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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