Carbon balance in grapevines (*Vitis vinifera* L.): effect of environment, cultivar and phenology on carbon gain, losses and allocation

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Abstract

Background and Aims: Measuring the carbon assimilation and respiration during vine phenology can provide an understanding of the dynamics of carbon fluxes from different organs and their relationship. Most field studies to date do not consider the respiratory losses of different plant organs and their variability under environmental, genetic and phenological changes. The aim of this study was to investigate the effect of genotype and water regime on carbon assimilation, respiration and allocation during vine phenology.

Methods and Results: Field trials were carried out during 2013 and 2014 to study the effect of genotype and water status on carbon assimilation, respiratory losses from leaves, shoots, fruits and roots during the vine phenological cycle, and on biomass production. Carbon respiration varied during plant phenology and represented a significant proportion of the total vine carbon assimilation. The integrated carbon respiratory loss in leaves, fruits and roots was greater in irrigated vines than in non-irrigated vines. Tempranillo recorded the highest carbon assimilation, leaf and stem respiration, as well as the highest above-ground biomass. Garnacha showed a higher root respiration loss and allocated more biomass to the permanent organs. Accumulation of above-ground biomass was influenced by plant carbon budgets during the growing season.

Conclusions: Vine phenology, cultivar and plant water status affected carbon assimilation, carbon loss and carbon allocation. Non-irrigated vines had a higher respiratory carbon loss in respect to the total carbon assimilation by photosynthesis. Above- and below-ground carbon fluxes were coupled during vine phenology.

Significance of the Study: The present work illustrates the importance of respiratory processes on the carbon balance and the relationship among different carbon balance components during vine phenology.

Keywords: *carbon assimilation, CO_2 balance, irrigation, respiration,* Vitis vinifera

Introduction

Plant carbon balance integrates the CO_2 fluxes from photosynthesis and respiration, which results in the accumulation of plant biomass. There has been a growing interest in the quantitative assessment of the carbon allocation in plants because of its contribution to carbon sequestration and CO_2 emission control (Scandellari et al. 2016), and more specifically in crops (Buwalda 1991, Lakso and Poni 2005, Iglesias et al. 2013). In grapevines, measuring the changes in carbon assimilation and respiration during the growing season can provide an understanding of the dynamics of carbon fluxes from different organs and their relationship during vine phenology. Nowadays, the quantitative understanding of how plants gain and allocate their resources would help to make predictions under particular climate change conditions, such as higher temperature (Greer 2017), drought and salinity (Flexas et al. 2006), under specific cultural practices (Brunori et al. 2016) or under the effect of plant diseases (Montero et al. 2016, El Aou-Ouad et al. 2018). Plant carbon balance in the field, however, represents important experimental limitations and difficulties because of a large number of factors that interact along plant phenology in natural vegetation and crop systems (Mirás-Avalos et al. 2018). Thus, to estimate changes of the carbon balance in crops, it is necessary to study the contribution of plant phenology, environmental conditions and management practices to plant carbon sequestration and respiration. These estimations could contribute to cultivar qualification and the development of adaptation and mitigation strategies to maximise the CO_2 sequestration and minimise the CO_2 emissions in response to climate change (Medrano et al. 2016).

Plant carbon balance can be determined by measuring photosynthetic and respiratory fluxes of different plant organs, which means that considerable technical limitations must be overcome because most gas exchange methods have focused on leaves. In grapevines, carbon gain has been largely studied considering the genetic, environmental and phenological variations (De Souza et al. 2003, Schultz 2003, Baeza et al. 2005, Weyand and Schultz 2006, Escalona et al. 2012). The number of studies evaluating respiratory cost are, however, scarce despite its importance. Respiratory carbon loss has been reported to account for about 50% of the total carbon fixed by photosynthesis, and could reach 90% depending on environmental conditions and the plant phenological stage (Amthor 2000). Some studies have developed models that integrate the aerial respiratory component in the plant carbon balance, considering the temperature as the main factor that influences respiratory

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processes (Wermelinger et al. 1991, Poni et al. 2006, Weyand and Schultz 2006). It has been reported, however, that temperature exerts a much lower control over respiration under conditions of water stress (Escalona et al. 2012) and of elevated CO_2 levels (Martínez-Lüscher et al. 2015), suggesting that consideration of multiple factors may lead to a more accurate estimation of the whole plant carbon balance. Recent studies by our research group reported the variation of root, leaf and fruit respiration under field conditions (Hernández-Montes et al. 2017, 2019, 2020), showing a significant effect of plant water status, genotype and phenology on respiratory losses. Despite these studies, not enough information was found regarding the contribution of different plant organs to the whole plant respiration and the whole plant carbon balance under field conditions. During the last few decades whole plant chambers have been used to estimate the whole plant gas exchange fluxes and the influence of genotype and environment (Poni et al. 2009, Tarara et al. 2011, Douthe et al. 2018). Most of these studies exclude, however, the below-ground respiration component from the determination of whole plant gas exchange, despite it being responsible for the greatest respiratory losses (Escalona et al. 2012). Because of the difficulties involved in accurately measuring plant carbon balance under field conditions, total dry mass accumulation per year (Poni et al. 2006, Greer et al. 2011, Greer 2017) and shoot biomass (Miller et al. 1996, Greer and Sicard 2009) have been used to estimate carbon accumulation in several cultivars of grapevines. Additionally, several models have been developed to estimate overall above-ground non-permanent biomass (Vivin et al. 2002) and permanent dry matter production (Lakso et al. 2008). In fact, for permanent structures, the difficulty of measuring biomass under field conditions was overcome by applying allometries for these organs based on the ratio between root and trunk biomass. In a more recent contribution, different ways have been tested to estimate above-ground and below-ground total grapevine biomass using a range of cultivars, vine age and environmental conditions (Miranda et al. 2017). A few studies have integrated CO_2 fluxes (photosynthesis and respiration) and biomass accumulation in order to complete an understanding of plant carbon balance during the phenological cycle (Palliotti et al. 2004, Lakso et al. 2008, Escalona et al. 2012), but none have been undertaken under field conditions.

Genotype and water status effects on carbon assimilation have been extensively and intensively studied (Escalona et al. 2012, Tomás et al. 2014, Martorell et al. 2015, Bota et al. 2016). Also, during recent years, the effect of genotype and plant water status on the respiration rate of different plant organs was also evaluated (Hernández-Montes et al. 2017, 2019, 2020). There is a lack of information, however, concerning the variation of the carbon balance components during the phenological cycle, studying the effect of genotype and water status on carbon balance from an integrative perspective, considering carbon gains, respiratory costs and biomass production. Therefore, a field trial was carried out during two consecutive years with the aim to: (i) study the variation of carbon assimilation and carbon respiratory losses during vine phenology; (ii) evaluate the effect of genotype and water regime on the respiratory components and carbon allocation; and (iii) study the relationship between carbon assimilation, respiration and allocation in field-grown grapevines along the plant phenological cycle.

Materials and methods

Plant material and treatments

The experiment was conducted during 2013 and 2014 in the experimental field at the University of the Balearic Islands (Majorca, Spain). Garnacha and Tempranillo vines were grafted on 110-Richter rootstocks and planted in 2009 in a NE–SW orientation separated by 1 m between plants and 2.5 m between rows. The vines were trained to a bilateral cordon with 12 shoots per vine. The vineyard has clay-loamy soil with 1.5 m of maximum depth. Two irrigation treatments were established on each cultivar: (i) irrigation and (ii) non-irrigation, which consisted of withholding irrigation during the whole vegetative cycle. Weekly irrigation application was calculated from the ET_o registered by a meteorological station (Meteodata 3000, Geónica SA, Madrid, Spain) at the experimental site. The crop coefficient for the irrigation treatment (I) was first fixed at 30% of ET_o recorded during 2013; during 2014 the crop coefficient was increased to 40% of ET_o to better differentiate the treatments. The irrigation period was applied from June to September in both years using three drippers per plant of 4 L/h on a single pipe for each row. Predawn leaf water potential (Ψ_{pd}) was measured every 2 weeks from budburst to harvest, using a Scholander pressure chamber (Soil Moisture Equipment, Santa Barbara, CA, USA). Measurement of Ψ_{pd} was made about 1 h before sunrise on four leaves chosen from four different plants per treatment.

Leaf net carbon assimilation rate

Leaf gas exchange measurements were made using a portable gas exchange analyser (Li-6400; LI-COR Biosciences, Lincoln, NE, USA) equipped with a transparent chamber. Environmental conditions in the chamber were higher than 1000 µmol photon/($m^2 \cdot s$) (saturation light), at a CO_2 concentration of 400 µmol/mol and ambient air temperature. Leaf gas exchange was measured in four plants per treatment at five stages: flowering, pea size, veraison, ripening and postharvest.

In order to measure plant net carbon assimilation, five leaves per plant located in five different locations in the canopy were selected according to Escalona et al. (2003): (i) bottom east; (ii) bottom west; (iii) top east; (iv) top west; and (v) inner canopy zone. The bottom positions (i and ii) are related to adult fully expanded leaves, whereas the top positions (iii and iv) are related to younger and/or expanding leaves. The inner canopy zone (v) is referred to leaves covered by at least one layer of leaves and shaded for most of the day except for occasional sun flecks. Measurements were made five times during the day (0800, 1030, 1300, 1630, 1930) to obtain the daily net photosynthesis of each type of leaf.

Net daily carbon assimilation per plant was calculated by summing the fraction of daily carbon gain of each zone. It was calculated by multiplying the net leaf carbon assimilation rates (A_n) by the leaf area of each plant zone. Whole plant leaf area was calculated every 2 weeks following the methodology described by Sanchez-de-Miguel et al. (2011). The proportion of leaves from each canopy zone was calculated at the end of the experiments using a biomass approach. Each canopy zone was visually identified and defoliated independently in all Control plants (four plants per treatment and cultivar). Leaves from each position were kept in paper envelopes and then dried in an oven at 70°C until they reached a constant mass. The dry mass from the

sample of each leaf position was referred to the total leaf dry mass to obtain the proportion of leaves associated to each canopy zone. A constant daily leaf area rate of change was assumed along the period between consecutive leaf area measurements. Leaf carbon assimilation rate was considered constant between measuring dates, assuming a similar leaf activity all along that period (most of the days were sunny).

Night leaf respiration

Leaf night respiration was measured using the same equipment as for leaf net carbon assimilation rate (Li-6400) in two different types of leaves during the vine vegetative period: (i) young expanding leaves, close to the apex; and (ii) adult leaves placed in mid-shoot, to have, respectively, rates referring to growth and maintenance costs (Hernández-Montes et al. 2019). All measurements were made in four vines (two leaves per vine) per treatment and cultivar at night, between 2300 and 0200, during budburst, flowering, pea size, veraison, ripening and postharvest.

At each phenological stage, the proportion of 'growing' and 'mature'' leaf area was estimated by measuring the shoot length and leaf number that corresponded to each leaf type. Total leaf respiration loss during the night was estimated by integrating the respiratory rate, the proportion of leaf area associated (growing and mature) at each phenological stage, and the duration of the night considering respiration rate almost constant during the night (Escalona et al. 2012). Also, the respiratory rate associated to different types of leaves was assumed constant during the period between measurements, on which the plants maintained similar plant water status. A constant daily leaf area rate of change was assumed between measurements.

Stem respiration

Stem respiration rate was measured using a modified chamber which 'embraces' the stem connected to the Li-6400 gas exchange analyser. Two different parts of the stem were selected: (i) apical zone; and (ii) mid to bottom zone, to establish the growth and maintenance respiratory losses, using a similar procedure as for the leaves. All measurements were made in four vines (one shoot per vine) per treatment and cultivar at mid-morning, during the phenological stages of flowering, pea size, veraison, ripening and postharvest. The head of the Li-6400 gas exchange analyser was equipped with a light source (Li-6400-02B LED, LI-COR) to measure under light and dark conditions to simulate the respiration during the day and night.

Length and diameter of four shoots per vine were measured every 2 weeks. Moreover, the proportion of growing and mature shoots was estimated to integrate the respiratory rate and the length of shoot at each time of measurement. The total respiratory losses were calculated considering the number of shoots per vine.

Fruit respiration

Fruit respiration was measured in a home-made methacrylate fruit chamber connected to a Li-6400 gas exchange analyser, as described by Hernández-Montes et al. (2020). Measurements were made in four plants (one bunch per vine) per treatment and cultivar. The carbon efflux of entire bunches was measured at three times during berry development: pea size, veraison and ripening stage. Measurements were taken between 1030 and 1230 local time to minimise differences in the fruit microclimate (temperature and light) that could affect fruit respiration. Bunch respiration rate was measured under light [PAR around 1000–1200 μmol/(m^2 · s)] and dark conditions (by covering the fruit chamber with an isothermal reflective sheet) to simulate the fruit respiration at night. The total bunch carbon loss per vine was estimated considering the bunch carbon flux at pea size, veraison and ripening stages, the number of bunches per vine and the daily change in bunch mass until harvest. Daily change in bunch mass was estimated using the correlation between bunch volume (water displaced technique) and fresh and dry mass described by Hernández-Montes et al. (2020).

Root respiration

Root respiration was estimated by measuring soil respiration at three different positions 'along the row' and 'between rows' during different phenological stages as described by Hernández-Montes et al. (2017). Soil CO_2 efflux was measured using a CO_2 flux chamber connected to a Li-6400 portable gas exchange analyser. Measurements were made at six phenological stages, from budburst until postharvest. Soil CO_2 efflux was measured in three vines per treatment and at five different locations in the soil surrounding each vine (positions 1, 2 and 3 located 'along the row', and positions 4 and 5 located 'between rows'), in order to estimate the root-dependent respiratory activity and basal respiratory activity. Root respiration was estimated assuming that soil respiration measured between rows represents mainly the heterotrophic respiration component. The ratio between soil respiration measured in the positions 'between rows' and 'along the row' resulted in the estimated root respiration component for each replicate. Total soil carbon loss per day and per vine was calculated from the sum of the CO_2 efflux emission measured in the different locations multiplied by their respective soil surface and by the number of hours in the day. Seasonal integration of root respiration was calculated at each phenological stage to obtain the total carbon loss.

Biomass production

For each experiment (2013 and 2014), before leaf fall, all vines were divided into three main organ types: stems (main and laterals), leaves (including petioles) and fruit (harvested according to quality specifications). Vine parts were dried in an oven at 70°C until they reached a constant mass. During the winter 2014/15, after the experiments were completed, the Control vines were uprooted, and the root system carefully extracted from the soil. Roots were excavated as described by de Herralde et al. (2010). The bulk volume predefined by previous experiments was dug out with a small excavator equipped with a backhoe. Width within the row was half the plant distance to both sides (0.75 m) and the same distance towards the inter-row space. Then, borders of the soil hole were manually finished with the help of tools. Below-ground trunk and main coarse roots were manually retrieved. All recovered roots were gently washed and wiped to eliminate soil particles (Figure S1). Then, trunk, cordons and fine, medium and main roots were separated, oven-dried at 70°C for 10 days and weighed.

Statistical analysis

The effect of irrigation, cultivar, phenological stage, year and their interaction was analysed using ANOVA procedures, and Tukey's test was used for post hoc means comparisons using JMP 14.0 (SAS Institute, Cary, NC, USA). Multiple regressions and correlation analyses were made using SigmaPlot 12.0 (Systat Software, Chicago, IL, USA).

Results

Environmental conditions

Environmental conditions were recorded during the 2013 and 2014 seasons. Total rainfall from April to October (growing season) during 2013 and 2014 was 149 and 163 mm, and the evaporative demand (ET_o) was 823 and 814 mm, respectively (Table S1). The growing degree days, accumulated from April to October, were 2354°C days in 2013 and 2474°C days in 2014. In both years, the mean daily temperature of the growing cycle of grapevines was compressed between 20 and 30°C having a maximum daily average of 32.4°C in July 2013 and 30.1°C in August 2014. Precipitation was almost nil during these 2 months, registering 0.6 mm in 2013, and 8.7 mm in 2014. On average, the irrigation applied during 2013 and 2014 was 95 mm and 177 mm, respectively.

Plant water status

The Ψ_{pd} was measured in irrigated (I) and non-irrigated (NI) Garnacha and Tempranillo vines at different phenological stages during 2013 and 2014 seasons (Table 1). Plant water status was similar for both cultivars and treatments until the irrigation started (fourth week of June 2013 and first week of June 2014). Irrigation significantly affected Ψ_{pd} values from pea-size stage (first week of July) until the end of grape ripening (September). Cultivar did not affect vine water status, except for the pea-size stage in 2013, where the effect of irrigation, cultivar and their interactions was significant. On average, irrigated plants maintained a Ψ_{pd} higher than −0.4 MPa during the 2013 and 2014 growing seasons. The soil water depletion in the NI treatment from both cultivars was reflected by a progressive decline in Ψ_{pd} from veraison to ripening during 2013, and from veraison to postharvest in 2014.

Carbon assimilation and carbon losses along the phenological cycle

Carbon fixation by photosynthesis and respiratory carbon losses were calculated from gas exchange measurements in different plant organs at different phenological stages (Table S2). Table 2 shows the averaged plant carbon balance components during phenology. In general, a progressive rise in leaf carbon assimilation was found until 90 days after budburst (DAB), reaching up to 611 g of fixed C per plant from 61 to 90 DAB, and followed by a continuous decline until postharvest. The changes in total carbon respiratory losses during phenology followed a similar pattern, with the maximum respiratory loss registered between 61 and 90 DAB (berry at green hard stages) for all measured organs, with fruit respiration the origin of the highest carbon losses (86 g C/vine) during this period.

Changes in plant carbon assimilation and respiratory carbon losses were analysed in detail to evaluate the effect of irrigation, cultivar and phenological stage on vine carbon balance. Irrigation and cultivar effects and their interactions during phenology were examined with a repeat measures ANOVA analysis. In terms of water regime effects (Figure 1a,b), carbon assimilation was significantly different between treatments from flowering until postharvest. The significance and intensity of the irrigation effect, however, on carbon respiratory losses varied across phenological stages and vine organs. Irrigation increased root respiration from the green stages of the fruit (60–90 DAB, start of irrigation) until harvest (150 DAB). The irrigation effect on fruit respiratory losses was significant only at the ripening stage. Also, differences between irrigation treatments were found in the integrated leaf respiratory losses from the periods 91–120 DAB to 151–180 DAB (postharvest).

Tempranillo fixed more C than Garnacha from flowering to veraison (Figure 1c,d), and both cultivars fixed a similar amount of carbon at the beginning of vegetative growth, ripening and postharvest. Also, the cultivar affected root respiratory losses before budburst, between budburst and flowering and at veraison. Garnacha had the highest root respiration values before budburst. Root respiratory loss, however, was lower for Garnacha than for Tempranillo during the remaining phenological cycle. Fruit respiration was higher in Tempranillo than in Garnacha until ripening, having similar fruit respiratory costs thereafter (around 60 g C/vine). Tempranillo had higher leaf respiratory losses than Garnacha during the vine vegetative cycle. Stem respiratory losses were affected by cultivar, but significant interactions were found between cultivar and irrigation effects.

Table 1. Predawn leaf water potential measured at different phenological stages during the 2013 and 2014 seasons.

			Ψ_{pd} (MPa)				
Year	**Cultivar**	**Irrigation**	**Flowering**	**Fruitset**	**Pea size**	**Veraison**	**Ripening**
2013	Tempranillo	I	−0.24 ± 0.03 a	−0.43 ± 0.04 a	−0.24 ± 0.01 a	−0.33 ± 0.02 a	−0.33 ± 0.02 a
		NI	−0.28 ± 0.02 a	−0.49 ± 0.01 a	−0.28 ± 0.05 a	−0.62 ± 0.10 b	−0.51 ± 0.02 c
	Garnacha	I	−0.25 ± 0.05 a	−0.40 ± 0.05 a	−0.27 ± 0.03 a	−0.36 ± 0.01 a	−0.41 ± 0.03 ab
		NI	−0.32 ± 0.05 a	−0.40 ± 0.01 a	−0.50 ± 0.03 b	−0.74 ± 0.05 b	−0.48 ± 0.04 bc
	Significance	Cultivar	n.s.	n.s.	***	n.s.	n.s.
		Treatment	n.s.	n.s.	***	***	***
		Cultivar × Treatment	n.s.	n.s.	***	n.s.	n.s.
			Flowering	**Fruitset**	**Pea size**	**Veraison**	**Ripening**
2014	Tempranillo	I	−0.29 ± 0.03 a	−0.35 ± 0.04 a	−0.23 ± 0.01 a	−0.22 ± 0.04 a	−0.39 ± 0.04 a
		NI	−0.33 ± 0.04 a	−0.41 ± 0.03 a	−0.41 ± 0.05 b	−0.55 ± 0.08 b	−0.63 ± 0.01 b
	Garnacha	I	−0.33 ± 0.03 a	−0.37 ± 0.01 a	−0.26 ± 0.01 a	−0.35 ± 0.01 ab	−0.39 ± 0.03 a
		NI	−0.36 ± 0.03 a	−0.40 ± 0.02 a	−0.43 ± 0.03 b	−0.59 ± 0.10 b	−0.74 ± 0.05 b
	Significance	Cultivar	n.s.	n.s.	n.s.	n.s.	n.s.
		Treatment	n.s.	n.s.	***	***	***
		Cultivar × Treatment	n.s.	n.s.	n.s.	n.s.	n.s.

*, $P < 0.1$; **, $P < 0.05$; ***, $P < 0.001$; n.s., not significant; values are means ± SE ($n = 4$). Different letters denote a significant difference ($P < 0.05$) among cultivars and treatments at each phenological stage and year.

Table 2. Integrated leaf carbon assimilation and carbon losses from leaves, shoots, fruits and roots across vine phenology (a 10-day period before budburst and 30-day periods from budburst to postharvest).

Days after budburst (DAB)	Leaf C assimilation (g C/plant)	Leaf C respiration (g C/plant)	Stem C respiration (g C/plant)	Fruit C respiration (g C/plant)	Root C respiration (g C/plant)	Net C assimilation (g C/plant)
10 DBB	–	–	–	–	15.21 ± 1.23	−15.21 ± 1.23 b
1–30	110.33 ± 10.2 d	6.56 ± 0.44 d	1.19 ± 0.26 c	–	26.02 ± 2.32 bc	76.71 ± 10.43 b
31–60	409.23 ± 27.15 c	26.49 ± 1.45 c	11.81 ± 2.18 ab	–	32.44 ± 4.22 abc	338.83 ± 24.01 a
61–90	611.1 ± 36.29 a	41.24 ± 2.32 a	16.92 ± 3.04 a	85.72 ± 12.76 a	39.02 ± 3.55 ab	427.34 ± 32.44 a
91–120	559.89 ± 44.62 ab	37.23 ± 2.56 ab	6.29 ± 1.17 bc	51.92 ± 8.82 b	46.24 ± 5.1 a	419.42 ± 39.3 a
121–150	439.26 ± 38.03 bc	34.75 ± 2.97 abc	3.41 ± 0.77 c	61.11 ± 5.6 ab	34.71 ± 4.25 abc	306.1 ± 31.09 a
151–180	324.75 ± 35.79 c	30.79 ± 2.62 bc	1.72 ± 0.38 c	–	21.34 ± 2.14 c	270.35 ± 33.82 a

Values are means of all replicates ± SE (n = 24–32); – denotes no available value for that period. Different letters denote a significant difference ($P < 0.05$) among periods. DBB, days before budburst.

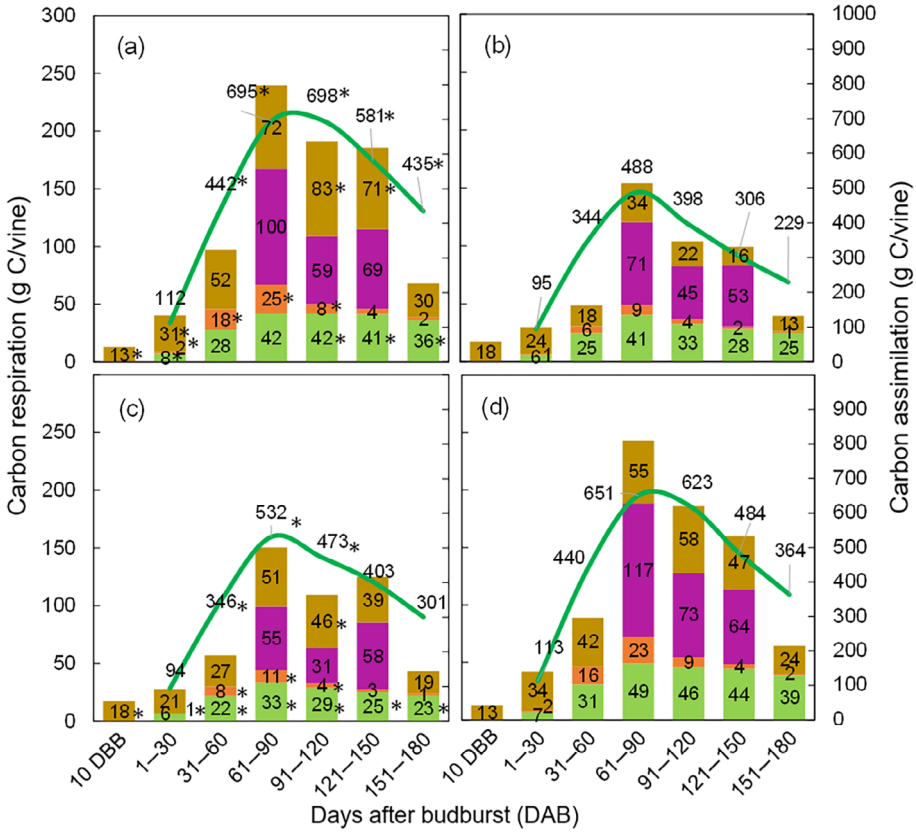

Figure 1. Changes in the integrated carbon assimilation (——) and losses from leaves (■), shoots (■), fruits (■) and roots (■) during vine phenology (days after budburst, from pre-budburst to postharvest) for (a) irrigated and (b) non-irrigated vines, and for (c) Garnacha and (d) Tempranillo cultivars. Values represent averaged carbon assimilation and carbon respired from 2013 to 2014 at each phenological stage (n = 7–8). Asterisks after values in (a) represent a significant difference between irrigated and (b) non-irrigated treatments. Asterisks after values in (c) represent a significant difference between (a) Garnacha and (d) Tempranillo.

Annual net vine carbon assimilation and carbon losses

Net vine carbon assimilation was calculated from the leaf carbon fixation minus carbon respiration from leaves, stems, fruit and roots. Leaf carbon fixation was calculated by integrating the photosynthetic rates from leaves located at five different positions in the vine canopy (Escalona et al. 2003) measured at five different times during the day. Additionally, an exhaustive measurement of the total leaf area in each Control vine allowed the estimation of the leaf area corresponding to each position where photosynthesis was measured. Total plant carbon assimilation resulted from the integration of leaf area measurements and photosynthesis rates at different times of the phenological cycle (flowering, pea size, veraison, ripening and postharvest). The integrative carbon assimilation values per vine and year are presented in Table 3. Irrigation increased total carbon assimilation in Garnacha (up to 70%) and Tempranillo (around 60%) compared to non-irrigated conditions. Also, Tempranillo assimilated a higher amount of carbon by photosynthesis than Garnacha during both 2013 and 2014.

Table 3 shows carbon costs of leaves, shoots, fruits and roots expressed on a per vine and per year basis, which were calculated by integrating the measured respiratory rates during plant phenology. The carbon losses of aerial organs (leaves, shoots and fruit) accounted for about 20–30% of the total carbon fixed by photosynthesis. Irrigation affected the annual respiratory costs, and irrigated plants registered higher respiratory losses in all organs compared to the losses of non-irrigated vines. The effect of plant water

status, however, on respiratory losses was not equal among vine organs. Although the integrative carbon losses due to leaf respiration in non-irrigated vines were significantly lower than in irrigated plants, the maximum effect of irrigation was registered in root respiratory carbon losses. Consequently, the proportion of root respiratory losses in respect to the total fixed carbon increased under irrigation. The remaining respiratory components (leaves, stems and fruits), however, did not show significant changes under irrigation, in terms of the proportion of the total carbon fixed by photosynthesis. Total carbon losses derived from leaf respiration represented a 7–8% of the total carbon fixed by photosynthesis in irrigated vines, and 9–11% in non-irrigated vines. The carbon loss from the respiratory activity of stems was the lowest in relation to the remaining organs (Table 3). From the total carbon fixed by photosynthesis, only 1–2% was lost by stem respiration. The highest fruit carbon respiratory losses were recorded in irrigated vines, both in Garnacha and Tempranillo. Although no significant difference was found between treatments in fruit respiration rates per dry mass unit, irrigated plants recorded higher yield per vine than non-irrigated vines (Table 3). These facts together made the integrated values of fruit respiratory carbon losses higher in irrigated vines than in non-irrigated vines. Even so, the respiratory losses of non-irrigated Garnacha and Tempranillo vines were about 18% of the total carbon fixed by photosynthesis, and for irrigated vines about 13%. The carbon loss because of root respiration represented 20–40% of the total losses, and 8–14% of the total carbon fixed by photosynthesis. Significant differences were found between cultivars in leaves and stems, with Tempranillo having the highest respiratory carbon losses.

Annual biomass production and carbon allocation

Biomass production of irrigated and non-irrigated Garnacha and Tempranillo vines is presented in Table 4. Water stress clearly reduced the total biomass accumulated, and the difference between cultivars and irrigation treatments in non-permanent and permanent organs was significant. On a dry mass basis, leaves, stems and fruits accounted for 18, 25 and 50%, respectively, of the total aerial biomass produced during the year. Likewise, leaves, stems, fruits and permanent organs (arms, trunk and roots) accounted for 12, 15, 27 and 46%, respectively, of the total vine biomass, including permanent organs. The fruit was the most important carbon sink, and in this study represented around 50% of the total aerial biomass produced (Table 4). In general, the biomass of the non-permanent structures of Tempranillo was higher than that of Garnacha.

The permanent structures of the vines were weighed to measure the biomass of those organs during the winter of 2014. Permanent organs (trunk, arms and roots) accounted for the major proportion of dry matter of the total accumulated in vines, representing 55 and 45% in Garnacha and Tempranillo, respectively. The total biomass of permanent structures was significantly higher in Garnacha than in Tempranillo (around 1900 g dry mass/vine in Garnacha and 1700 g dry mass/vine in Tempranillo). Those differences were mainly because of biomass of trunk and arms that represented around 20% for Garnacha, and around 30% for Tempranillo. Conversely, the biomass of aerial organs (leaves, shoots and fruits) was significantly lower in Garnacha than in Tempranillo, accounting for about 45 and 55% of the total vine biomass for Garnacha and Tempranillo, respectively. On a dry mass basis, reproductive organs formed during the season accounted for 20–30% of the total biomass, and newly formed vegetative organs (leaves and shoots) represented a similar proportion.

Relationship between annual carbon assimilation, carbon loss and biomass accumulation

Correlations were done to evaluate the relationship between plant biomass accumulation and carbon gains and losses calculated from gas exchange measurement (Figure 2). Significant linear relationships were found between aerial biomass and aerial carbon respired (Figure 2a, $R^2 = 0.91$, $P < 0.001$), carbon fixed by photosynthesis (Figure 2b, $R^2 = 0.69$, $P = 0.01$) and aerial net carbon assimilation (fixed carbon − respired carbon; Figure 2c, $R^2 = 0.54$, $P = 0.04$). Additionally, estimated carbohydrate reserve was calculated from the difference between net carbon fixed and aerial biomass. A significant linear regression was found between estimated carbohydrates reserve and the fruit to shoot ratio (Figure 3).

Above-ground and belowground carbon coupling

A strong positive linear relationship was found between carbon assimilation and soil respiration during phenology (data not shown). Likewise, the relationship became more significant when carbon assimilation was related to root respiration, showing different linear regressions for irrigated (Figure 4, $R^2 = 0.73$, $P = 0.002$) and non-irrigated (Figure 4, $R^2 = 0.91$, $P < 0.001$) vines during phenology. The relationship between carbon assimilation and root respiration during budburst followed a significant linear regression (Figure 4, $R^2 = 0.8$, $P = 0.1$) that applied only to this phenological stage.

Discussion

Leaf carbon fixation

Carbon fixation by the canopy is the subject of controversy because of the difficulty involved in its measurement. In this study, the complexity of the canopy arranged on a trellis system was taken into account by measuring leaves standing at five locations, basal east oriented, basal west oriented, apical east oriented, apical west oriented and internal leaf, and the leaf area corresponding to each leaf position, according to Escalona et al. (2003). The rate of photosynthesis throughout the season was similar to that reported by Martorell et al. (2015) in a similar experiment using Garnacha and Tempranillo under well-watered and water-stressed conditions, finding the same decay in photosynthesis throughout the summer. The integrated photosynthesis values all along the canopy and time clearly indicated that Garnacha and Tempranillo vines assimilated more than 1.8 kg C/year and 2.5 kg C/year, respectively. These values did not differ from those previously reported for vines under field conditions (Poni et al. 2006, Weyand and Schultz 2006). The data presented confirm a clear effect of genotype and irrigation on carbon balance components during vine phenology (Figure 1). The total carbon assimilation per vine varied between cultivars, probably because of the accumulated difference in photosynthesis rates and leaf area (Bota et al. 2001, Escalona et al. 2012), as expected because of the well-known physiological behaviour of the cultivars Garnacha and Tempranillo (Tomás et al. 2014, Martorell et al. 2015). Significant variation of vine carbon balance was induced by the irrigation treatment. As expected, water stress reduced both the leaf area and photosynthesis. In

Table 3. Average of final leaf area, annual leaf carbon assimilation, leaf carbon respiration, stem carbon respiration, fruit carbon respiration, root carbon respiration and net carbon fixed during the growing season for irrigated and non-irrigated Garnacha and Tempranillo vines during the growing seasons 2013 and 2014.

Year	Cultivar	Treatment	Leaf area (m^2/plant)	Yield (kg/plant)	Leaf C fixation (g C/year)	R_{leaf} (g C/year)	R_{stem} (g C/year)	R_{fruit} (g C/year)	R_{root} (g C/year)	Net C fixed† (g C/year)
2013	Garnacha	I	4.1 ± 0.4 ab	4.74 ± 0.9 ab	2190.5 ± 259 ab	148.6 ± 13 bc	26.6 ± 7.2 ab	202.6 ± 45 b	289.03 ± 2 ab	1812.7 ± 235 ab
		NI	3.6 ± 0.1 b	3.08 ± 0.4 b	1238.2 ± 106 b	104.2 ± 5 c	9.8 ± 0.8 b	201.2 ± 21 b	132.98 ± 2 c	922.5 ± 77.9 b
	Tempranillo	I	5.1 ± 0.6 a	6.36 ± 1.9 a	3435.2 ± 734 a	264.1 ± 24 a	52.8 ± 9.0 a	358.5 ± 52 a	296.17 ± 11 a	2759.6 ± 684.2 a
		NI	4.1 ± 0.3 ab	5.42 ± 1.3 ab	1915.5 ± 280 b	210.9 ± 4 ab	12.2 ± 3.3 b	343.4 ± 32 ab	150.73 ± 5 bc	1348.9 ± 170.6 b
2014	Garnacha	I	6 ± 0.4 ab	4.41 ± 0.7 a	2643.8 ± 220 ab	211.0 ± 21 ab	40.8 ± 7.7 b	515.5 ± 148 ab	218.22 ± 10 a	1876.5 ± 321.7 ab
		NI	4.3 ± 0.2 b	2.65 ± 0.6 ab	1596.0 ± 193 b	134.0 ± 14 b	27.2 ± 2.4 b	375.5 ± 119 c	123.11 ± 5 b	1059.3 ± 290.8 b
	Tempranillo	I	6.7 ± 0.4 a	3.04 ± 0.2 ab	3214.1 ± 253 a	246.9 ± 39 a	108.6 ± 19.9 a	415.9 ± 55 a	281.17 ± 6 a	2442.7 ± 191.5 a
		NI	5.1 ± 0.6 ab	2.17 ± 0.7 b	2212.8 ± 450 ab	226.2 ± 42 ab	31.5 ± 9.4 b	274.8 ± 55 bc	167.63 ± 13 b	1680.3 ± 377.1 ab
Cultivar			*	*	**	***	**	n.s.	n.s.	***
Treatment			**	*	***	**	***	n.s.	***	***
Cultivar × Treatment			n.s.	n.s.	n.s.	n.s.	***	n.s.	n.s.	n.s.
Year			**	*	n.s.	n.s.	***	*	***	n.s.
Year × Cultivar			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Year × Treatment			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

*, $P < 0.1$; **, $P < 0.05$; ***, $P < 0.001$; n.s., not significant; values are means ± SE ($n = 4$). Different letters denote a significant difference ($P < 0.05$) among cultivars and treatments in each year. †Net C fixed = Leaf $C_{fixation} - (R_{leaf} + R_{stem} + R_{fruit})$; I, irrigated; NI, non-irrigated; R_{fruit}, fruit carbon respiration; R_{leaf}, leaf carbon respiration; R_{root}, root carbon respiration; R_{stem}, stem carbon respiration.

consequence, non-irrigated vines fixed 30–40% less carbon than irrigated vines in both cultivars, similar to other studies under the same climate conditions (Escalona et al. 2003, Medrano et al. 2003).

Carbon losses

Carbon loss derived from the respiratory activity of leaves, stems, fruits and roots was estimated by integrating the respiration rate of different organs along the vine phenological cycle, indicating grape ripening stage as the most limited time for the vine, when photosynthates may be limiting because of the maximum respiration losses from all organs (Vivin et al. 2002). Calculated total respiratory losses during phenology, ranged from 30 to 40% of the total net carbon assimilation (Table 3), similar to that obtained from whole-vine gas exchange measurements (Lakso et al. 1997, Poni et al. 2000) and that calculated by Weyand and Schultz (2006), and lower compared to the results of Escalona et al. (2012) in a potted experiment using fruitless vines. Seasonal growth and maintenance costs of the above-ground vegetative organs represented between 18 and 34% of the net C assimilated through the growing season. It is important to clarify that in this study photorespiration was not calculated but included in the net carbon assimilation measurements during the day. Previously it was estimated that photorespiration could account for 20–50% of the carbon fixed by photosynthesis depending on vine water status (Düring 1988), and this fact could explain the values obtained in this work that resulted in a lower total respiration costs of the above-ground organs (leaves and stems) compared to those simulated by Vivin et al. (2002). The respiration losses from leaves at night showed differences between irrigated and non-irrigated vines, with the irrigated Tempranillo vines reaching the greatest loss, probably because of higher leaf area and respiratory rate of growing leaves recorded in Tempranillo (Hernández-Montes et al. 2019).

From the total carbon fixed by photosynthesis, only 1–2% was lost by stem respiration, although it represented on average 25% of the aerial biomass produced (Table 4). Stem respiratory loss in our study was much lower than that modelled and the calculated value reported by Palliotti et al. (2004) in Sangiovese vines. These differences could be explained because in our study respiration measurements in stems and fruits were made under light and dark conditions considering the re-fixation of CO_2 in green vine organs under light conditions. This improvement should be considered in the current C balance models (Palliotti et al. 2004, Poni et al. 2006) which are based only on dark respiration measurements of the different organs (Palliotti et al. 2004).

Bunch respiration represents one of the major carbon budgets from the total plant carbon losses. Irrigation and genotype affected the bunch respiration losses per vine until berries started to soften, and until veraison, respectively. These results confirmed the findings reported by our research group, where plant water status and genotype affected bunch respiration during the first stages of grape development.

Root respiration was influenced by the vine phenology as clearly shown in both cultivars (Figure 1). This temporal variation corresponds to the effect of phenology on soil respiration previously found by Hernández-Montes et al. (2017). Additionally, irrigation increased root respiration, likely because of the positive effect of moisture on root growth (Franck et al. 2011). Annual root respiration costs represented 20–40% of the total loss, similar to root respiration costs simulated by Lakso et al. (2008) and lower than the root respiration expenses estimated by Escalona et al. (2012).

Table 4. Total annual biomass production from leaves, stems, bunches, roots, trunk and arms from irrigated and non-irrigated Grenache and Tempranillo vines during 2013 and 2014 seasons.

Year	Cultivar	Treatment	Leaves (g)	Shoots (g)	Bunches (g)	Total aerial organs (g)	Trunk and arms (g)	Roots (including main axis) (g)	Total permanent organs (g)
2013	Grenache	I	367.8 ± 37 a	454.6 ± 53 b	828.1 ± 177 ab	1559.6 ± 88 bc	–	–	–
		NI	239.5 ± 11 b	302.8 ± 21 b	581.5 ± 58 b	1123.8 ± 70 c	–	–	–
	Tempranillo	I	420.9 ± 49 a	690.0 ± 85 a	1462.5 ± 327 a	2573.4 ± 440 a	–	–	–
		NI	356.1 ± 37 ab	478.2 ± 80 ab	1357.7 ± 144ab	2192.0 ± 246 ab	–	–	–
2014	Grenache	I	333.6 ± 37 b	480.8 ± 48 a	1322.0 ± 204 a	2469.9 ± 205 a	1098.9 ± 175 a	1096.4 ± 101 a	2195.3 ± 259 a
		NI	323.3 ± 61 b	461.7 ± 83 a	794.0 ± 179 b	1902.2 ± 103 ab	860.6 ± 64 ab	830.4 ± 114 b	1691.0 ± 165 ab
	Tempranillo	I	469.2 ± 18 a	618.9 ± 63 a	1001.6 ± 64 ab	2558.7 ± 88 a	662.0 ± 25 b	1098.9 ± 65 a	1760.9 ± 85 ab
		NI	297.0 ± 69 b	462.7 ± 99 a	716.9 ± 216 b	1721.9 ± 285 b	602.1 ± 17 b	849.7 ± 106 ab	1451.8 ± 92 b
Cultivar			**	**	*	**	**	n.s.	*
Treatment			**	**	*	***	n.s.	*	*
Cultivar × Treatment			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Year			n.s.	n.s.	n.s.	*	–	–	–
Year × Cultivar			n.s.	n.s.	***	***	–	–	–
Year × Treatment			n.s.	n.s.	n.s.	n.s.	–	–	–

*, $P < 0.1$; **, $P < 0.05$; ***, $P < 0.001$; n.s., not significant; values are means ± SE ($n = 4$); – denotes not available value for that year. Different letters denote a significant difference ($P < 0.05$) among cultivars and treatments in each year. I, irrigated; NI, non-irrigated.

Root respiration is not included in most of carbon balance models in grapevines (Palliotti et al. 2004, Poni et al. 2006, Mirás-Avalos et al. 2018) because of the technical difficulties involved in its estimation. Respiration losses are dependent on carbon availability and, in consequence, above- and below-ground respiration should be linked to leaf C assimilation. To test this hypothesis, the linear regression among these rates was studied at different phenological stages (Table S2) which implicates significant variation in plant carbon uptake. Figure 4 shows a clear linear correspondence with regression coefficients of 0.73 and 0.91, confirming the dependency of below-ground respiration rates from the carbon supply by leaves. Such correspondence can be used to improve the current models to estimate root respiration based on non-destructive measurements in leaves, because their relationship was consistent throughout phenology for irrigated ($R^2 = 0.73$) and non-irrigated ($R^2 = 0.91$) vines (Figure 4). These results confirmed the relationship between maximum photosynthesis and estimated root respiration found by Escalona et al. (2012) in potted vines, supporting the link between photosynthesis and carbohydrate export to roots.

The methodological difficulties encountered in the study of root growth and activity explains the limited work on this subject reported in the literature. Among these few reports, Franck et al. (2011) measured lower values of root respiration during the growing season using a trenching approach (not destructive) in a drip-irrigated field experiment. Related to this issue, our study integrated a novel estimation of root respiration comparing the soil respiration along the row and between rows (Hernández-Montes et al. 2017) and the destructive technique of root biomass at the end of the experiment, reinforcing the value of the present data and the relationships found.

Biomass accumulation—coupling of above-ground and below-ground carbon allocation

It is widely assumed that biomass production is determined by carbon gain through photosynthesis, but also by carbon losses through respiration (Valentini 2000, Griffis et al. 2004), and this was confirmed by the strong correlation found between aerial respiration and aerial biomass accumulation (Figure 2a). Net plant C assimilation was calculated (leaf C assimilation minus leaf, stem, fruit and root respiration) during phenology in order to estimate the amount of C turned into biomass. Early spring growth is supported by the stored reserves (Holzapfel et al. 2010) as shown by the low net C assimilation found in this study until 30 DAB (Table 2). Interestingly, after that time, the net carbon assimilation remained constant until 180 DAB, indicating a constant biomass accumulation during the growing season despite the difference in leaf carbon assimilation and respiration losses found during phenology. Above-ground dry mass values were in line with the dry mass accumulation in Tempranillo grapevines under two different irrigation conditions in Spain (Mirás-Avalos et al. 2018). Our study confirms the significance of respiratory processes to the carbon balance in field-grown grapevines, including a relationship between above- and below-ground activity during phenology (Figure 4). Also, biomass accumulation was affected by genotype and plant water status, directly influencing the inputs (photosynthesis) and the outputs (respiration) of the carbon balance of the vine. Additionally, the comparison between the net C fixed during the growing season (excluding roots) and the aerial biomass accumulated during the growing season could provide information about the

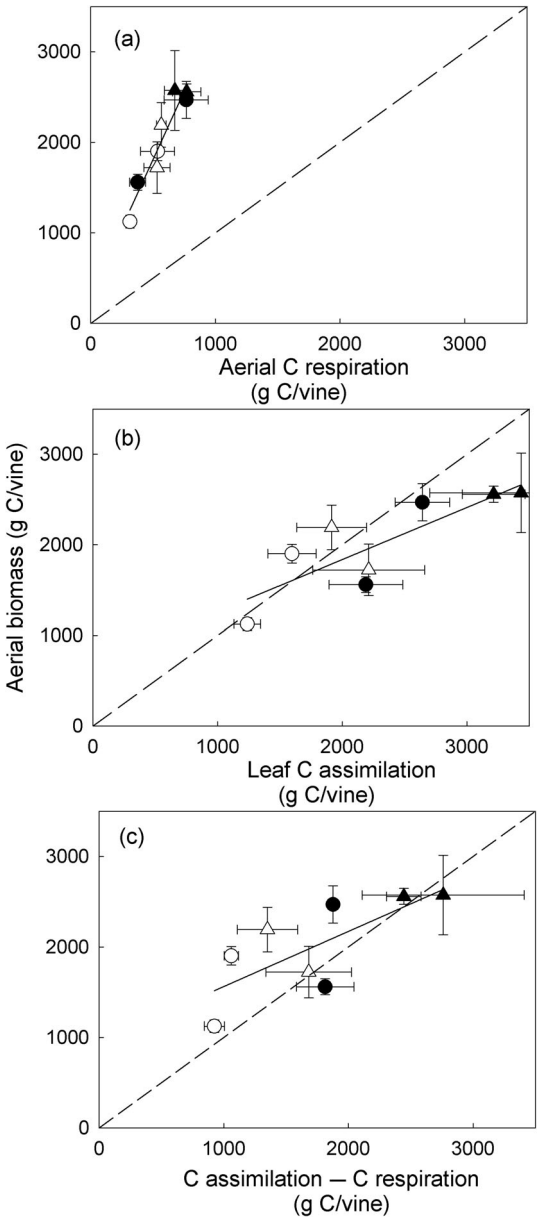

Figure 2. Relationship between the above-ground (aerial) annual biomass (respired carbon) and (a) annual respiratory carbon losses ($R^2 = 0.91$, $P < 0.001$), (b) annual leaf carbon assimilation ($R^2 = 0.69$, $P < 0.01$) and (c) the difference between assimilated carbon and respired carbon ($R^2 = 0.54$, $P < 0.04$) for Garnacha (●, ○) and Tempranillo (▲, △) and under irrigated (●, ▲) and non-irrigated (○, △) conditions in 2013 and 2014 ($n = 3$–4).

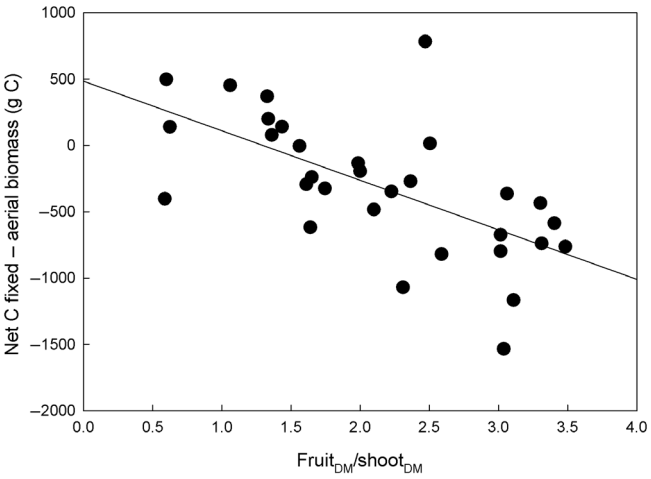

Figure 3. Relationship between the ratio of fruit dry mass to shoot dry mass and estimated carbohydrate reserves (net carbon fixed minus aerial biomass) ($R^2 = 0.4$). Circles represent each replicate from 2013 to 2014.

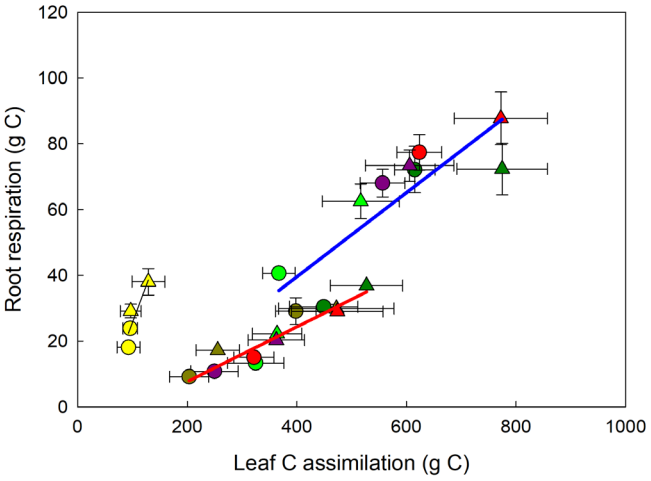

Figure 4. Relationship between monthly leaf net carbon assimilation and root respiration per plant for Garnacha (●) and Tempranillo (▲) from April to September. The periods represented are for 1 to 30 days after budburst (DAB) (○), for 31 to 60 DAB (●), for 61 to 90 DAB (●), for 91 to 120 DAB (●), for 121 to 150 DAB (●) and for 150 to 180 DAB (●). Symbols represent averages from 2013 to 2014 ($n = 7$–8) and the lines represent a linear regression for water-stressed plants ($R^2 = 0.91$, $P < 0.001$) (——), for irrigated plants ($R^2 = 0.73$, $P = 0.002$) (——) and for the period 1 to 30 DAB ($R^2 = 0.8$, $P = 0.1$) (——).

effect of cultivar and irrigation on storage reserves. Non-irrigated plants (both Garnacha and Tempranillo) accumulated higher aerial biomass compared to the net C fixed during the growing season. Such results confirm the idea of reserves acting as a buffer by providing a temporary carbon supply that can be used during water stress periods (Holzapfel et al. 2010). Furthermore, carbohydrate mobilisation and storage can be influenced by seasonal variation in yield, canopy size or climatic conditions (Holzapfel and Smith 2012), affecting reproductive and vegetative development. The fruit to shoot ratio affected the carbohydrate reserves (Figure 3), confirming that crop load can alter carbohydrate reserve storage and mobilisation by changing the relationship between the main carbon sink and source during the ripening period (Holzapfel and Smith 2012).

The proportion of carbon allocated to fruit in relation to the remaining organs is usually represented by the harvest index. Despite the positive effect of irrigation recently reported (Torres et al. 2021), no significant effect of irrigation on harvest index was found in our study, likely because of the difference in the irrigation applied between the studies. Despite the significant effect of genotype on the accumulation of total aerial biomass, both cultivars allocated a similar proportion of carbon to the fruit compared to the remaining aerial biomass. The root/shoot ratio is used in grapevines and other woody plants to provide a quantitative relationship between below- an above-ground growth (Williams 1996). In this study the root/shoot (including leaves) ratio in Garnacha (0.8–1.0) was higher than in Tempranillo (0.6–0.62). Irrigation decreased the root/shoot ratio in Tempranillo, contrary to Garnacha, suggesting that the amount of carbon allocated to the roots decreased or increased under irrigation depending on the genotype. The relationship between root respiration and leaf carbon assimilation during vine phenology (Figure 4) suggests that measurements in the aerial part could provide a better

understanding of root activity through non-destructive techniques that help to understand root growth and the C allocation into the roots during the vine phenological cycle, including the different root growth pulses along vine phenology.

The seasonal increment in trunk biomass accumulation varies with growing conditions and genotype (Williams 1996). In our study, Garnacha allocated more C into the root system than Tempranillo under drought conditions. Moreover, Garnacha respired less C from the total fixed by photosynthesis than Tempranillo. This result reinforces the reputation of Garnacha as a more drought-resistant cultivar than Tempranillo, because the greater C allocation to roots and lower respiration rate could represent higher C stored, and higher root extension capacity, even though such activity needs to be confirmed. All these results may also be interesting in the context of climate change, because of the growing interest in locating species and genotypes with a high C storage potential.

Conclusions

The present study highlighted the variation in carbon respiration during plant phenology that represents a significant proportion of the total carbon assimilation in field-grown vines, as found in previous studies. Plant phenology, cultivar, and plant water status affected the several components of carbon balance, indicating a significant variation among the different organs of the vine. Accumulation of aerial biomass was influenced by plant carbon budgets during the growing season. Tempranillo recorded the highest values of carbon fixation, leaf and stem respiration, as well as the highest values of aerial biomass. Garnacha, however, had higher values of root respiration loss, and was the cultivar that allocated a higher biomass to the permanent organs. Therefore, the respiratory losses varied significantly between vine organs and environmental conditions, but also between cultivars. All these factors should be considered to gain a better understanding of carbon balances in grapevines as well as for a better assessment of management techniques for a more sustainable viticulture.

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References

Amthor, J.S. (2000) The McCree-de Wit-Penning de Vries-Thornley respiration paradigms: 30 years later. Annals of. Botany **86**, 1–20.

Baeza, P., Ruiz, C., Cuevas, E., Sotés, V. and Lissarrague, J.R. (2005) Ecophysiological and agronomic response of Tempranillo grapevines to four training systems. American Journal of Enology and Viticulture **56**, 129–138.

Bota, J., Flexas, J. and Medrano, H. (2001) Genetic variability of photosynthesis and water use in Balearic grapevine cultivars. Annals of Applied Biology **138**, 353–361.

Bota, J., Tomás, M., Flexas, J., Medrano, H. and Escalona, J.M. (2016) Differences among grapevine cultivars in their stomatal behavior and water use efficiency under progressive water stress. Agricultural Water Management **164**, 91–99.

Brunori, E., Farina, R. and Biasi, R. (2016) Sustainable viticulture: the carbon-sink function of the vineyard agro-ecosystem. Agriculture, Ecosystems & Environment **223**, 10–21.

Buwalda, J.G. (1991) A mathematical model of carbon acquisition and utilisation by kiwifruit vines. Ecological Modelling **57**, 43–64.

Coombe, B.G. (1995) Growth stages of the grapevine. Adoption of a system for identifying grapevine growth stages. Australian Journal of Grape and Wine Research **1**, 104–110.

de Herralde, F., Savé, R., Aranda, X. and Biel, C. (2010) Grapevine roots and soil environment: growth, distribution and function. Delrot, S., Medrano, H., Or, E., Bavaresco, L. and Grando, S., eds. Methodologies and results in grapevine research (Springer Netherlands: Dordrecht, The Netherlands) pp. 1–20.

De Souza, C.R., Maroco, J.P., Dos Santos, T.P., Rodrigues, M.L., Lopes, C.M., Pereira, J.S. and Chaves, M.M. (2003) Partial rootzone drying: regulation of stomatal aperture and carbon assimilation in field-grown grapevines (*Vitis vinifera* cv. Moscatel). Functional Plant Biology **30**, 653–662.

Douthe, C., Medrano, H., Tortosa, I., Escalona, J.M., Hernández-Montes, E. and Pou, A. (2018) Whole-plant water use in field grown grapevine: seasonal and environmental effects on water and carbon balance. Frontiers in Plant Science **871**, 1540.

Düring, H. (1988) CO_2 assimilation and photorespiration of grapevine leaves: responses to light and drought. Vitis **27**, 199–208.

El Aou-Ouad, H., Bota, J., Obata, T., Montero, R., Fernie, A.R., Medrano, H., Pou, A. and Florez-Sarasa, I. (2018) Combined drought and virus infection trigger aspects of respiratory metabolism related to grapevine physiological responses. Journal of Plant Physiology **231**, 19–30.

Escalona, J.M., Flexas, J., Bota, J. and Medrano, H. (2003) Distribution of leaf photosynthesis and transpiration within grapevine canopies under different drought conditions. Vitis **42**, 57–64.

Escalona, J.M., Tomàs, M., Martorell, S., Medrano, H., Ribas-Carbo, M. and Flexas, J. (2012) Carbon balance in grapevines under different soil water supply: importance of whole plant respiration. Australian Journal of Grape and Wine Research **18**, 308–318.

Flexas, J., Bota, J., Galmés, J., Medrano, H. and Ribas-Carbó, M. (2006) Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. Physiologia Plantarum **127**, 343–352.

Franck, N., Morales, J.P., Arancibia-Avendaño, D., García de Cortázar, V., Perez-Quezada, J.F., Zurita-Silva, A. and Pastenes, C. (2011) Seasonal fluctuations in *Vitis vinifera* root respiration in the field. New Phytologist **192**, 939–951.

Greer, D.H. (2017) Responses of biomass accumulation, photosynthesis and the net carbon budget to high canopy temperatures of *Vitis vinifera* L. cv. Semillon vines grown in field conditions. Environmental and Experimental Botany **138**, 10–20.

Greer, D.H. and Sicard, S.M. (2009) The net carbon balance in relation to growth and biomass accumulation of grapevines (*Vitis vinifera* cv. Semillon) grown in a controlled environment. Functional Plant Biology **36**, 645–653.

Greer, D.H., Weedon, M.M. and Weston, C. (2011) Reductions in biomass accumulation, photosynthesis in situ and net carbon balance are the costs of protecting *Vitis vinifera* 'Semillon' grapevines from heat stress with shade covering. AoB Plants **11**, 1–13.

Griffis, T.J., Black, T.A., Gaumont-Guay, D., Drewitt, G.B., Nesic, Z., Barr, A.G., Morgenstern, K. and Kljun, N. (2004) Seasonal variation and partitioning of ecosystem respiration in a southern boreal aspen forest. Agricultural and Forest Meteorology **125**, 207–223.

Hernández-Montes, E., Escalona, J.M., Tomás, M. and Medrano, H. (2017) Influence of water availability and grapevine phenological stage on the spatial variation in soil respiration. Australian Journal of Grape and Wine Research **23**, 273–279.

Hernández-Montes, E., Escalona, J.M., Tomàs, M. and Medrano, H. (2020) Plant water status and genotype affect fruit respiration in grapevines. Physiologia Plantarum **169**, 544–554.

Hernández-Montes, E., Tomás, M., Escalona, J.M., Bota, J. and Medrano, H. (2019) Leaf growth rate and nitrogen content determine respiratory costs during leaf expansion in grapevines. Physiologia Plantarum **165**, 746–754.

Holzapfel, B.P. and Smith, J.P. (2012) Developmental stage and climatic factors impact more on carbohydrate reserve dynamics of Shiraz than cultural practice. American Journal of Enology and Viticulture **63**, 333–342.

Holzapfel, B.P., Smith, J.P., Field, S.K. and Hardie, W.J. (2010) Dynamics of carbohydrate reserves in cultivated grapevines. Horticultural Reviews **37**, 143–211.
Iglesias, D.J., Quiñones, A., Font, A., Martínez-Alcántara, B., Forner-Giner, M.Á., Legaz, F. and Primo-Millo, E. (2013) Carbon balance of citrus plantations in eastern Spain. Agriculture Ecosystems and Environment **171**, 103–111.
Lakso, A.N. and Poni, S. (2005) 'Vitisim'—a simplified carbon balance model of a grapevine. Proceedings of the XIV international GESCO viticulture congress; 23–27 August 2005; Geisenheim, Germany (Groupe d'Etude des Systemes de Conduite de la Vigne: Montpellier, France) pp. 478–484.
Lakso, A.N., Intrigliolo, D. and Eissenstat, D.M. (2008) Modeling Concord grapes with 'Vitisim', a simplified carbon balance model: understanding pruning effects. Acta Horticulturae **803**, 243–250.
Lakso, A.N., Denning, S.S., Dunst, R., Fendinger, A. and Pool, R.M. (1997) Comparisons of growth and gas exchange of conventionally and minimally pruned 'Concord' grapevines. Proceedings for the 4th international symposium on cool climate viticulture and enology; 16–20 July 1996; Rochester, NY, USA (Vitis Information Resources: Lancaster, PA, USA) pp. 11–12.
Martínez-Lüscher, J., Morales, F., Sánchez-Díaz, M., Delrot, S., Aguirreolea, J., Gomès, E. and Pascual, I. (2015) Climate change conditions (elevated CO_2 and temperature) and UV-B radiation affect grapevine (*Vitis vinifera* cv. Tempranillo) leaf carbon assimilation, altering fruit ripening rates. Plant Science **236**, 168–176.
Martorell, S., Diaz-Espejo, A., Tomàs, M., Pou, A., El Aou-Ouad, H., Escalona, J.M., Vadell, J., Ribas-Carbó, M., Flexas, J. and Medrano, H. (2015) Differences in water-use-efficiency between two *Vitis vinifera* cultivars (Grenache and Tempranillo) explained by the combined response of stomata to hydraulic and chemical signals during water stress. Agricultural Water Management **156**, 1–9.
Medrano, H., Escalona, J.M., Cifre, J., Bota, J. and Flexas, J. (2003) A ten-year study on the physiology of two Spanish grapevine cultivars under field conditions: effects of water availability from leaf photosynthesis to grape yield and quality. Functional Plant Biology **30**, 607–619.
Medrano, H., Perez Peña, J., Prieto, J., Tomás, M., Franck, N. and Escalona, J.M. (2016) Carbon balance in grapevine under a changing climate. Gerós, H., Chaves, M.M., Medrano, H. and Delrot, S., eds. Grapevine in a changing environment: a molecular and ecophysiological perspective (John Wiley: Chichester, England) pp. 109–134.
Miller, D.P., Howell, G.S. and Flore, J.A. (1996) A whole-plant, open, gas-exchange system for measuring net photosynthesis of potted woody plants. HortScience **31**, 944–946.
Miranda, C., Santesteban, L.G., Escalona, J.M., De Herralde, F., Aranda, X., Nadal, M., Intrigliolo, D.S., Castel, J.R., Royo, J.B. and Medrano, H. (2017) Allometric relationships for estimating vegetative and reproductive biomass in grapevine (*Vitis vinifera* L.). Australian Journal of Grape and Wine Research **23**, 441–451.
Mirás-Avalos, J.M., Uriarte, D., Lakso, A.N. and Intrigliolo, D.S. (2018) Modeling grapevine performance with 'VitiSim' a weather-based carbon balance model: water status and climate change scenarios. Scientia Horticulturae **240**, 561–571.
Montero, R., El Aou-Ouad, H., Flexas, J. and Bota, J. (2016) Effects of grapevine leafroll associated virus 3 (GLRaV-3) on plant carbon balance in *Vitis vinifera* L. cv. Giró Ros. Theoretical and Experimental Plant Physiology **28**, 1–10.
Palliotti, A., Cartechini, A., Nasini, L., Silvestroni, O., Mattioli, S. and Neri, D. (2004) Seasonal carbon balance of 'sangiovese' grapevines in two different central Italy environments. Acta Horticulturae **652**, 183–190.
Poni, S., Intrieri, C. and Magnanini, E. (2000) Seasonal growth and gas exchange of conventionally and minimally pruned Chardonnay canopies. Vitis **39**, 13–18.
Poni, S., Palliotti, A. and Bernizzoni, F. (2006) Calibration and evaluation of a STELLA software-based daily CO_2 balance model in *Vitis vinifera* L. Journal of the American Society for Horticultural Science **131**, 273–283.
Poni, S., Bernizzoni, F., Civardi, S., Gatti, M., Porro, D. and Camin, F. (2009) Performance and water-use efficiency (single-leaf vs. whole-canopy) of well-watered and half-stressed split-root Lambrusco grapevines grown in Po Valley (Italy). Agriculture Ecosystems and Environment **129**, 97–106.
Sanchez-de-Miguel, P., Junquera, P., De la Fuente, M., Jimenez, L., Linares, R., Baeza, P. and Lissarrague, J.R. (2011) Estimation of vineyard leaf area by linear regression. Spanish Journal of Agricultural Research **9**, 202–212.
Scandellari, F., Caruso, G., Liguori, G., Meggio, F., Palese, A.M., Zanotelli, D., Celano, G., Gucci, R., Inglese, P., Pitacco, A. and Tagliavini, M. (2016) A survey of carbon sequestration potential of orchards and vineyards in Italy. European Journal of Horticultural Science **81**, 106–114.
Schultz, H.R. (2003) Extension of a Farquhar model for limitations of leaf photosynthesis induced by light environment, phenology and leaf age in grapevines (*Vitis vinifera* L. cvv. White Riesling and Zinfandel). Functional Plant Biology **30**, 673–687.
Tarara, J.M., Peña, J.E.P., Keller, M., Schreiner, R.P. and Smithyman, R.P. (2011) Net carbon exchange in grapevine canopies responds rapidly to timing and extent of regulated deficit irrigation. Functional Plant Biology **38**, 386–400.
Tomás, M., Medrano, H., Brugnoli, E., Escalona, J.M., Martorell, S., Pou, A., Ribas-Carbó, M. and Flexas, J. (2014) Variability of mesophyll conductance in grapevine cultivars under water stress conditions in relation to leaf anatomy and water use efficiency. Australian Journal of Grape and Wine Research **20**, 272–280.
Torres, N., Martínez-Lüscher, J., Porte, E., Yu, R. and Kurtural, K. (2021) Impacts of leaf removal and shoot thinning on cumulative daily light intensity and thermal time and their cascading effects of grapevine (*Vitis vinifera* L.) berry and wine chemistry in warm climates. Food Chemistry **343**, 128447.
Valentini, R. (2000) Respiration as the main determinant of carbon balance in European forests. Nature **404**, 861–865.
Vivin, P., Castelan, M. and Gaudillère, J.P. (2002) A source/sink model to simulate seasonal allocation of carbon in grapevine. Acta Horticulturae **584**, 43–56.
Williams, L.E. (1996) Grape, Photoassimilate distribution in plants and crops. Zamski, E. and Schaffer, A.A., eds. Source–sink relationships (Marcel Dekker: New York, NY, USA) pp. 851–881.
Wermelinger, B., Baumgärtner, J. and Gutierrez, A.P. (1991) A demographic model of assimilation and allocation of carbon and nitrogen in grapevines. Ecological Modelling **53**, 1–26.
Weyand, K.M. and Schultz, H.R. (2006) Light interception, gas exchange and carbon balance of different canopy zones of minimally and cane-pruned field-grown Riesling grapevines. Vitis—Journal of Grapevine Research **45**, 105–114.

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Supporting information

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Figure S1. Examples of root systems after uprooting the vines in 2014 of Garnacha (a) irrigated and (b) non-irrigated and of Tempranillo (c) irrigated and (d) non-irrigated.

Table S1. Environmental variables registered during 2013 and 2014.

Table S2. Phenological stages and corresponding dates for the years 2013 and 2014.