\text{At}} \). Average values of \( R_{\text{H}}/R_{\text{eco}} \), \( R_{\text{Ab}}/R_{\text{eco}} \) and \( R_{\text{Aa}}/R_{\text{eco}} \) amounted to 0.41, 0.14 and 0.45, respectively (Table 1).

3.4. Drivers of autotrophic and heterotrophic respiration

3.4.1. The effect of water pulses

\( R_{\text{s}} \) peaks recorded after rewetting the dry soil were mostly of heterotrophic origin. For example, on 5 September 2011, after a cumulated rain input of only 16 mm, there was an abrupt increment of \( R_{\text{H}} \) from 0.24 to 0.65 g CO\(_2\) m\(^{-2}\) h\(^{-1}\), an increment of 170% relative to pre-rain values (Fig. 3d). On this occasion, \( R_{\text{H}} \) contributed up to 95% of \( R_{\text{s}} \) (Fig. 4), and \( R_{\text{eco}} \) reached its maximum annual value (1.5 g CO\(_2\) m\(^{-2}\) h\(^{-1}\)). \( R_{\text{H}} \) decreased to pre-rain rates as soon as the uppermost soil layers dried out again.

\( R_{\text{Ab}} \) peaks after rain pulses were considerably lower. It is worth highlighting the fact that while \( R_{\text{H}} \) and \( R_{\text{s}} \) showed a synchronous response, \( R_{\text{Ab}} \) peaks were out of phase, lagging behind \( R_{\text{H}} \) peaks approximately a week after the rain event (Fig. 5).

In Fig. 6, the difference in respiration rates between consecutive sampling dates was used as a measure of the short-term variability of CO\(_2\) pulses. We found that during the dry season, sudden \( R_{\text{H}} \) changes were linearly related to variations of superficial SWC \((r^2 = 0.88; p < 0.001)\) (Fig. 6a). However, changes in superficial soil temperature did not explain \( R_{\text{H}} \) variations (Fig. 6b). A different pattern was observed during the colder and wetter part of the observation period (November 2011–March 2012), when variations in soil temperature were the best descriptors of \( R_{\text{H}} \) changes.
3.4.2. The decoupled control of SWC, temperature and GPP

Partitioned respiration data are plotted separately against SWC and temperature in Fig. 7. SWC was the best environmental driver from May to October 2011 (dry season), explaining 53% and 76% of $R_A$ and $R_H$, respectively (Fig. 7a). As expected, respiration rates generally increased with increasing temperatures. Nonetheless, the relationship with temperature was significant only during the cold period (November 2011–March 2012) when the confounding effect of SWC was minimal (Fig. 7c–d and Table 2). $R_S$ and $R_A$ were the components with the greatest fit ($r^2 = 0.93$ and 0.88, respectively), while both $R_{Ab}$ and $R_{H}$ showed a weaker relationship with temperature.

Despite the fact that the apparent annual temperature sensitivity coefficient ($Q_{10}$) of all partitioned fluxes was close to the theoretical value of 2 expected from biochemical assumptions (Raich and Schlesinger, 1992; Lloyd and Taylor, 1994), temperature dependencies showed a strong seasonality (Table 2). Very low and statistically non-significant $Q_{10}$ values were calculated for both $R_H$ and $R_{Ab}$ during periods of severe drought (shaded areas in Fig. 8). $Q_{10}$ became significant and grew as soon as precipitation relieved the water stress (as in September 2011, DOY 242–270) and during the coldest and wettest part of the year (Fig. 8).

Throughout the whole observation period (16 May 2011–31 March 2012), $R_{Ab}$ had a strong asymptotic relationship with recently assimilated carbon ($r^2 = 0.60$) (Fig. 9). $R_{Ab}$ increased steeply with GPP up to 1 g CO$_2$ m$^{-2}$ h$^{-1}$, while at higher GPP rates there was little increment. On the contrary, GPP explained only 28% of $R_H$ variability.

### Table 1

| Climate | Whole period | May–October | November–March |
|---------|--------------|-------------|----------------|
| Mean T air (°C) | 15 | 21 | 9 |
| Mean T soil (°C) | 14 | 19 | 9 |
| Mean SWC (%) | 6.2 | 5.1 | 6.6 |
| PPT (mm) | 367 | 109 | 258 |
| Integrated fluxes (g C m$^{-2}$) | 1600 | 1083 | 517 |

* T air, air temperature; T soil, soil temperature; SWC, superficial (10 cm) soil water content; PPT, total precipitation; GPP, gross primary productivity; $R_{eco}$, ecosystem respiration; $R_{H}$, total soil respiration; $R_{Ab}$, heterotrophic respiration; $R_{Ab}$, belowground autotrophic respiration; $R_{Ab}$, aboveground autotrophic respiration; $R_{H}$, total autotrophic respiration.

The relationship between measurements ($r^2 = 0.80$; p < 0.001) (Fig. 6c,d). Interestingly, short-term changes in respiration of autotrophic origin ($R_{Ab}$ and $R_{H}$) were not explained by changes in SWC or temperature.

4. Discussion

4.1. Soil respiration partitioning by tree girdling

In this study we used the tree-girdling method to estimate heterotrophic and belowground autotrophic respiration in a Mediterranean pine forest in Central Italy during an anomalous dry period. Our partition study clearly demonstrated that, under these peculiar eco-climatic conditions, $R_S$ was driven by $R_{H}$, which accounted for 74% of total belowground respiration. The 30% suppression of soil respiration that we measured on girdled plots 20 days following the treatment was within the 24–65% range reported by other authors across different biomes and ecosystem types (Hogberg et al., 2001; Bhupinderpal-Singh et al., 2003; Andersen et al., 2005; Olsson et al., 2005; Binkley et al., 2006; Frey et al., 2006; Scott-Denton et al., 2006; Johnsen et al., 2007; Hogberg et al., 2009; Chen et al., 2010; Subke et al., 2011; Levy-Varon et al., 2012; Bloemen et al., 2014).

It is commonly acknowledged that tree girdling underestimates $R_{Ab}$ because root starch reserves can still be respired after the
treatment, and enhanced decomposition of dead roots is expected over time (Hogberg et al., 2001; Bhupinderpal-Singh et al., 2003; Olsson et al., 2005; Ekberg et al., 2007). As these biases are also inherent to other partitioning approaches, an ideal partitioning method does not exist (Hanson et al., 2000; Kuzyakov, 2006). Nonetheless, some considerations can be drawn. If root-starch consumption accounts for a significant part of the CO2 flux from the girdled plots (and thus erroneously included in \( R_{H} \)), the relative contribution of \( R_{Ab} \) should substantially increase over time as starch reserves are depleted (Hogberg et al., 2001; Bhupinderpal-Singh et al., 2003). However, we found that after an initial phase lasting approximately one month, the \( R_{Ab} \) contribution stabilised to an average of 30% \( R_{S} \) throughout the growing season, and gradually decreased thereafter. This suggests that root-starch reserves were respired during the first weeks following the girdling treatment. Although the microbial decomposition of roots is expected to increase as roots starve and die, thus also increasing apparent \( R_{H} \), this is not likely to happen within a short time (Noel, 1970; Frey et al., 2006). For instance, Ekberg et al. (2007) showed that enhanced organic matter decomposition occurred after trees began to show major signs of decay one year after the girdling treatment. However, we found that after an initial phase lasting approximately one month, the \( R_{Ab} \) contribution stabilised to an average of 30% \( R_{S} \) throughout the growing season, and gradually decreased thereafter. This suggests that root-starch reserves were respired during the first weeks following the girdling treatment. Although the microbial decomposition of roots is expected to increase as roots starve and die, thus also increasing apparent \( R_{H} \), this is not likely to happen within a short time (Noel, 1970; Frey et al., 2006). For instance, Ekberg et al. (2007) showed that enhanced organic matter decomposition occurred after trees began to show major signs of decay one year after the girdling treatment. Moreover, it should be taken into account that conifer roots are rich in recalcitrant carbon compounds such as lignin, condensed tannins and terpenes, which are responsible for slow decomposition rates (Silver and Miya, 2001). Despite the fact that these potential biases cannot be ruled out, we can hypothesise that, in contrast to other partitioning techniques where roots are abruptly excised (i.e. trenching experiments), girdling does not produce large confounding effects in the months immediately following the girdling treatment. Our conclusions are also supported by an independent partitioning study performed by means of stable carbon isotopes within the same experimental site by Albanito et al. (2012) in spring 2011, which showed that roots contributed to 30% \( R_{S} \), while litter-humus and SOM contributed 33% and 37%, respectively.

4.2. Environmental drivers of partitioned fluxes

The analysis of the seasonal course of temperature sensitivities highlighted the fact that drought masked the temperature sensitivity of all partitioned components of \( R_{eco} \) during most of the growing season, and that the temperature control was restored as soon as the water stress was relieved (i.e. at the end of the growing season and during the cold season). Davidson et al. (2006) suggested that a high variability in temperature sensitivity indicates that additional factors confound the temperature response, thus causing inflated or suppressed apparent \( Q_{10} \). These may include: (1) the effect of water content on substrate diffusivity in soil water films, which is expected to affect, above all, microbial respiration, (2) the availability of substrates supplied by photosynthesis, and (3) the seasonality of carbon allocation patterns within plant tissues (Hansen et al., 1997; Bhupinderpal-Singh et al., 2003; Tang et al., 2005; Davidson et al., 2006). \( Q_{10} \) values well above the commonly accepted range of 2–2.5 (Raich and Schlesinger, 1992; Atkin et al., 2000) should therefore be carefully interpreted (Bhupinderpal-Singh et al., 2003; Olsson et al., 2005; Davidson et al., 2006). For example, it has been suggested by several authors that rhizosphere respiration apparently has greater temperature sensitivity than microbial respiration (Boone et al., 1998; Epron et al., 2001; Ruehr and Buchmann, 2010), as we also have observed during the interval DOY 242–270 (Fig. 8). Nevertheless, it should be noted that \( R_{Ab} \) estimated by means of the girdling approach also accounts for the respiration of rhizosphere heterotrophs that are closely associated with and influenced by roots (such as, for example, ectomycorrhizas (Heinemeyer et al.,...
Moreover, autotrophic components of respiration are also strongly dependent on substrates supplied by photosynthesis (Hogberg et al., 2001; Tang et al., 2005). This might also explain why \( R_{At} \) largely contributed to \( R_{eco} \ (>60\%) \), especially during the dry growing season.

Table 2

| Source of Respiration | \( Q_{10} \) (±SE) | \( r^2 \) | p value |
|----------------------|-------------------|--------|---------|
| **Whole period (n = 41)** | | | |
| \( R_S \) | 1.62 (0.11) | 0.57 | *** |
| \( R_H \) | 1.51 (0.10) | 0.49 | *** |
| \( R_{Ab} \) | 1.99 (0.26) | 0.61 | *** |
| \( R_{Aa} \) | 1.75 (0.16) | 0.49 | *** |
| \( R_{At} \) | 1.75 (0.14) | 0.57 | *** |
| **May–October (n = 23)** | | | |
| \( R_S \) | – | – | ns |
| \( R_H \) | – | – | ns |
| \( R_{Ab} \) | – | – | ns |
| \( R_{Aa} \) | – | – | ns |
| \( R_{At} \) | – | – | ns |
| **November–March (n = 18)** | | | |
| \( R_S \) | 3.53 (0.31) | 0.93 | *** |
| \( R_H \) | 3.17 (0.34) | 0.88 | *** |
| \( R_{Ab} \) | 5.12 (1.62) | 0.62 | ** |
| \( R_{Aa} \) | 2.53 (0.71) | 0.41 | ** |
| \( R_{At} \) | 2.55 (0.58) | 0.52 | ** |

\( R_S \), soil respiration; \( R_H \), heterotrophic respiration; \( R_{Ab} \), belowground autotrophic respiration; \( R_{Aa} \), aboveground autotrophic respiration; \( R_{At} \), total autotrophic respiration \( (R_{Ab} + R_{Aa}) \); ns \( p > 0.05 \); ** \( p < 0.01 \); *** \( p < 0.001 \).

Fig. 7. Relationship between partitioned components of respiration and superficial (3 cm) soil water content (SWC) (a, c) and temperature (b, d) during the warm and dry period (16 May–31 October 2011, a and b) and the cold period (1 November 2011–31 March 2012, c and d). See also Table 2.

Fig. 8. Temporal evolution of the apparent temperature sensitivity coefficient \( (Q_{10}) \) for heterotrophic \( (R_H) \) and total autotrophic \( (R_{At}) \) respiration. Black symbols indicate \( Q_{10} \) values derived from significant \( (p < 0.05) \) regressions (Eq. (5)). Light grey shaded areas show extremely dry periods with SWC consistently < 5%. The horizontal dotted line indicates the global median value of 2.4 for \( Q_{10} \) reported for soil respiration by Raich and Schlesinger (1992). DOY is day of year.
4.3. Water pulse effect on carbon fluxes

The San Rossore conifer forest has a typical Mediterranean climate characterised by extended periods of summer drought with scattered precipitation events. We found that water pulses > 10 mm day\(^{-1}\) had a quick stimulating effect on \(R_\text{H}\), that lasted approximately two to three weeks. This phenomenon has been observed in several water-limited ecosystems, and is generally referred to as the “Birch effect” (Birch, 1958, 1959, 1964; Orchard and Cook, 1983; Jarvis et al., 2007; Inglima et al., 2009; Unger et al., 2010). Although the biological mechanisms behind the CO\(_2\) pulses are still not fully understood, it is generally acknowledged that they originate from the rapid increment in the mineralisation of labile carbon compounds of microbial origin (i.e. osmoregulatory solutes or dead microbial biomass) and enhanced mineralisation of labile SOM. After rewetting is clearly of heterotrophic origin. This is not surprising, since microbial activity is expected to be concentrated in the upper layers of the soil profile, and it is therefore exposed to sudden variations of water availability (Davidson et al., 2006; Heinemeyer et al., 2007).

Precipitation pulses also exerted a stimulating effect on \(R_{\text{AB}}\). Nonetheless, \(R_{\text{AB}}\) peaks were considerably weaker than those of \(R_\text{H}\) and, more importantly, reached their maximal intensity approximately one week after the precipitation event. A similar delay (three to four days) in root vs. microbial respiration was recently observed by Carbone et al. (2011) during a water addition experiment in a Central California pine forest with a Mediterranean-type climate. This lag could be due to: (1) the time required for water to infiltrate into the soil and reactivate plant photosynthesis (Ogle and Reynolds, 2004); (2) the time required to transfer photosynthates recently assimilated after the water stimulus to the roots (Kuzyakov and Gavrichkova, 2010). In a recent review, Kuzyakov and Gavrichkova (2010) found that, for mature trees, the time lag between photosynthesis and soil CO\(_2\) efflux is in the order of four to five days, and that the transport of assimilates in the phloem is the rate-limiting process which, in turn, is a function of plant height.

5. Conclusions

In this study we present the results of a novel approach that combines data from a tree-girdling experiment and from eddy covariance in order to partition soil and ecosystem respiration into heterotrophic/autotrophic and above/belowground components. The anomalous drought during the observation period gave us the opportunity to disentangle the effects of SWC, temperature and rain inputs on the partitioned respiratory fluxes. Altogether, our data highlight that, although the processes responsible for the release of CO\(_2\) from the ecosystem may be common across different biomes and ecosystem types, the magnitude by which biotic and environmental controls affect the ecosystem response may be significantly different. In addition, even within a single ecosystem, their relative importance is not constant over time but varies according to the current ecological conditions and the recent meteorological-climatic history. A mechanistic understanding of these processes and their temporal dynamics is therefore fundamental to predict the climatic impacts at larger temporal and spatial scales, and to improve the representation of ecosystem responses in climate change scenarios.

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