Characterisation of substrate pools supplying leaf growth of Lolium perenne

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Introduction

The growth of a grass leaf depends on carbon (C) substrate supply to the leaf growth zone (LGZ) (**Fehler! Verweisquelle konnte nicht gefunden werden.**), which may originate from current photosynthesis or from stores. The substrate is partly incorporated in new tissue and partly respired. During undisturbed growth, export and respiration are balanced by substrate import (LATTANZI et al., 2004).

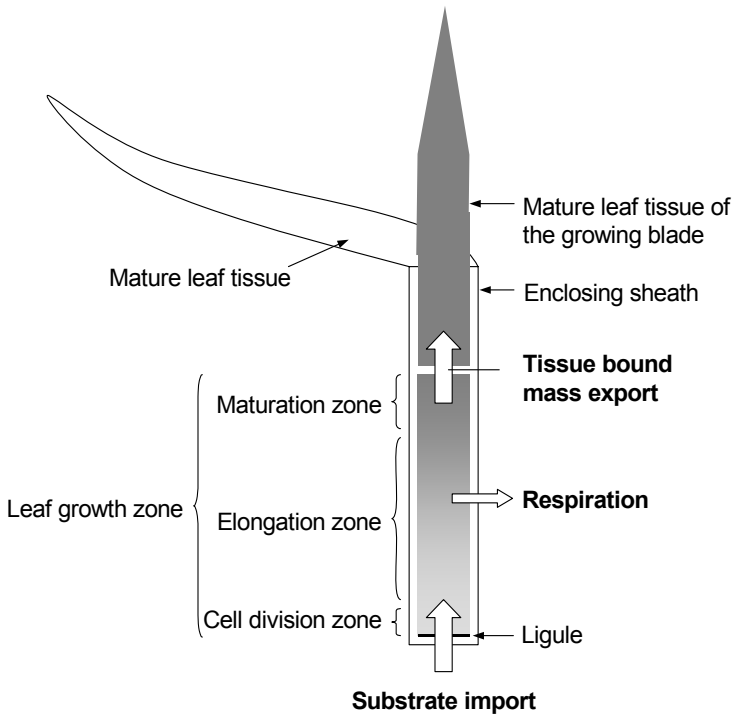

Fig. 1: Schematic view of a growing grass leaf. Cells are produced in the cell division zone and shifted away from it as a result of the production of new cells. At the same time cells elongate and mature. The cell division, elongation and maturation zone together are called the ´leaf growth zone´ (LGZ). As cells divide, elongate and mature, they incorporate C substrate that is imported into the LGZ (adapted from LATTANZI et al., 2004)

Until now only little is known about the relative importance of current photosynthesis and stores in supplying C to leaf growth. LATTANZI ET AL. (2005) found that long-term stores supplied little C to leaf growth of a C_3 and a C_4 grass, and that this was true in different situations of competition. Nothing is known about the role of short-term stores and on how nitrogen (N) nutrition might affect the relationships.

Thus, the aim of this study was to determine the importance of fast C pools (transport and short-term storage pools) in relation to long-term stores in supplying leaf growth in *Lolium perenne* growing with low and high N supply. The number and characteristics of pools supplying leaf growth was assessed by steady-state $^{13}CO_2/^{12}CO_2$ labelling and compartmental modelling of the time-course of tracer incorporation in C imported into the LGZ.

Materials and Methods

Plants of ryegrass (*Lolium perenne,* cv. Acento) were grown in four growth chambers in continuous light with an irradiance of 275µmol m^{-2} s^{-1} PPFD, a temperature of 20°C and a relative humidity of 85%. Stands were watered eight times per day with modified Hoagland nutrient solution. Two N treatments were established. Low N (N-) plants received 1.0mM NO_3^- and high N (N+) plants 7.5mM NO_3^-. The growth chambers formed part of an open $^{13}CO_2/^{12}CO_2$ gas exchange and labelling system described by SCHNYDER ET AL. (2003). In each N treatment, one chamber received CO_2 with a $\delta^{13}C$ of –28.8‰, and the other CO_2 with $\delta^{13}C$ of –1.7‰. For labelling plants were swapped between chambers. Plants were harvested at different times following swapping (from 2 h up to 39 d), and LGZ and newly exported tissue (NT) were sampled as explained by LATTANZI ET AL. (2005).

The isotopic composition of LGZ and NT was analysed in an isotope-ratio mass spectrometer and the fraction of labelled C (f_{labC}) and unlabelled C (f_{unlabC}=1-f_{labC}) in tissues calculated using a two-component-mixing model (SCHNYDER und DE VISSER, 1999). The tracer kinetics in imported substrate was estimated according to LATTANZI ET AL. (2005).

Results and Discussion

Import kinetic of growth substrateFig. 2 shows the time course of f_{unlabC} in C substrates imported into the LGZ. In both N treatments, three different phases were detected. The first phase showed a rapid decrease, which lasted about 4h in N+, and 8 h in N-. In the second phase f_{unlabC} decreased at a slower rate in both N treatments, but more so in N+. Both treatments reached similar values of f_{unlabC} at 48 h. Thereafter the kinetics of N- and N+ were virtually the same and f_{unlabC} gradually approached zero.

Three-term exponential functions described this tracer time-course very well in both N treatments, giving evidence for three distinct pools supplying the LGZ with substrates. A two-term exponential was not describing the data points well, and a four-term exponential did not improve the goodness of fit. N did not have an effect on the number of pools.

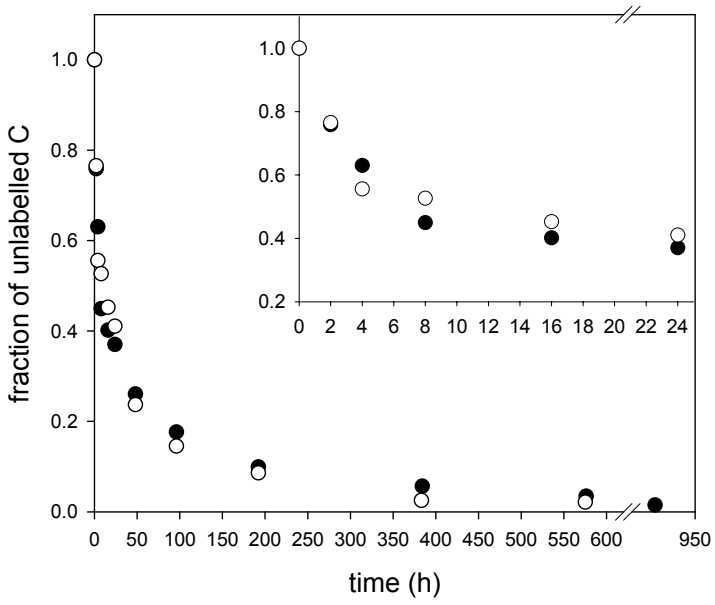

Fig. 2: Kinetic of the fraction of unlabelled carbon imported into the LGZ over time. Comparison of the low N (●) and the high N treatment (○)

Structure of the pool model: half-times and importance of the pools

We then constructed a 3-pool model (Fig. 3) and implemented it in ModelMaker (Version 3.0.4; Cherwell-Scientific, Oxford, UK) to estimate the pool characteristics and exchange fluxes between pools (LATTANZI et al., 2005).

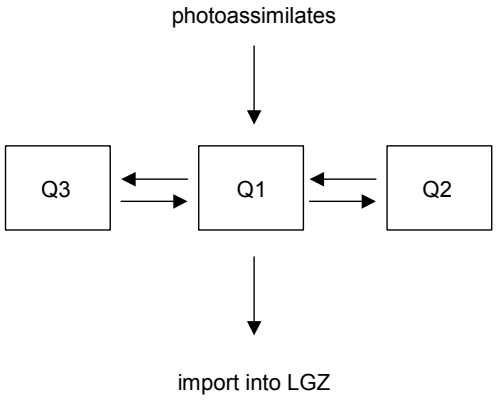

Fig. 3: Structure of the used model. Photoassimilates from photosynthesis are entering the first pool Q_1 (transport pool). From there substrates are either transported directly to the LGZ or are fist cycling through one of the two storage pools (Q_2 and Q_3).

Rate constanst, describing the fluxes between pools, were optimized to fit the model to the experimental data points (R^2 of the fit was 0.99 for N- and N+). From rate constants half-times ($Q_x t_{0.5}$) and relative sizes of the pools (Q_x/tillerC) were derived (Tab. 1). High N reduced the half-times of the two fast pools Q_1 and Q_2 to about half of the values of low N, but did not change the half-time of the long-term storage pool Q_3. N had only an effect on the relative size of Q_2; it increased with N+. The relative size of the other pools was not influenced.

The result of a higher N status of the plant was that less photoassimilates were passed directly to the LGZ (p_{10} in Tab. 1), and more were cycled first through the short-term storage pool Q_2 (p_{12}).

Tab. 1: Results of the pool model. Half-times of the pools (Q_1 $t_{0.5}$, Q_2 $t_{0.5}$ and Q_3 $t_{0.5}$), the relative size of the pools (Q_x/ tillerC, in mgC per g tillerC) are shown. The probability ,p, of C flowing directly to the LGZ (p_{10}) or being stored in Q2 or Q3 before (p_{12}, p_{13}) entering the LGZ is also given (± standard error, SE).

	N-	N+
Q_1 $t_{0.5}$	71 min	40 min
Q_1/ tillerC	2.6 mg g^{-1}	2.5 mg g^{-1}
Q_2 $t_{0.5}$	21 h	13 h
Q_2/ tillerC	17.5 mg g^{-1}	25.4 mg g^{-1}
Q_3 $t_{0.5}$	7.2 - 8.2 d	6.4 - 6.7 d
Q_3/ tillerC	36.9 mg g^{-1}	35.3 mg g^{-1}
p_{10}	0.53 ± 0.07	0.43 ± 0.07
p_{12}	0.38 ± 0.07	0.51 ± 0.07
p_{13}	0.09 ± 0.06	0.06 ± 0.04

Discussion

We found that three distinct pools supplied C to leaf growth in both N treatments. High N decreased the half-times of the two fastest pools (Q_1 and Q_2), and increased the relative size of Q_2.

Substrate in Q_1 was labelled very quickly (~1 h). For this, Q1 was titled "transport pool". Precursors of sucrose, sucrose and amino acids in the cytosol and apoplast are possibly included in this pool, because the half-times of these are reported to be about 1 h (FARRAR, 1989). This pool represented less than 1% of tiller C, but about half of C substrates for leaf growth was directly supplied via this pool. With more N the importance of this pool decreased slightly.

The second pool, Q_2, probably consists of a mixture of vacuolar sucrose, fructans and amino acids, which have a reported half-time of 7-25h (FARRAR, 1989). For this we termed it "short-term storage pool". This pool was affected most by N. The relative size of the pool increased with N, as did the importance in supplying the LGZ. Together the two fast pools provided more than 90% of the substrate, in both N treatments. Only the relation between the relative size of Q_1:Q_2 changed. High N reduced the half-times of the two pools by about one half. This meant that the flux through the pools must have increased with N, since the relative size of the pools did not decrease. In fact it increased by 45%.

The long-term storage pool, Q3, contributed less than 10% of the C flux to the LGZ. The half-time of this pool indicated that it contained substrates with a slow turnover. This could include C from protein turnover or fructans in leaf sheaths. The half-times, the relative size and the importance of this pool was not affected by N.

In conclusion we have shown that leaf growth in undisturbed *Lolium perenne* is primarily based on recent assimilates with a half-time less than 1 d, and largely independent of long-term stores. N did not change these relationships. This extends the findings of LATTANZI ET AL. (2005) and shows the rigidity of this pattern also under different N levels.

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