Characterization of substrate pools involved in respiratory carbon release of *Lolium perenne*

C. A. Lehmeier, F. A. Lattanzi, M. Wild, R. Schäufele und H. Schnyder

Lehrstuhl für Grünlandlehre, Technische Universität München-Weihenstephan

Introduction

Grassland ecosystems play a significant role in the global terrestrial C (carbon) budget. Large amounts of CO2 are fixed during photosynthesis and are partitioned to organic substrate pools with possible differences in both chemical and functional identity. Part of the C is eventually released back to the atmosphere by way of autotrophic respiration. To improve our understanding of whole plant C metabolism and also of ecosystem CO2 fluxes, it is important to know which substrate pools are involved in respiratory C release.

Here, we applied steady-state 13CO2/12CO2 labelling in conjunction with gas exchange measurements and followed the labelling kinetics of respired CO2 of individual ryegrass plants, grown in controlled environments. We analyzed the tracer time-course of respired CO2 with compartmental analysis to identify and characterize substrate pools that are involved in respiratory C release at the whole plant level.

Materials & Methods

Seeds of perennial ryegrass (Lolium perenne, cv. Acento) were sown individually in plastic pots and arranged in two growth chambers, where plants experienced continuous irradiance at 275 µmol m-2 s-1 PPFR, temperature of 20ºC and relative humidity near 85%. Stands were irrigated every 3 hours with an excess of nutrients. The two growth chambers formed part of the 13CO2/12CO2 gas exchange and labelling system described by SCHNYDER et al. (2003). Both CO2 concentration of 360 ppm and C isotope composition (δ13C) of CO2 in the chambers was controlled online by an infrared gas analyser (IRGA) and a continuous-flow 13CO2/12CO2 isotope-ratio mass spectrometer (CF-IRMS). One chamber received CO2 with δ13C -28.8‰, while the other received CO2 with δ13C – 1.7‰. Once homogeneous stands were established, labelling was performed by swapping randomly selected, individual plants between the chambers. At the end of target labelling times of 1, 2, 4, 8, 16 hours and 1, 2, 4, 8, 12, 17 and 25 days, plants were removed from the stands and used in respiration measurements. For this, plants were fixed in cuvettes and arranged in a growth cabinet, held at 20ºC. The system allowed a separation of shoot and root respiratory CO2 fluxes. Both plant parts were connected to an open gas exchange system which consisted of an IRGA and a CF-IRMS (see LÖTSCHER et al. 2004 for further details). Respiration rates as well as the isotopic signatures of respired CO2 of shoots and roots of labelled and non-labelled plants were measured for about 5-6 hours in the dark. After measurements, plants were harvested, dried and aliquots of ground sample material were combusted and analysed for total C and 13C content with the CF-IRMS.

The proportions of C in respired CO_2 that were assimilated before ($f_{unlabelled}$) and during labelling ($f_{labelled}$) were estimated as given by SCHNYDER & DE VISSER (1999):

$f_{unlabelled} = (\delta_S - \delta_{labelled}) / (\delta_{unlabelled} - \delta_{labelled})$, and $f_{labelled} = 1 - f_{unlabelled}$,

where δ_S, $\delta_{unlabelled}$ and $\delta_{labelled}$ are the isotopic signatures of the respired CO_2 of a sample plant, and of plants growing continuously in the chamber of origin (unlabelled) and in the labelling chamber, respectively.

The labelling kinetics of respired CO2 was evaluated with compartmental analysis. The underlying idea of this approach is that photosynthetically assimilated tracer may move through a number of metabolic pools before it is released in respiration. In a compartmental system, there are characteristic transfer coefficients that determine the rates of exchange between pools and their respective sizes. Separate pools appear in the labelling kinetics, if they show marked differences in their turn-over rates, which can be detected by application of exponential decay-functions. Compartmental analysis has been used to study tracer export from source leaves (e.g. MOORBY & JARMAN 1975). Recently, it was also applied to characterize substrate pools supplying leaf growth of ryegrass (LATTANZI et al. 2005), another main sink for photoassimilate in grasses.

Results

Figure 1 shows the fraction of non-labelled C in respired CO_2 of shoots and roots of ryegrass plants with increasing labelling time. The kinetics reveals several distinct features.

In the shoot, new C already accounted for 17% of total shoot respired CO2 after only 1h of labelling. This was followed by a lag period of about 3 hours in which the degree of labelling did not change. Then, the proportion of unlabelled C decreased fast, but the rate of decrease slowed down strongly after about 1d and, again, after about 12d of labelling.

Fig. 1 (left): Fraction of un-labelled carbon in CO_2, respired by shoots (closed symbols) and roots (open symbols) of ryegrass plants, labelled for different time intervals. Error bars denote 1 SE (n = 4). The cross denotes t = 0, where no tracer was incorporated.

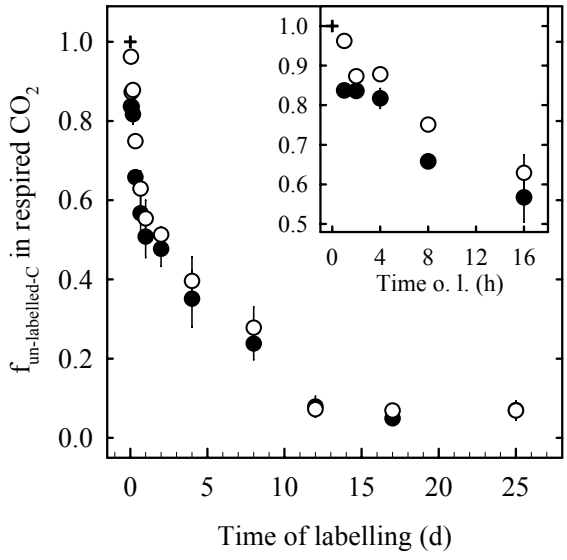
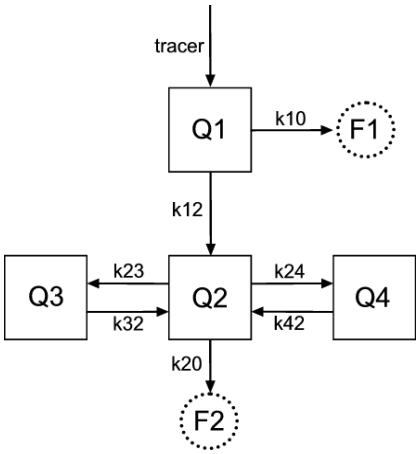


Fig. 2 (right): 4-pool compartmental model of tracer assimilation in photosynthesis. The model is based on the assumptions of a steady-state system and first-order kinetics, that is, pool sizes are constant in time and fluxes are the product of pool size (Qx, Qy) times a rate constant (kxy; numbers refer to donor and receptor pools, respectively). Respiratory C release in CO_2 via F1 and F2.



The labelling kinetics of roots was very similar to that of shoots, except that initial labelling was somewhat slower (~1h delay) and weaker. From 2h until 8d, the proportion of tracer in root respired CO_2 was generally about 5% less than in shoot respired CO_2. After that, there was no difference between shoots and roots until 25d when the investigation stopped.

The labelling kinetics of shoot respired CO_2 served as the basis for a compartmental analysis. First, we determined how many pools with different turnover times were involved in creating the tracer signal time course. 1- or 2-exponential decay functions did not provide a good fit. Also, the use of more than four exponential terms did not improve the fit, which was similar for 3- and 4-exponential functions. This indicated that the system included three or four major pools. Thereafter, we implemented a wide range of 3- and 4- compartment systems in the software ModelMaker (version 4.0; Cherwell Scientific, Oxford, UK). None of the 3-pool models was able to provide a satisfactory prediction of the data.

Among the huge number of possible 4-pool models, we finally selected the arrangement shown in Figure 2. This selection was based on two main arguments: (i) it was the simplest model which gave a good prediction of the data, and (ii) it provides a physiologically meaningful interconnection of metabolic C flows in the plant.

This model predicts that tracer is first incorporated in pool Q1 where it can be respired directly (via F1) or transferred to Q2, which is regarded as a "transport pool". Once in Q2, tracer may be respired directly (via F2) or it may exchange with Q3 and/or Q4 (temporary storage pools) before it is finally respired via F2 (Figure 2).

Model parameters were estimated numerically by ModelMaker, and optimised rate constants were used to derive the parameters shown in Table 1, which characterize the different pools.

Tab. 1: Model optimisation results; the relative importance to CO_2 release is calculated as the probability of tracer leaving a pool in a certain flux (e.g. k10 / (k10 + k12))

	Q1	Q2	Q3	Q4
half-time of pool ($t_{0.5}$)	11 min	2.5h	21h	4d
pool-size in mg C g^{-1} plant C	<1	14	50	89
relative importance in shoot	17%	26%	39%	18%
contribution to total CO_2, released via	F1	F2		
shoot	17%	83%		
root	12%	88%		

Import of tracer = Export = F1 + F2 = specific respiration rate = 1.5mg C * g^{-1} plant C * h^{-}

Discussion

This is the first comprehensive and quantitative analysis of the properties of metabolic pools that provide substrate for shoot and root respiration. The study was based on following ^{13}C tracer incorporation in respiratory CO_2 in time steps ranging from 1 h to almost 1 month. This led to the identification of four major substrate pools which together supplied 95% of all C released in respiration (Figures 1 + 2).

The results demonstrate that the size of a pool is not a proper measure for its importance as a source of substrate for respiration. This is most evident in the case of Q1, which was a very small pool. Its short half-time (~11 min) meant that the flux of C through it was very

high, resulting in a contribution of 17% to total dark respiration in the shoot (F1 in Figure 2, Table 1). We believe that Q1 is located in photosynthesizing cells of mature tissue, and includes primary photosynthetic products such as triosephosphates or organic acids. Interestingly, Q1 also supplied root respiration (Figure 1, Table 2), demonstrating that C from Q1 was also translocated below-ground.

Q2 is best seen as a transport pool for C, in which all fixed C that is not released *via* F1, must enter. The half-time of 2.5h for Q2 is near the upper limit of reported half-times for transport sucrose, which is the most abundant product of photosynthesis (cf. FARRAR 1989). But, other fast labelled substrates like some amino-acids could also contribute to Q2. In studies of C export from source leaves, a compartmentation of sucrose was reported repeatedly. Here, the estimated sizes of 14 and 50 mg C g^{-1} plant C for Q2 and Q3 (thus 22:78%) is in line with the reported sucrose compartmentation (cf. FARRAR 1989). The half-time of 21h for Q3 is close to that expected for short-term (diurnal) vacuolar sucrose storage.

Q4 was the biggest pool and had the slowest turn-over. This may be regarded as a "long-term" storage pool. The half-time of 4d lies in the range of half-times found for protein turn-over (e.g. DUNKEY & DAVIES 1982). Also, stored fructans could contribute to Q4, but relatively little is known about its involvement in respiration. In spite of the large size of Q4, its contribution to respiratory C release was relatively low (ca. 18%). Interestingly, LATTANZI et al. (2005) found that long-term stores supply only about 5% of C to leaf growth in grasses. This suggests different importance of C stores in supplying different C sinks, such as growth and respiration.

In conclusion, we demonstrate, that about 43% of respiratory C release of *Lolium perenne* originated from (very) recent assimilation, whereas 57% of the substrate was stored temporarily before it was released in respiration (Table 1). The whole set of respiratory substrate pool was almost completely exchanged within 2 weeks. Notably, the proposed model was able to predict the labelling kinetics of root respired CO_2 without implementation of additional pools for the roots. This would suggest that the labelling kinetics of substrate transported below-ground is very similar to that of root respired CO_2 and that the storage pools supplying respiration are mainly located in the above-ground parts of the grass plant.

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