Rapid report

Day-length effects on carbon stores for respiration of perennial ryegrass

Christoph Andreas Lehmeier, Fernando Alfredo Lattanzi, Ulrike Gamnitzer, Rudi Schäufele and Hans Schnyder
Lehrstuhl für Grünlandlehre, Department für Pflanzenwissenschaften, Technische Universität München, Alte Akademie 12, D-85350 Freising-Weihenstephan, Germany

Summary

- The mechanism controlling the use of stored carbon in respiration is poorly understood. Here, we explore if the reliance on stores as respiratory substrate depends on day length.
- *Lolium perenne* (perennial ryegrass) was grown in continuous light (275 μmol photons m⁻² s⁻¹) or in a 16 : 8 h day : night regime (425 μmol m⁻² s⁻¹ during the photoperiod), with the same daily photosynthetic photon flux density (PPFD). Plants in stands were labelled with \(^{13}\text{C}\)O₂ : \(^{12}\text{CO}_2\) for various time intervals. The rates and isotopic signatures of shoot- and root-respired CO₂ were measured after labelling, and water-soluble carbohydrates were determined in biomass. The tracer kinetics in respired CO₂ was analysed with compartmental models to infer the sizes, half-lives and contributions of respiratory substrate pools.
- Stores were the main source for respiration in both treatments (c. 60% of all respired carbon). But, continuous light slowed the turnover (+270%) and increased the size (+160%) of the store relative to the 16 : 8 h day : night regime. This effect corresponded with a greatly elevated fructan content. Yet, day length had no effect on sizes and half-lives of other pools serving respiration.
- We suggest that the residence time of respiratory carbon was strongly influenced by partitioning of carbon to fructan stores.

Introduction

Dark respiration consumes between 30 and 80% of the gross primary production of plants (Amthor, 2000; Gifford, 2003; Van Iersel, 2003). Partly, this is met by carbon derived directly from recent photosynthesis, which therefore resides only briefly in the plant (Pärnik et al., 2007; Lehmeier et al., 2008; Priault et al., 2009). However, a large part of respiratory carbon first cycles through storage pools (Prosser & Farrar, 1981; Carbone & Trumbore, 2007; Lehmeier et al., 2008; Mortazavi et al., 2009). Temporary stores effectively buffer the continuous carbon demand of respiration from the discontinuous supply from photosynthesis (see Smith & Stitt, 2007). But to date, what controls carbon partitioning between immediate consumption and storage for later use in respiration is not well understood. This lack of knowledge translates into uncertainties about the residence time of respiratory carbon in plants and, hence, ecosystems.

Carbohydrates are considered as the main storage form of respiratory substrate (ap Rees, 1980; Tcherkez et al., 2003). Most plants store carbohydrates as transitory starch in chloroplasts or as sucrose and fructans (fructose-based oligosaccharides) in vacuoles. Experimental alterations...
of day length demonstrated a striking ability of starch-storing species to adjust the fluxes of daytime deposition and night-time mobilization so that the starch pool was nearly exhausted at the end of the night (Chatterton & Silvius, 1981; Fondy et al., 1989; Lu et al., 2005; Gibon et al., 2009). This suggested an efficient use of transitory stores as supplies of terminal sinks and minimal ‘excess’ storage.

Similar knowledge does not exist for sucrose- and fructan-storing species (including the C3 cereals and forage grasses). Previous investigations using carbon isotopes to partition storage- and current assimilation-derived substrate supply of respiration showed that the contribution of stores depends on environmental conditions like temperature (Prosser & Farrar, 1981), nitrogen supply (Lehmeier et al., 2010) and the growing season (Carbone & Trumbore, 2007; Gamnitzer, 2010). Recently, we have shown that about half of all carbon respired by perennial ryegrass (Lolium perenne, a sucrose/fructan-storing grass) first cycled through stores when plants were grown in continuous light (Lehmeier et al., 2008). One should expect that plants growing in day : night cycles exhibit a greater reliance on stores, since these are needed to fuel respiration in the dark period when current assimilate supply is nil.

In the present study, we test the hypothesis that the quantitative significance of temporary stores for respiration is higher in day : night cycles than in continuous light. To this end, we grew perennial ryegrass in a 16 : 8 h day : night regime (Gamnitzer, 2010). Recently, we have shown that about half of all carbon respired by perennial ryegrass (Lolium perenne, a sucrose/fructan-storing grass) first cycled through stores when plants were grown in continuous light (Lehmeier et al., 2008). One should expect that plants growing in day : night cycles exhibit a greater reliance on stores, since these are needed to fuel respiration in the dark period when current assimilate supply is nil.

In the present study, we test the hypothesis that the quantitative significance of temporary stores for respiration is higher in day : night cycles than in continuous light. To this end, we grew perennial ryegrass in a 16 : 8 h day : night cycle with otherwise identical environmental conditions to those in continuous light. That is, plants in these two treatments received the same total daily photosynthetic photon flux density (PPFD), not to confuse day-length effects with total irradiances. Plants were labelled with 13CO2 : 12CO2 for 1 h up to several weeks, and the 13C : 12C ratios of respired CO2 of shoots and roots were measured in darkness immediately after labelling. Then, the time course of tracer in respired CO2 was analysed with compartmental models.

**Materials and Methods**

Continuous light data have been presented before (Lehmeier et al., 2008). In this work these data served as the reference against which the effect of day length on respiratory substrate stores was tested.

In both treatments, Lolium perenne L. cv Acento was grown from seeds in growth chambers which formed part of a custom-made 13CO2 : 12CO2 gas exchange and labelling facility (Schnyder et al., 2003). In the continuous-light treatment, plants were grown with constant illumination at a PPFD of 275 μmol m−2 s−1. Day : night plants were grown in alternating 16 h light (PPFD, 425 μmol m−2 s−1) and 8 h dark periods. Further chamber settings were the same in both treatments, including air temperature (20°C), CO2 concentration (360 μl l−1) and nutrient supply (see Supporting Information, Methods S1).

In each treatment, half of the plants were grown in a chamber with 13C-enriched CO2, and the other half in 13C-depleted CO2. When closed stands were established, the δ13C of CO2 was changed for intervals ranging from 1 h to 25 d (13C-enriched → 13C-depleted atmosphere or vice versa). At the end of each labelling interval, the rates of shoot and root respiration as well as the δ13C of shoot- and root-respired CO2 of individual plants were measured in the dark (Lötscher et al., 2004; Klumpp et al., 2005). After 5–6 h (continuous-light treatment) or 6–7 h (day : night treatment) of respiration measurements, plants were harvested. Carbon and nitrogen elemental contents and water-soluble carbohydrate fractions were determined in shoot and root biomass. Respiration of nonlabelled control plants was measured likewise. This determined the end members for a two-source mixing model which was used to calculate the fractions of unlabelled carbon in CO2 respired by the labelled plants (Fig. 1).

The tracer time courses contain information about the respiratory carbon supply system: carbon fluxes in the system, pool sizes and half-lives, and the contribution of pools to respiration. This information was extracted with compartmental analysis. In plants of the day : night treatment metabolite pool sizes and fluxes must have fluctuated during the day as a result of the discontinuous photosynthesis (Farrar & Farrar, 1986). To account for this nonsteadiness, we restricted the compartmental analysis to the day-by-day timescale (Lattanzi et al., 2005), for

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**Fig. 1** The fraction of unlabelled carbon (funlabelled C) in CO2 evolved in dark respiration of shoots (a) and roots (b) of Lolium perenne (perennial ryegrass) plants grown in continuous light (closed symbols) or in a 16 : 8 h day : night regime (open symbols) during labelling. Each value is the mean of four to six replicate plants (± 1 SE). Data points at 2 h of labelling duration in root-respired CO2 overlap. Insets expand the first 34 h. The continuous-light data are taken from Lehmeier et al. (2008).
which relevant features were approximately steady in both treatments. For this, we calculated the mean fraction of unlabelled carbon in CO2 respired during a 1-d-long period (or multiples thereof) as respiration-weighted averages of the fraction of unlabelled carbon of a given day (or multiples thereof, depending on the available time resolution of the data shown in Fig. 1). The mean fraction of unlabelled carbon in respired CO2 in both treatments (Fig. 2) then served to infer differences and similarities in the respiratory carbon supply systems using compartmental analysis.

For the day : night treatment, the shoot respiration rate in light ($R_{\text{day}}$) may have been lower than that in darkness ($R_{\text{night}}$; Peisker & Apel, 2001; Pärnik et al., 2007). This could modify the relative contributions of pools to respiration. To assess the possible effects, we calculated the mean fraction of unlabelled carbon in respired CO2 in assuming that $R_{\text{day}} : R_{\text{night}}$ was 1, 0.7 or 0.35. See Methods S1 for a comprehensive description of materials and methods.

Results

General growth and respiration parameters

All plants remained vegetative during the entire experimental period. Average specific growth rates of shoots and roots, obtained by a regression line to log$_e$-transformed carbon mass observed over time, were similar in both light treatments, as were the plants’ shoot : root ratios of c. 4 : 1 (Table 1). However, specific shoot respiration rate was 23% higher in plants grown in day : night cycles than in continuous light. Overall, the photosynthetic carbon-use efficiency ($= \text{growth rate}/(\text{growth rate} + \text{respiration rate})$) was similar: 0.66 for plants in day : night cycles and 0.68 for plants in continuous light (Table 1).

Table 1 Experimental and growth parameters of perennial ryegrass (Lolium perenne) grown in continuous light or in a 16 : 8 h day : night regime

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Continuous light</th>
<th>Day : night</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiance (photosynthetic photon flux density)</td>
<td>275</td>
<td>425</td>
</tr>
<tr>
<td>Specific dark respiration rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant, mg plant-respired C g$^{-1}$ plant h$^{-1}$</td>
<td>1.50 ± 0.02</td>
<td>1.67 ± 0.03*</td>
</tr>
<tr>
<td>Shoot, mg shoot-respired C g$^{-1}$ plant h$^{-1}$</td>
<td>0.97 ± 0.02</td>
<td>1.19 ± 0.03*</td>
</tr>
<tr>
<td>Root, mg root-respired C g$^{-1}$ plant h$^{-1}$</td>
<td>0.53 ± 0.01</td>
<td>0.48 ± 0.01*</td>
</tr>
<tr>
<td>Specific growth rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant, mg C g$^{-1}$ plant C h$^{-1}$</td>
<td>3.23 ± 0.21</td>
<td>3.19 ± 0.54 ns</td>
</tr>
<tr>
<td>Shoot, mg C g$^{-1}$ shoot C h$^{-1}$</td>
<td>3.53 ± 0.23</td>
<td>3.42 ± 0.56 ns</td>
</tr>
<tr>
<td>Root, mg C g$^{-1}$ root C h$^{-1}$</td>
<td>3.00 ± 0.28</td>
<td>2.40 ± 0.54 ns</td>
</tr>
<tr>
<td>Shoot : root ratio</td>
<td>3.84 ± 0.14</td>
<td>4.15 ± 0.08 ns</td>
</tr>
<tr>
<td>Nitrogen content (mg g$^{-1}$ DW)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot</td>
<td>18.3 ± 0.5</td>
<td>29.4 ± 0.8*</td>
</tr>
<tr>
<td>Root</td>
<td>10.7 ± 0.3</td>
<td>12.6 ± 0.5*</td>
</tr>
<tr>
<td>Photosynthetic carbon-use efficiency</td>
<td>0.68 ± 0.05</td>
<td>0.66 ± 0.13 ns</td>
</tr>
</tbody>
</table>

Values are means of 60 (continuous light) and 121 (day : night cycles) replicate plants ± 1 SE. $n$ for respiration rates and nitrogen content in the day : night treatment was 55. Some of the continuous-light data have been published previously (Lehmeier et al., 2008, 2010).

*, $P \leq 0.05$; ns, not significant, $P > 0.05$.

Contents of water-soluble carbohydrates and nitrogen in shoot and root biomass

Total water-soluble carbohydrates accounted for 183 mg C g$^{-1}$ total plant C in day : night cycles. This was almost 40% less than in plants grown in continuous light.
The tracer kinetics of shoot respired CO2 in both treatments, there was a small residual respiratory (asymptotic) activity which was not exchanged by tracer within the timeframe of labelling. In essence, a very similar pattern was observed in both treatments for root-respired CO2 (Fig. 1b).

Compartmental modelling of respiratory carbon pools

The transformation of the tracer kinetics in Fig. 1 to the mean fraction of unlabelled carbon in respired CO2 per interval of 24 h (or multiples thereof; Fig. 2), conserved the two-phase pattern seen in Fig. 1 and the differences between treatments. Assumptions about the degree of light inhibition of shoot respiration in day : night cycles had little effect on the results of compartmental modelling (Table S1) and did not affect any conclusions of the present work.

The two-pool model depicted in Fig. 2(a) was a simple model with biological significance able to fit the tracer time courses of shoots and roots of both light treatments (Fig. 2). One-pool models were statistically inferior, and more complex variations of the two-pool model were overparameterized. The two-pool model consisted of a pool Q1 which received tracer from current photosynthesis, conveyed it to the respiratory supply system (i.e. the sum of Q1 and Q2 of shoot dark respiration rate during light was 0.7 times the shoot respiration rate measured in darkness. Values ± 95% CI were calculated from model-optimized rate constants (and the measured respiration rates for the size estimation).

Substrate pool sizes, half-lives and contributions to respiration

The light regime had a large effect on the size of the total respiratory supply system (i.e. the sum of Q1 and Q2 of shoot and root; Table 3). In day : night plants, it accounted for 74 mg C g⁻¹ C, only about half that of plants in continuous light. This was because of a smaller size of the temporary store Q2 in day : night plants.

The kinetic properties of Q1 were similar in both treatments, exhibiting a half-life between 3 and 7 h (Table 3). By contrast, the half-life of Q2 was c. 0.5 d in day : night.
and c. 2 d in continuous light. Within a treatment, half-lives of pools supplying shoot and root respiration were almost the same.

More than 50% of all respired carbon first cycled through the store Q2. This was true for shoots and roots in both treatments (Table 3). Current assimilation supplied c. 40% of all respired carbon, except for root respiration of plants in day : night cycles (26%; Table 3).

Discussion

The fractional contribution of stores as a substrate for respiration was independent of day length

The hypothesis of this investigation was that stores become a greater source of carbon for respiration when day length gets shorter. The work falsified this hypothesis: the relative contributions of stores to respiration were virtually identical in continuous-light and day : night cycles.

We believe that any responses observed here were true day-length responses, since environmental conditions were the same in the two treatments, including the daily dose of PPFD. Plant respiration rates in day : night cycles were slightly higher, but relative growth rates were the same in both treatments (Table 1). This suggests that daily rates of photosynthesis were not very different between the treatments, as irradiance was well below light saturation levels for photosynthesis (Gay & Thomas, 1995; Spiering et al., 2006). Nevertheless, day length affected respiratory pool sizes and fluxes, and, possibly, also the metabolic identity of respiratory stores.

Perhaps the most striking result of the present work was that such a large fraction (50–60%) of respired carbon first cycled through a store, and that this occurred in both treatments (Table 3). The size of the store was much larger than was required to sustain respiratory activity even in the day : night cycle. Thus, the size of Q2 in day : night cycles of 46 mg g⁻¹ C would have allowed sustaining current dark respiration rates of those plants theoretically for 28 h (Tables 1, 3), much longer than the ‘normal’ dark period of 8 h. The surplus was even higher in continuous light (Tables 1, 3), where it was sufficient to supply respiration for 80 h. Even larger contributions were found for ryegrass grown in nitrogen-limited conditions (Lehmeier et al., 2010).

Clearly, the sucrose/fructan-storing grass stored more carbon in its respiratory storage pool (Q2) than was ‘needed’ to buffer day : night fluctuations in respiratory substrate. This differs from reports of plants that store transient starch: these plants adjusted storage fluxes to environmental changes such as the length of the dark period to deposit only as much starch during the daytime as they ‘anticipated’ using during the following night (Chatterton & Silvius, 1981; Fondy et al., 1989; Lu et al., 2005; Smith & Stitt, 2007; Gibon et al., 2009). Our findings may not be directly comparable, as these studies did not characterize the respiratory substrate supply system. But such an apparently fundamental difference makes it worth pursuing labelling studies to characterize sink supply systems in plants with other forms of carbon storage.

Did day length affect the metabolic identity of temporary stores supplying respiration?

While the fractional contribution of stores to respiration was similar in both light regimes, their half-life was considerably shorter in day : night (0.5 d) than in continuous-light (2 d) treatments. Both sucrose and fructan may form part of vacuolar stores in grasses (Wagner et al., 1983; Borland & Farrar, 1988). Indeed, these were the only fractions whose size was sufficient to accommodate the size of Q2 in both treatments (Tables 2, 3). The much lower fructan : sucrose ratio in plants grown in day : night cycles than in continuous light (Table 2) suggests that sucrose was a quantitatively more important storage carbohydrate for respiration in day : night cycles.

This interpretation agrees with the half-life of Q2 in day : night plants being close to values reported for vacuolar sucrose (Bell & Incoll, 1982; Farrar, 1989), and suggests that the longer half-life of Q2 in continuous-light plants reflects a slower turnover of vacuolar fructans as compared with vacuolar sucrose. If this is true, the residence time of respiratory carbon was mainly influenced by the ratio of carbon partitioning between sucrose and fructan in storage deposition fluxes.

Why did day length elicit differences in shoot fructan content and in the size of stores (despite, in principle, equal resource supply in both treatments)? We suggest these are linked responses. Fructan and sucrose concentrations are thought to be associated, fructan synthesis kicking in before sucrose concentration reaches values which could cause osmotic imbalances or a feedback inhibition of photosynthesis (Pollock & Cairns, 1991; Cairns et al., 2000). In day : night cycles, sucrose concentration probably remained below critical values for part of the day (Sicher et al., 1984; Borland & Farrar, 1985). But in continuous light, a continuous provision of assimilates would have led to the larger fructan : sucrose ratio. Since fructans turn over more slowly than sucrose, the similar proportion of carbon diverted to storage (Table 3) would have led to a bigger storage pool size. This strategy of temporary storage use appears to have been effective, as growth rates and photosynthetic carbon-use efficiency were similar in both treatments (Table 1).

Day length affected shoot respiration rates and nitrogen content but not plants’ growth performance

Plants in day : night cycles exhibited a 23% higher shoot respiration rate than those in continuous light. One reason for this was likely posed by the 61% higher nitrogen...
content in shoot biomass (Table 1), since requirements of respiration for de novo synthesis of amino-compounds and turnover of proteins are closely associated with nitrogen content (Amthor, 2000). The probable cause of the difference in shoot nitrogen content between treatments is less clear. Part of it may have been a dilution effect: the carbohydrate content was lower in day : night plants (Table 2), so that shoot nitrogen content per unit carbohydrate-free dry matter was only 33% higher.

Possibly, continuous illumination lowered the capacity of plants to assimilate nitrate, as compared with plants in day : night cycles. The tricarboxylic acid (TCA) cycle, an important carbon source for nitrate assimilation, is known to have reduced activity in illuminated leaves (Atkin et al., 2000; Nunes-Nesi et al., 2007). With ample nitrogen supply, as used here, nitrate assimilation occurs mainly in the shoots of temperate grasses (Andrews et al., 1992). Recent findings suggest that the alternation of light and dark periods may be important for a balanced functioning of nitrogen metabolism (Gauthier et al., 2010). However, despite the lower shoot nitrogen content, it is hard to argue that plants in continuous illumination experienced a nitrogen limitation compared with plants in day : night cycles, since growth rates and photosynthetic carbon-use efficiency were similar (Table 1).

In conclusion, this work highlights a remarkable flexibility of the respiratory substrate supply system of perennial ryegrass in response to day length. The adjustment occurred not in the proportion of current assimilates that were stored, but in the rate with which the carbon store was turned over. This, in turn, was probably linked to a change in the biochemical nature of the stored substrate, specifically the fructan : sucrose ratio. Such an adjustment would serve plants to sustain high growth rates in a changing environment. While this may be of general validity for plants (cf. Smith & Stitt, 2007), there seems to be a substantial difference regarding how this is achieved between sucrose/fructan- and starch-storing species.

Acknowledgements

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References


Lehmeier CA, Lattanzi FA, Schäufele R, Wild M, Schnyder H. 2008. Root and shoot respiration of perennial ryegrass are supplied by the same substrate pools: assessment by dynamic 13C labeling and


Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Optimized parameters of the two-pool compartmental model shown in Fig. 2(a), applied to the mean fraction of unlabelled carbon in CO2 respired by shoots of plants grown in day : night cycles with either 0 or 65% reduced shoot respiration rate in light as compared with the shoot respiration rate in darkness

Methods S1 A comprehensive description of all materials and methods used for the present study.

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