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Two distinct plant respiratory physiotypes might exist which correspond to fast-growing and slow-growing species

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ABSTRACT

The origin of the carbon atoms in CO_2 respired by leaves in the dark of several plant species has been studied using $^{13}C/^{12}C$ stable isotopes. This study was conducted using an open gas exchange system for isotope labeling that was coupled to an elemental analyzer and further linked to an isotope ratio mass spectrometer (EA–IRMS) or coupled to a gas chromatography–combustion-isotope ratio mass spectrometer (GC–C-IRMS). We demonstrate here that the carbon, which is recently assimilated during photosynthesis, accounts for nearly ca. 50% of the carbon in the CO_2 lost through dark respiration (R_d) after illumination in fast-growing and cultivated plants and trees and, accounts for only ca. 10% in slow-growing plants. Moreover, our study shows that fast-growing plants, which had the largest percentages of newly fixed carbon of leaf-respired CO_2, were also those with the largest shoot/root ratios, whereas slow-growing plants showed the lowest shoot/root values.

Introduction

In leaves, the CO_2 fixed by the Calvin cycle is subsequently converted to triose phosphates which are partitioned between (i) glycolysis and mitochondrial respiration, (ii) processes leading to sucrose synthesis and export, and (iii) starch synthesis and temporary storage. The reducing power provided by photosynthesis drives C and N assimilation, whereas in tissues which remain unexposed to light and in non-green tissues such as roots, the necessary reducing power is mainly supplied by the oxidative pentose phosphate pathway (Foyer et al., 2011). Previous studies (Atkin et al., 1996; Loveys et al., 2002) showed that 30–70% of CO_2 fixed by photosynthesis during the day is respired by the plants and is therefore lost to the atmosphere. Moreover, according to Atkin et al. (2007), 50–70% of whole plant respiration takes place in the leaves. The assimilation of N by plants involves the reduction of nitrate to nitrite by nitrite reductase using NADH produced by the malate shuttled from the mitochondria or chloroplasts (Foyer and Noctor, 2002). Estimates of C partitioning in roots suggest that about 5% of root C catabolism is coupled to soil nitrate absorption, 15% to nitrate assimilation and 3% to ammonium assimilation (Bloom et al., 2002).

Whereas plants use photosynthesis to produce the carbohydrate substrate on which they depend, glycolysis and respiration are the processes whereby the energy stored in these carbohydrates is released. Respiration is a key physiological process in sustaining growth and biomass production of plants and ecosystems (Tcherkez et al., 2012). Respiratory metabolism provides ATP, reducing equivalents and metabolic intermediates used in biosynthesis elsewhere in the cell (Araújo et al., 2014). As observed by Araújo et al. (2014), although historically photosynthesis and respiration have been considered to be independent pathways, over the last decade, the functioning of chloroplasts and mitochondria has been described to be coordinated and tightly interact through intracellular metabolite pools. The metabolic interaction of respiratory metabolism with other pathways is being determined, particularly its relationship to Calvin cycle reactions in photosynthesis, photorespiration and nitrate assimilation (Araújo et al., 2014 and references therein).

Plant respiration has been described as differing from one species to another (Wright et al., 2001; Turnbull et al., 2003). It was hypothesized that slow-growing plants (with lower growth and ion transport rates) would have lower respiration rates than fast-growing plants. However, as observed by previous studies (Poorter et al., 1991; Scheurwater et al., 1998), differences in respiration

Abbreviations: $\delta^{13}C$, carbon isotope composition; $\Delta^{13}C$, carbon isotope discrimination; A_n, net photosynthetic rate; C_{new}, labeled C in respired CO_2; IRMS, isotope ratio mass spectrometry; PPFD, photosynthetic active photon flux density; R_d, dark respiration; RQ, respiration quotient.

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between fast- and slow-growing plants were much smaller than theoretically expected. According to Scheurwater et al. (1998) such differences could be explained by the fact that in fast-growing species (species with 3-fold higher relative growth rates, RGR, than slow-growing species; Poorter et al., 1990) respiratory costs are 70% lower than in slow-growing plants (Scheurwater et al., 1998). However, according to other authors (Atkin and Tjoelker, 2003), alpine plants, for example, show higher respiratory rates than lowland species. Those studies suggest that a greater amount of photoassimilates are invested in maintenance than growth processes in slow-growing plants when compared to fast-growing plants. Respiration can be broken down into three components, i.e. the respiratory cost of root growth and of ion uptake, and maintenance respiration. Protein turnover and the maintenance of ion gradients are regarded as the two most important maintenance processes in terms of energy requirements (Bouma et al., 1994; Scheurwater et al., 2000). With respect to the maintenance costs, a previous study conducted with the fast-growing *Dactylis glomerata* and the slow-growing *Festuca ovina* showed that both plant species spent between 22 and 30% of their daily ATP production dedicated to maintenance on protein turnover, which corresponds to 11–15% of total root ATP production per day (Scheurwater et al., 2000).

The origin of C used in respiratory processes is another matter of controversy. Even if respired C is derived from compounds such as malate, pyruvate, isocitrate and α-ketoglutarate, such C may proceed from recently fixed photoassimilates and/or remobilization of C storage forms such as starch, sugar and fructans (Lehmeier et al., 2008, 2010). The residence time of respired C varies from compounds that are rapidly (seconds to minutes) transferred to respiration centers to long lived compounds (such as proteins or storage carbohydrates) with long residence times (days to months) (Lehmeier et al., 2008). Lehmeier et al. (2008) showed that 43% of respiration in perennial ryegrass was supported by recently fixed photosynthates whereas the 57% was supported by the remobilization of storage compounds. Those studies highlighted the fact that carbohydrate respiration is supported by a heterogeneous mixture of molecules that cycle, more or less extensively, through a network of biochemical compounds and compartments.

Carbon residence time has been previously analyzed through the use of stable isotopes (Schnyder et al., 2003; Nogués et al., 2004; Lötscher and Gayler, 2005; Lehmeier et al., 2008, 2010; Aranjuelo et al., 2009). $^{13}CO_2$ labeling carried out by Lötscher and Gayler (2005) in *Medicago sativa* plants showed two phases in the appearance of enriched $^{13}CO_2$ in respiration. The largest amount of labeled ^{13}C (corresponding to current photosynthate activity) appeared in the first phase, whereas in the second phase the contribution of non-labeled C exceeded the labeled ^{13}C. The second respiration phase was fueled by one (or more) C stores.

Plants have several contrasting and complementary strategies for optimizing C and N uptake. However, these strategies do not occur randomly across terrestrial environments. Slow-growing plants usually grow in harsh and constraining conditions that might favor the recycling of reserves. In contrast, fast-growing species have to sustain rapid growth that requires higher respiration and metabolic activity (compared with slow-growing species). Therefore, for a given amount of assimilated carbon, it would be expected that slow-growing plants would invest a lower amount of recently fixed carbon in respiration processes than fast-growing plants (Baptist et al., 2009; Aranjuelo et al., 2011).

Although respiration metabolism has been extensively characterized over the last decade, important gaps remain to be elucidated. To date, the different contributions of C pools to leaf respiration in fast- and slow-growing plants are still unknown. Here, the $^{13}C/^{12}C$ isotope labeling technique was used to study the respiratory metabolism of recently fixed carbon in the leaves of ten different plant species. We used a system that consists of an LI-6400 open gas-exchange system coupled to an elemental analyzer (EA) and to an isotope ratio mass spectrometer (IRMS, for a recent review see Ghashghaie and Tcherkez, 2013). This system takes advantage of the difference in carbon isotope composition ($\delta^{13}C$) between atmospheric CO_2 (ca. – 9.5‰, see Section "Material and methods") and commercially available CO_2 (^{12}C-enriched e.g. ca. – 51.2‰). This allows one to calculate the contribution of stored carbon versus current photoassimilates to the production of CO_2 through respiration (Schnyder et al., 2003). It is noteworthy that this would not have been possible if heavily labeled carbon had been used (i.e. several percent more of ^{13}C would have blurred the contribution made by non-labeled carbon). The abundance of ^{13}C in the CO_2 used for labeling is in the same order of magnitude as that found in nature, thereby allowing us to calculate the proportion of 'new' (i.e. recently fixed) carbon in CO_2 respired in the dark (Nogués et al., 2004). Furthermore, this system also allowed us to estimate leaf metabolic fluxes *in vivo*.

The aim of this study was to determine the origin of the carbon atoms in the CO_2 respired by leaves of several C3 and one C4 plant species. Some data presented in this paper is in part a re-analysis of previously published data (Nogués et al., 2004, 2006a,b; Aranjuelo et al., 2009), such that this study brings together this previously published data along with new data for a more complete and in-depth analysis of plant respiration.

Material and methods

Plant material

In the present study, a large number of plants grown in different environments were used (i.e. from wild-grown to greenhouse-grown plants):

(i) Alpine *Ranunculus glacialis* (L.) plants were collected from the Galibier Pass in the French Alps, at an elevation of 2700 m, as previously described (Nogués et al., 2006a). For the measurements, the petiole was cut and maintained under water throughout the experiments, otherwise leaves were floated on water.

(ii) Two slow-growing Mediterranean plants (*Chamaerops humilis* L. and *Cycas revoluta* Thunb., gymnosperm) were grown in pots for 20 months in a greenhouse at the Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Cabrils, Barcelona, as previously described (Pardo et al., 2009; Aranjuelo et al., 2009). The plants were grown in 4-L pots (one plant per pot) containing a mixture of peat and perlite in a 2:1 ratio (v:v) and they were continuously watered with ca. 1-L day^{-1} of Hoagland complete nutrient solution (pH 6.5) through a drip irrigation system (Hoagland and Arnon, 1950).

(iii) Sunflower (*Helianthus annuus* L.) plants were grown for 5 weeks in four growth chambers (E15, Conviron, Winnipeg, Canada). These four growth chambers formed part of the mesocosm $^{13}CO_2/^{12}CO_2$ open gas exchange system as described in detail by Schnyder et al. (2003). The plants were sown individually in plastic pots (5 cm diameter–35 cm depth) filled with washed quartz sand. Modified Hoagland nutrient solution (7.5 mol N m^{-3}) was supplied by an automatic irrigation system throughout the experiment. Irradiance during the 16 h photoperiod was supplied by cool white fluorescent tubes (16 × 160 W; Sylvania Germany GmbH, Erlangen, Germany) and incandescent lamps (12 × 100 W; General Electric Germany, München, Germany), and was maintained at ca. 500 μmol m^{-2} s^{-1} photosynthetic active photon flux density (PPFD) at the top of the canopy by adjusting the height of the lamps following plant development. Air temperature was

controlled at 20/16 °C and relative humidity at 75/80% during the photo and dark periods, respectively.

(iv) French bean (*Phaseolus vulgaris* L.) plants were grown from seeds in 1-L pots of potting mix for 5 weeks in a greenhouse at Université Paris Sud as described by Nogués et al. (2004). Minimum PPFD during a 16-h photoperiod was maintained at approximately 500 μmol m^{-2} s^{-1} by supplementary lighting. Temperature and vapor pressure deficit were maintained at ca. 25.5/18.5 °C and 1.4/1.2 kPa day/night, respectively.

(v) Three isolated 20-year-old beech (*Fagus sylvatica* L.) trees were grown at the campus of the Université Paris Sud (Orsay, France) as previously described (Nogués et al., 2006b). Mean annual precipitation, and minimum and maximum temperatures of the site are 685 mm, 7 °C and 16 °C, respectively.

(vi) *Medicago sativa*, *Triticum turgidum*, *Arundo donax* and the C4 *Zea mays* L. plants were grown in pots in a greenhouse at Servei de Camps Experimentals of the University of Barcelona. They were planted in pots filled with sand and watered daily with a complete Hoagland solution. Details of the growing conditions at the University of Barcelona can be found in Cabrera-Bosquet et al. (2007). Plants were grown in the greenhouse under mean day/night temperatures of ca. 25/15 °C and a maximum PPFD of ca. 1000 μmol m^{-2} s^{-1}.

All experiments were carried out on the fully expanded mature leaves for (at least) three plants for each species studied.

Gas exchange determinations

Plants were removed from the greenhouse or the field after a light period of about 8–10 h and the leaf was placed for ca. 50 min in a respiration chamber to take measurements of dark-respired CO_2 (unlabeled). After the initial measurement of dark-respired CO_2, the leaf was removed from the respiration chamber and placed in a specially designed gas-exchange labeling chamber built within the laboratory for online isotope labeling (see below; Nogués et al., 2004). After labeling, the leaf was removed from the labeling chamber, and the leaf was returned to the respiration chamber system for measurements of dark-respired CO_2 (labeled).

Gas-exchange measurements were carried out with the Li-6400 gas exchange portable photosynthesis system (LI-COR, Lincoln, NE, USA) on healthy and fully expanded leaves under conditions similar to growth conditions (500 μmol m^{-2} s^{-1} PPFD, 25 °C, 380 μmol mol^{-1} CO_2, 21% O_2). Plants were dark-adapted for 50 min before dark respiration (R_d) measurements were taken (Nogués et al., 2004; Florez-Sarasa et al., 2012).

The respiration quotient (RQ) was calculated from the ratio of carbon production [$v(CO_2)$] to oxygen consumption [$v(O_2)$] according Nogués et al. (2004): $RQ = v(CO_2)/v(O_2)$. CO_2 production in darkness was measured using the Li-6400 described above. Oxygen consumption of leaf discs was measured with an oxygen electrode (Hansatech, Norfolk, England). RQ values close to 1 indicate highly oxygenated substrates (e.g. carbohydrates) and RQ values close to 0.6 indicate weakly oxygenated substrates (e.g. fatty acids) which are used during respiratory processes. RQ values higher than 1 may be observed when metabolites such as malate or citrate is oxidized.

Isotope labeling measurements

As mentioned above, after the initial measurement of dark-respired CO_2, leaves were placed in a custom designed and built gas-exchange chamber for isotope labeling, as described by Nogués et al. (2004). The chamber was connected in parallel to the sample air hose of the LI-6400. This aluminum chamber (20 cm × 12 cm × 6 cm), fitted with a clear plastic lid, can hold two to four leaves (total leaf surface ca. 50 cm^2). Two fans were placed in the chamber which generated a boundary layer conductance to water of ca. 6.7 mol m^{-2} s^{-1}. Leaf temperature in the chamber was maintained at ca. 20 °C by water cooling the jacket around the leaf chamber, and was measured with a copper-constant an thermocouple plugged into the thermocouple sensor connector of the LI-6400 chamber/IRGA. Ingoing air was passed through the chamber at a rate of 1-L min^{-1} and monitored by the LI-6400. Molar fractions of CO_2 and humidity were measured with the LI-6400 infrared gas analyzer (IRGA). The PPFD inside the chamber was maintained at 500 μmol m^{-2} s^{-1} during the labeling period. Light was supplied by a 500-W halogen lamp (Massive N.V., Kontich, Belgium). The lamp was placed at approximately 30 cm above the chamber and 5 cm of deionized water and 1 cm of glass in the container were used to filter the radiation. The PPFD at the leaf level inside the chamber was maintained at 500 μmol m^{-2} s^{-1} during the labeling period. For labeling, CO_2 was supplied from a bottle (Air Liquide, Grigny, France) with a $\delta^{13}C$ value depleted in ^{13}C relative to the air (for example −51.2 ± 0.1‰; see Table 1).

All species assimilated the same amount of labeled CO_2 (i.e. ca. 350 mmol C m^{-2}) but the assimilation time varied between the different plant species studied according to their net assimilation rates (Table 2). For example, *P. vulgaris* had a net photosynthetic rate (A_n) of ca. 12.4 μmol C m^{-2} s^{-1} (Table 2), so it was labeled as ca. 8 h which is the time needed to reach ca. 350 mmol C m^{-2} (12.4 μmol C m^{-2} s^{-1} × 8 h × 3.6); and so on.

After photosynthetic measurements, the outgoing air of the chamber was shunted and the air with a CO_2 concentration of ca. 300 μL L^{-1} was collected in 50-mL glass balloons or in 10-mL vacutainers and analyzed in order to measure photosynthetic carbon isotopic discrimination ($\Delta^{13}C$). The $\Delta^{13}C$ measured varied between 20.4‰ and 5.3‰ for C3 and C4 plants, respectively.

Table 1
$\delta^{13}C$ of the CO_2 respired before and after labeling (‰), $\delta^{13}C$ of the CO_2 used during labeling (‰), $\Delta^{13}C$ measured during the labeling period (‰) and the % of new carbon in the CO_2 respired after labeling are shown for several C3 and one C4 (*Z. mays*) plant species. All species assimilated the same amount of labeled CO_2 (i.e. ca. 350 mmol C m^{-2}). Data is the mean of (at least) three replicates ± SE. *Ranunculus glacialis* data is from Nogués et al. (2006b). *Chamaerops humilis* and *Cycas revoluta* (gymnosperm) data is from Aranjuelo et al. (2009). *Phaseolus vulgaris* data is from Nogués et al. (2004). *Fagus sylvatica* data is from Nogués et al. (2006a).

Plant species	$\delta^{13}C$ (‰)			$\Delta^{13}C$ during labeling (‰)	New C in CO_2 (%)
	Before	After	Used during labeling		
Ranunculus glacialis	−23.4 ± 0.7	−28.0 ± 0.8	−51.2 ± 0.1	20.4 ± 0.7	9.6 ± 1.1
Chamaerops humilis	−21.8 ± 0.5	−23.2 ± 0.3	−22.9 ± 0.1	15.5 ± 0.9	6.7 ± 0.7
Cycas revoluta	−19.2 ± 0.3	−23.3 ± 0.2	−22.9 ± 0.1	13.1 ± 1.1	17.6 ± 0.6
Helianthus annuus	−15.6 ± 0.7	−38.1 ± 1.0	−45.4 ± 0.1	17.9 ± 1.0	47.0 ± 0.8
Phaseolus vulgaris	−22.0 ± 0.6	−44.8 ± 0.8	−51.2 ± 0.1	20.1 ± 0.2	50.3 ± 0.9
Fagus sylvatica	−18.8 ± 0.7	−44.5 ± 0.9	−51.2 ± 0.1	17.0 ± 0.6	56.8 ± 1.0
Zea mays	−15.4 ± 0.5	−25.9 ± 0.4	−37.9 ± 0.1	5.3 ± 0.5	46.7 ± 0.9
Medicago sativa	−24.8 ± 0.5	−31.3 ± 1.3	−37.9 ± 0.1	17.2 ± 1.1	49.6 ± 0.9
Triticum turgidum	−18.8 ± 0.3	−27.0 ± 1.3	−37.9 ± 0.1	18.1 ± 0.9	43.0 ± 1.8
Arundo donax	−20.9 ± 0.8	−28.5 ± 1.1	−37.9 ± 0.1	18.9 ± 1.2	44.7 ± 1.5

Table 2
Net assimilation rates (A_n, μmol m^{-2} s^{-1}), respiration rates in the dark (R_d, μmol m^{-2} s^{-1}), R_d/A_n ratios (%) and respiration quotients (RQ) are shown for mature leaves of the studied plant species. Measurements were done under atmospheric conditions at PPFD 1000–1200 μmol m^{-2} s^{-1} or in the dark. Data is the mean of (at least) three replicates ± SE. *Ranunculus glacialis* data is from Nogués et al. (2006b). *Chamaerops humilis* and *Cycas revoluta* (gymnosperm) data is from Aranjuelo et al. (2009). *Phaseolus vulgaris* data is from Nogués et al. (2004). *Fagus sylvatica* data is from Nogués et al. (2006a).

Plant species	A_n (μmol m^{-2} s^{-1})	R_d (μmol m^{-2} s^{-1})	R_d/A_n (%)	RQ
Ranunculus glacialis	12.5 ± 0.9	2.2 ± 0.3	17.0 ± 1.3	1.1 ± 0.1
Chamaerops humilis	3.12 ± 0.2	0.3 ± 0.1	9.6 ± 0.3	0.9 ± 0.1
Cycas revoluta	1.33 ± 0.1	0.2 ± 0.1	15.1 ± 0.2	1.0 ± 0.1
Helianthus annuus	17.9 ± 3.4	1.0 ± 0.2	5.6 ± 1.5	1.0 ± 0.1
Phaseolus vulgaris	12.4 ± 0.2	0.9 ± 0.1	7.3 ± 0.2	1.0 ± 0.1
Fagus sylvatica	7.2 ± 1.5	0.9 ± 0.5	13.0 ± 1.7	n.d.
Zea mays	24.4 ± 0.3	3.0 ± 0.4	12.3 ± 0.5	1.0 ± 0.1
Medicago sativa	25.8 ± 3.0	2.3 ± 0.3	8.9 ± 1.5	1.0 ± 0.1
Triticum turgidum	18.8 ± 2.4	1.7 ± 0.9	9.0 ± 1.6	1.0 ± 0.1
Arundo donax	23.4 ± 1.0	1.8 ± 0.8	7.7 ± 1.2	1.2 ± 0.1

After labeling, leaves were removed from the labeling chamber and replaced in the respiration chamber for measurements of dark-respired CO_2 (labeled). Gas samples containing the air with a CO_2 concentration of ca. 300 μL L^{-1} were collected for analysis in 50-mL glass balloons (Scott Glass, Mainz, Germany) or in 10-mL vacutainers (BD vacutainers, Plymouth, UK). The gas inside the balloon was then introduced into an elemental analyzer (EA) NA-1500 (Carlo-Erba, Milan) and pumped through a 15-mL loop as described by Tcherkez et al. (2003). On the other hand, the gas inside the vacutainers was directly injected into a gas chromatography–combustion-isotope ratio mass spectrometer (GC–C-IRMS) as previously described (Nogués et al., 2008). It is worth noting that the CO_2 respired in the dark after labeling comes from old carbon that was already in the leaf before labeling and new carbon that was recently fixed during labeling.

C isotope composition

Leaves samples were dried in an oven at 60 °C for 48 h and ground to a fine powder. Then 1 mg samples were weighed in tin capsules to determine the carbon isotope composition ($\delta^{13}C$) using an Elemental Analyzer Flash 112 (Carbo Erba, Milan) coupled to an isotope ratio mass spectrometry IRMS Delta C Conflo III Interface (Termo Finnigan, Germany). Results of carbon isotope ratio analyses are reported per mil (‰) on the relative δ-scale as $\delta^{13}C$ and are referred to the international standard V-PDB (Vienna Pee Dee Belemnite) according to the following equation:

$$\delta^{13}C = \left(\frac{R_{sample}}{R_{standard}}\right) - 1 \quad (1)$$

where R is the $^{13}C/^{12}C$ ratio. The standard PDB is a belemnite fossil coming from geological formation Pee Dee in South Carolina, USA. The PDB is slightly ^{13}C-enriched (R_{PDB} = 0.0112372) compared to almost all organic and inorganic materials but remains close to them so that $\delta^{13}C$ values of nearly all biological samples are very small and are thus expressed in per mil (‰, i.e. 10^{-3}) and are negative (Ghashghaie and Tcherkez, 2013).

Calculation of the percentage of new carbon in respired CO_2

The proportion of new carbon (derived from the labeling) in CO_2 respired in darkness after illumination was calculated following Nogués et al. (2004).

$$x = 100 \times \frac{\delta Y' - \delta Y}{\delta_0 - \Delta Y - \delta Y} \quad (2)$$

where $\delta Y'$ and δY are the isotope compositions from labeled and non-labeled, respectively; δ_0 is the isotope composition of the outlet air and ΔY is the isotopic discrimination.

Plant biomass analysis

At the end of the experiment, plants were harvested and oven dried at 60 °C for 48 h, and analyses of biomass of shoots and roots were carried out. Relative growth rate (RGR) was calculated as described by Wolfe et al. (1998). Total plant leaf area was estimated prior to drying using a flat-bed scanner.

Results and discussion

As is shown in Table 1, in the analyses of the labeled C percentage in respired CO_2 (C_{new}) two different groups were detected. Carbon which had been recently assimilated during photosynthesis accounted for ca. 50% of the carbon in CO_2 lost through dark respiration (R_d) after illumination in *Helianthus annuus*, *Phaseolus vulgaris*, *Fagus sylvatica*, *Zea mays*, *M. sativa*, *Triticum turgidum and Arundo donax*; and ca. 10% in *Ranunculus glacialis*, *Chamaerops humilis* and *Cycas revoluta* (Table 1; Nogués et al., 2004, 2006a,b; Aranjuelo et al., 2009). The analyses of both groups highlighted the fact that the first group was composed of fast-growing plants with C3 and C4 photosynthetic machinery while the second group was made up of slow-growing ones. These differing values indicate that in nature there might be two distinct "respiratory physiotypes". Therefore, for a given amount of assimilated carbon, parsimonious plants have a slow turnover in the respiratory pool, perhaps favoring the recycling of reserves (i.e. *R. glacialis* and *C. humilis*) and as a result, have a small proportion of 'new' carbon after illumination (Table 1, Nogués et al., 2006b). In contrast, other plants such as *H. annuus*, *P. vulgaris*, *F. sylvatica*, *Z. mays*, *M. sativa*, *T. turgidum and A. donax* invest more recently assimilated carbon in respiration. Presumably, this is due to the fact that plant respiratory strategies are dictated by the biological constraints of their environment. In general, Q_{10} (the proportional change in respiration per 10 °C rise in temperature) values are lower in tissues where respiratory flux is limited by substrate availability, and as a result, Atkin and Tjoelker (2003) have proposed that overall Q_{10} values will be lower in plants where photosynthesis and the subsequent synthesis of sugars is limited. On the other hand, increases in the availability of respiratory substrate that are produced as a consequence of sustained higher rates of photosynthesis might result in an increase in the Q_{10} of respiration. Interestingly, Atkin and Tjoelker (2003) identified two types of respiratory acclimation to temperature in plants, one of which is underpinned by adjustments in the Q_{10} (called Type I) and the other by changes in the enzymatic capacity of the respiratory system (called Type II); Type I acclimation allows for rapid changes to occur in the respiratory flux at high temperatures following changes in the thermal environment, whereas Type II acclimation is likely to be maximal upon the development of new leaves and roots following a change in temperature.

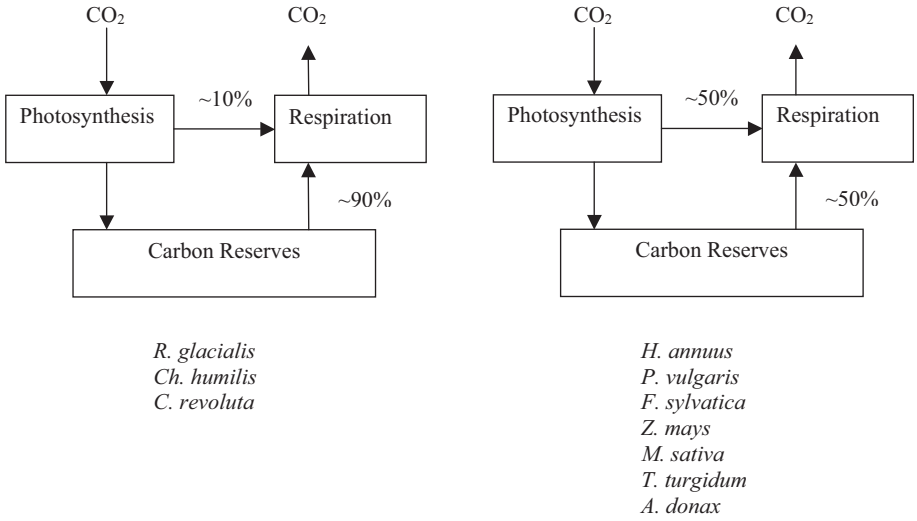

Fig. 1. Schematic representation of the contribution of new photoassimilates to respiration and to carbon reserves in mature leaves of several plant species. All the species assimilated the same amount of labeled CO_2 (i.e. ca. 350 mmol C m^{-2}).

Although in previous studies it has been observed that plants invest a large amount of recently fixed C in respiration (Day et al., 1985; Scheurwater et al., 2000; Lehmeier et al., 2010), it has also been noted that depending on the plant which is being analyzed, this amount can be lower (Nogués et al., 2006a,b; Aranjuelo et al., 2009; Baptist et al., 2009). As mentioned above, respiration is fueled by different compounds derived from recently fixed photoassimilates and/or remobilization of storage compounds such as starch, sucrose and fructans (Lehmeier et al., 2008, 2010). The fact that C_{new} did not reach 100% demonstrated that carbohydrate respiration was supported by a heterogeneous mixture of molecules with different residency times.

For plants such as *R. glacialis*, *C. humilis* and *C. revoluta* which grow slowly due to the harsh environments in which they live, i.e. alpine conditions and, hot and dry conditions respectively, most of the newly assimilated carbon is not respired and these species could be denominated as "low respiratory turn-over plants" (Nogués et al., 2006a). In fast-growing and cultivated plant species (such as *H. annuus*, *P. vulgaris*, *Z. mays*, *M. sativa*, *T. turgidum* and *A. donax*); and trees (like *F. sylvatica*) nearly 50% of the respiratory CO_2 comes from recently assimilated carbon; these could be denominated as "high respiratory turn-over plants" (Fig. 1). However, further studies into the biodiversity of the leaf respiratory response are required to verify the validity of this hypothesis and to relate it to the Atkin and Tjoelker model. It is worth noting that the mean relative growth rate (RGR) ranged from ca. 4 mg g^{-1} d^{-1} for slow-growing plants like *Chamaeropos humilis* (Aranjuelo et al., 2009) to ca. 200 mg g^{-1} d^{-1} for fast-growing plants like *H. annuus* (data not shown).

The increase in the respiratory rate as a function of assimilated C (Table 2) makes it clear that there was a tight link between photosynthesis and respiration. The correlation between these two parameters (net photosynthetic rate (A_n) versus R_d) was significant (Fig. 2). Even if *C. humilis* and *C. revoluta* were also sensitive, for *R. glacialis*, changes in A_n had the closest correlation with increases in R_d. This could be due to the different growth strategies of both plants. Given the limited time that *R. glacialis* has for growth under natural conditions, the high photosynthetic and respiration activity could be explained by the growth strategy of these plants. Their high metabolic activity could be due to the fact that they maximize resource acquisition when growth conditions are optimal. Gas exchange measurements were conducted in July, a period of maximum activity for *R. glacialis* plants. The lower R_d rates detected in *C. humilis* and *C. revoluta* might be explained by the fact that those plants showed a conservative strategy that maximizes resource conservation. On the other hand, the low correlation observed in A_n versus R_d of fast-growing plants demonstrated that the respiration of these plants was less sensitive to their photosynthetic performance.

Bowling et al. (2008) suggested that high respiration rates could be correlated to natural ^{13}C-abundance in evolved CO_2. Our study did not detect any significant correlation between respiration rate and $\delta^{13}C$ in fast- and slow-growing plants. However, Tcherkez (2010) suggested that fast-growing plants with high respiration rates produce more ^{13}C-depleted CO_2 than slow-growing plants. It is possible that in slow-growing plants, a higher proportion of carbon could be diverted from the tricarboxylic acid cycle into secondary metabolism (Bowling et al., 2008; Werner et al., 2009). Using $^{13}CO_2$ labeling, Priault et al. (2009) found that (slow-growing) aromatic plants with low tricarboxylic acid cycle activity (i.e. they

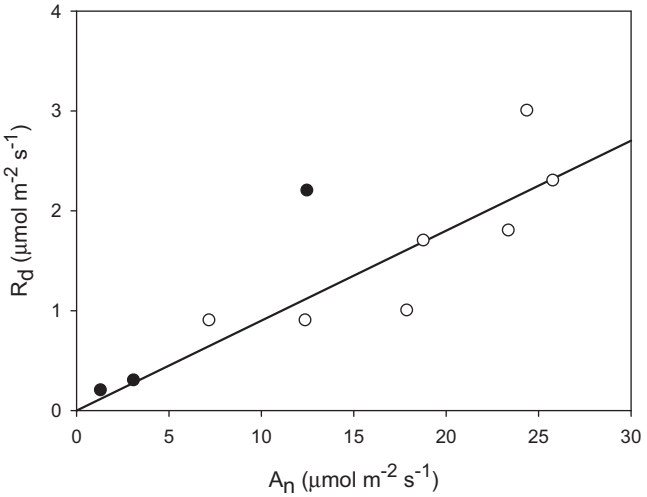

Fig. 2. The relationship between the dark respiration (R_d) and photosynthetic rates (A_n) of the studied plant species. Each point represents a single plant species (they are the means of three replicate plants for each species and the standard errors are lower than 10% in all cases). Closed circles are slow-growing and open circles are fast-growing plants, respectively. The line represents regression ($r^2 = 0.76$; $P < 0.05$). Data from Table 2.

invest more in secondary metabolites like isoprene) resulted in a pronounced increase in $\delta^{13}C$ of respired CO_2, but in fast-growing herbs with a high respiratory demand no changes were exhibited in respired $\delta^{13}CO_2$ since C was fully respired. Again, Tcherkez (2010) argued that this would have two consequences: (i) the metabolic flux throughout the pyruvate dehydrogenase would have to be very large and (ii) the metabolic commitment within the tricarboxylic acid cycle would be very small. Other explanations for these variations in isotopic patterns in respired CO_2 need to be investigated such as the involvement of other respiratory substrates (e.g. lipids which are ^{13}C-depleted; Tcherkez et al., 2003) or other metabolic pathways (e.g. the pentose-phosphate pathway as shown in roots; Bathellier et al., 2009; Ghashghaie and Badeck, 2014). The observation that all the plants studied had a respiratory quotient close to 1.0 highlighted the fact that they were mostly respiring carbohydrates.

Although our study showed that, with the exception of *R. glacialis*, the respiration rate of slow-growing plants was lower than that of fast-growing plants (Table 2), it should also be pointed out that respiration costs may not necessarily follow the same pattern. Respiration provides metabolic energy for growth, maintenance and ion transport (Mata et al., 1996). Scheurwater et al. (2000) found 3-fold higher specific costs for ion transport, expressed as mol O_2 per net mol NO_3^- taken up, in slow-growing plants. According to those authors, the higher costs were partly due to the higher efflux of N (per net NO_3^- uptake) and the larger maintenance costs for slow-growing plants than for fast-growing plants. According to Scheurwater et al. (2002), under optimal nutrient availability conditions, shoots are the main site of whole plant NO_3^- reduction in both fast- and slow-growing grasses. However, the mechanisms underlying such a shoot versus root pattern of N reduction may indicate large interspecific variability in non-optimal environmental conditions. As described by Baptist et al. (2009) in a study conducted with fast-growing (*Carex foetidea*) and slow-growing (*Kobresia myosuroides*) alpine plants, the fast-growing species displayed improved photosynthetic capacity and decreased N reduction capacity in leaves, which was compensated by the preferential C allocation to root growth and/or storage. These plants increased the percentage of reduced N translocated to above-ground organs so as to compensate for the lower N assimilation capacity. The high C flux allocated to the below-ground compartment in *Carex foetida* promoted significant levels of NO_3^- reduction in the roots. Interestingly, in this sense, our study shows that fast-growing plants, which had the largest percentages of newly fixed carbon of leaf-respired CO_2, were also those with the largest shoot/root ratios, whereas slow-growing plants showed the lowest shoot/root values (Fig. 3). These results suggest that fast-growing plants invested most of recently fixed C in shoot respiration, while in slow-growing plants, more C_{new} was partitioned toward roots to sustain N assimilation and transport.

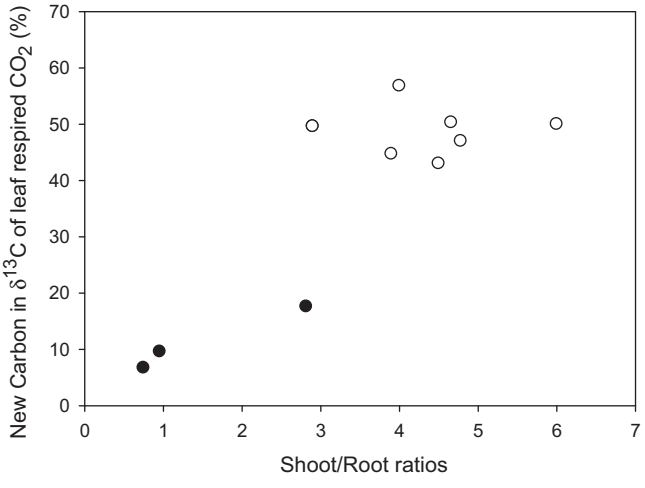

Fig. 3. The relationship between the percentage of new carbon in the $\delta^{13}C$ of leaf-respired CO_2 versus plant shoot/root ratios of the studied plant species. Each point represents a single plant species (they are the means of three replicate plants for each species and the standard errors are lower than 10% in all cases). Closed circles are slow-growing and open circles are fast-growing plants, respectively.

In conclusion, data presented in this study suggest that the two distinct respiratory physiotypes of plants exist and correspond to fast-growing (including trees) and slow-growing species. Slow-growing plants are likely invest more recently assimilated C in storage compounds than fast-growing plants. More specifically, the study revealed that in slow-growing plants, 90% of respired carbon was derived from "old" carbon which was remobilized for its respiration. The fact that those plants had low shoot/root ratio suggest that a large part of this C was partitioned toward roots. On the other hand, respiration in fast-growing plants (with large shoot/root) was composed of ca. 50% of recently assimilated C and of ca. 50% "old" C. However, more experiments are now needed in order to identify the nature of this both physiotypes and the role of roots in C partitioning.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jplph.2014.03.006.

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