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REGULAR ARTICLE

The above-belowground coupling of the C cycle: fast and slow mechanisms of C transfer for root and rhizomicrobial respiration

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Abstract

Background and aims The coupling of photosynthesis with belowground processes appears to be much faster than the time needed for assimilate translocation with the phloem flow. Pressure/concentration waves have been hypothesized to release belowground C already present in the phloem, resulting in a very fast feedback of rhizosphere processes to photosynthesis changes. We evaluate the speed of aboveground-rhizosphere coupling under maize by two mechanisms: pressure/concentration waves and direct phloem transport.

Methods We combined two isotopic approaches: 1) the speed of direct phloem transport was evaluated by labeling shoots in $^{14}CO_2$ and tracing ^{14}C in the nutrient solution and in the CO_2 flux, 2) pressure/concentration waves were evaluated by labeling the solution with [^{13}C] glucose and tracing the isotope dilution during photoassimilation.

Results ^{14}C shoot labeling of maize plants showed that 12 h were needed for ^{14}C to peak in root-derived CO_2. In contrast, in the solution labeling approach, CO_2 flux increased within 2 h after switching on the light. Pressure/concentration waves contributed 5 % to diurnal respiration efflux and affected only root respiration. Root exudation was independent of the fast mechanism of above-belowground coupling.

Conclusions Photosynthesis affected root and rhizomicrobial respiration on variable time-scales: root respiration within the first 2 h by pressure/concentration waves, whereas rhizomicrobial respiration may depend on internal circadian cycles in regulating exudation rather than on light directly.

Key words Phloem transport · Photosynthesis · Pressure/concentration waves · Rhizosphere · Soil respiration · Time lag

Abbreviations

OC	organic carbon
IC	inorganic carbon
TC	total carbon

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Introduction

Plants drive root and microbial respiration in the rhizosphere (together: "root-derived CO_2") and thus control one of the most fascinating fluxes of the C cycle (Janssens et al. 2001; Kuzyakov and Gavrichkova 2010; Barthel et al. 2011; Bahn et al. 2013; Makita et al. 2014). Nonetheless, the mechanisms of this above-belowground regulation of C fluxes remain uncertain (Kayler et al. 2010; Mencuccini and Hölttä 2010a, 2010b). The mechanism type determines the response speed of the C sinks – roots and rhizosphere microorganisms – to changes in photosynthetic activity aboveground. Assimilates are delivered belowground directly with the phloem flow. The response time of belowground processes to the photosynthetic activity is expected to be determined by the length of the path and by the velocity of phloem transport. A detailed literature overview enabled estimating an average time lag between C assimilation aboveground and release of this C as CO_2 by respiration from the rhizosphere: 4–5 d for mature trees and 12 h for grasses (Kuzyakov and Gavrichkova 2010; Epron et al. 2012; Burri et al. 2014). Nonetheless, faster mechanisms based on indirect transition of "information" on photosynthesis changes downward by electrical signaling and propagation of pressure and concentration waves have been proposed (Ferrier et al. 1975; Thomson and Holbrook 2004; Hölttä et al. 2006, 2009; Mencuccini and Hölttä 2010a).

According to the classical Münch hypothesis, sugar transport is driven osmotically by loading and unloading of assimilates in and out of the phloem. This creates a hydrostatic pressure gradient, which drives the assimilated molecules downward (Münch 1930; Minchin and Lacointe 2005). The resulting transport velocities, however, are low (0.01–0.3 m h^{-1}) and it takes days for assimilates to reach the destination organs in tall vegetation (e.g. trees: Wingate et al. 2010; Gavrichkova et al. 2011; Keel et al. 2012; Epron et al. 2012 for a review). Alternatively, it was proposed that phloem acts as a "high-pressure manifold", where the turgor pressure difference between the opposed phloem ends is low and the concentration is high (Fisher 2000; Thomson and Holbrook 2004). In conditions of a low pressure gradient, the sensitivity of the whole system to local turgor and concentration changes due to phloem loading increases. Accordingly, the time needed for the sap to move in order to re-establish pressure equilibrium in the whole system will be short, making access of multiple sinks to photoassimilates much faster. These sap displacements were termed pressure/concentration waves (Thomson and Holbrook 2004). Establishment of the new water potential equilibrium implies that the effect of wave propagation on root metabolism is constant unless a new perturbation of pressure and concentration occurs (Thomson and Holbrook 2004; Mencuccini and Hölttä 2010b). Compared with direct phloem flow, in which the time lag between photosynthesis and root-derived respiration is determined by the time needed for the assimilated molecules to reach belowground, pressure/concentration waves deliver C already present in the phloem at the moment of assimilate loading. Pressure/concentration waves will function efficiently and exceed the speed of direct phloem transport solely when the osmotic pressure is high relative to the source-sink turgor difference (Thomson and Holbrook 2003; Thomson 2006). Such conditions are common in short vegetation (e.g. grasses), whereas the length of sieve tubes in tall trees may hinder rapid above-belowground coupling. Given the importance of such coupling especially for tall vegetation, anatomic adjustments of the phloem structure are hypothesized, but require experimental confirmation (Thomson 2006).

Mencuccini and Hölttä (2010b) reported several examples of this type of signal propagation, strengthening the conclusion that very fast coupling exists. Such fast coupling was confirmed for example for xylem (Perämäki et al. 2001) where, although many days are necessary for the water molecules from the soil to reach the evaporation sites, transpiration rates recover almost immediately after cessation of the drought stress. For phloem, however, this type of fast coupling is merely hypothesized. Mencuccini and Hölttä (2010a) simulated the transition velocity time of phloem solution along with pressure and solute concentration waves. The authors quantify, for a 12 m-tall tree, a phloem transfer time of individual molecules of approximately 100 h, whereas less than 7.3 h was estimated for the wave propagation to increase the photoassimilate delivery belowground.

Pressure/concentration wave theory explains well the unexpectedly short time lags between photosynthesis and soil respiration observed in some experiments on mature tree stands (Ekblad et al. 2005; Tang et al. 2005; Baldocchi et al. 2006). Nonetheless, there is still no experimental confirmation for the relevance of this transport mechanism. The discussion therefore remains open on whether wave propagation is significant for

belowground metabolism as opposed to direct transport of assimilates (Kayler et al. 2010; Mencuccini and Hölttä 2010b; Gavrichkova and Kuzyakov 2012). We also cannot exclude that the measured extremely short time lags, completely absent in some cases (Liu et al. 2006), could be biased due to methodological shortcomings of time series analysis.

The main objectives of this study were 1) to test for the existence of a very fast transmission of photosynthetic signals belowground in addition to direct assimilate translocation with the phloem flow and 2) to evaluate the significance of pressure/concentration waves for belowground C transfer by maize. To compare two mechanisms – direct transport and pressure/concentration waves – we used two isotopic approaches: 1) labeling of plants in $^{14}CO_2$ atmosphere and tracing the appearance of ^{14}C in nutrient solution and in root-derived CO_2, and 2) isotope dilution of ^{13}C-enriched glucose in nutrient solution by unlabeled root exudates released by propagation of pressure/concentration waves. According to the literature review we hypothesized:

(1) Propagation of pressure/concentration waves induced by light variation results in a very fast response of root-derived CO_2 to changes in carbohydrate availability. In contrast, the newly loaded assimilates transported with the phloem flow reach belowground much later compared to that delivered by wave propagation. This difference between the speed of the two processes could be detected by the timing of appearance of labeled photoassimilates belowground compared with the response time of CO_2 and exudation fluxes to the light changes.
(2) The effect of wave propagation on belowground functioning (CO_2 flux and exudation) is not short-lived but constant in time bringing to a new steady state belowground fluxes and pools until light conditions and therefore, water potential equilibrium in the phloem, remain stable.
(3) Transient shading of the plants interrupts the phloem water potential equilibrium established by wave propagation, disturbing root respiration and the exudation steady state and thus lowering the respective CO_2 fluxes.
(4) High availability of sugars delivered with waves to the roots affects both root respiration and microbial metabolisation of exudates in the rhizosphere, leading to higher microbial respiration. Exudation of the sugars induced by waves will dilute the ^{13}C labeled solution, which will be reflected in a lower ^{13}C enrichment of microbial respiration and of the solution.

Material and methods

Plant growing conditions

Seeds of maize (*Zea mays* L.) were germinated on moist filter paper in Petri dishes for 2 days. Seedlings were then transplanted to transparent 250 mL plastic pots, with one seedling per pot. Pots were filled with Hoagland complete nutrient solution and covered with aluminum to prevent algal growth. Plants were grown under controlled environment conditions with a 12 h/12 h day/night period at day/night temperatures of 25/22 °C, and with a photosynthetically active radiation intensity of approximately 600 μmol m^{-2} s^{-1} at the top of the canopy. The Hoagland solution level was maintained at 250 mL and the solution was renewed every two days. To sufficiently aerate the roots, air was constantly pumped through the solution with a membrane pump.

After one month, the plants reached a height of approximately 90 cm (88.0 ± 3.0 cm) and were subjected to two labeling experiments. The first one (hereafter *Shoot Labeling*) was designed to estimate the velocity of direct transport of assimilates belowground. The second labeling (hereafter *Solution Labeling*) was designed to induce and measure pressure/concentration wave propagation belowground.

Shoot labeling

The *Shoot Labeling* experiment consisted of pulse labeling plants in $^{14}CO_2$ atmosphere and tracing the ^{14}C in the nutrient solution and in the respired CO_2. The ^{14}C labeling was done in the morning on five maize plants, shortly after the start of the photoperiod. Green stems of maize below the leaves were covered with aluminum to limit the photosynthetic uptake solely to the upper plant parts. The obtained non-photosynthesizing path (29.1 ± 4.4 cm) was used further to quantify the translocation velocity of the phloem flow. The ^{14}C pulse labeling of shoots has been described in detail by

Werth and Kuzyakov (2006) and Sanaullah et al. (2012). In brief, five replicates of solution-grown maize were first sealed with silicone paste (NG 3170, Thauer & Co., Dresden, Germany) to prevent diffusion of the label into solution and then placed in a Plexiglas chamber. $^{14}CO_2$ was introduced into the chamber by adding 1 mL of 5 M H_2SO_4 to a $Na_2{}^{14}CO_3$ (6.2 MBq) solution. This allowed complete evolution of $^{14}CO_2$ into the chamber atmosphere. The total CO_2 concentration in the chamber never exceeded 500 ppm. After a 40-min labeling period, the CO_2 from the chamber was trapped using 10 mL of 1 M sodium hydroxide (NaOH) solution to remove the remaining unassimilated $^{14}CO_2$. When the chamber was opened, pots with the plants were connected to membrane pumps. Air was circulated in a closed system loop from the pot to the vial with 3 mL of 1 M NaOH trap. The air was pumped from the bottom to the top of the pots and thus mixed and aerated the solution. Traps were changed nine times in the subsequent 48 h and analyzed for ^{14}C activity and total CO_2. At the same time, 2 mL of the nutrient solution were sampled for ^{14}C analyses. In order to increase the sampling frequency at the beginning of the chase period, two more solution samples were taken within the first 2 h after labeling. After the last sampling the plants were harvested, dried at 70 °C until constant weight, ground and stored until the further analyses.

Solution labeling

The *Solution Labeling* experiment was designed to study whether rhizomicrobial activity responds to photosynthesis prior to a direct delivery of recent assimilates belowground as estimated by *Shoot Labeling*. Here, we monitored a time response of root-derived respiration to variation of the light regime. Changes in exudation and microbial activity in the rhizosphere were assessed with isotope dilution of [^{13}C] glucose in nutrient solution. Release of non-enriched substrates, easily accessible for microbial functioning, will lower the ^{13}C enrichment of microbial respiration. However, alongside unlabeled exudates, root respiration may contribute to the dilution of the overall CO_2 flux. Combining $\delta^{13}C$ of CO_2 flux and solution was used to help discriminate these two processes.

After 15 h of a common dark period, the plants were divided into three groups (Fig. S1 in supplemental material). One group of five plants (Night treatment) was placed in an opaque box and maintained in the dark during the whole experiment duration (one day). Another group of five plants was maintained constantly in the light up to the end of the experimental day (Day treatment). The third group of plants was exposed to light for only 2 h, then darkened again for 1.5 h, then exposed again to light until the end of the experimental day (Day/Night treatment) (Fig. S1). Five pots were left unplanted and treated in the same way as the Day treatment. All treatments were kept at the same air temperature.

Labeling of nutrient solution with [^{13}C] glucose was performed 1.5 h prior to the termination of the common dark period. Glucose was prepared by mixing 99 atom% [^{13}C] glucose with the unlabeled one, so that $\delta^{13}C$ of the mixture was 800 ‰. 4.5 mg of the [^{13}C] glucose was added to each pot.

Immediately after labeling, the pots with plants were connected to membrane pumps to trap CO_2 as described for the *Shoot Labeling*. At the end of each light/dark phase (in total four, Fig. S1), NaOH traps were changed in all the treatments.

Nutrient solution was sampled three times at 30-min intervals before the end of the common dark period. In the successive light/dark phases (Fig. S1) the solution was sampled an additional seven times. In each sample, 15 mL of the solution was taken from each pot, transferred to a separate vial and sealed before the analyses. The nutrient solution in the pots was maintained at constant 250 mL level by adjusting the volume with non-labeled Hoagland solution at every sampling event. The dilution effect on solute concentration was accounted for. At the end of the last light phase, all the plants were harvested, carefully washed, dried at 70 °C until constant weight and ground for further analyses. The *Solution labeling* approach was repeated without [^{13}C] glucose addition to test whether modification of the solute gradient between root cells and growth media by glucose altered exudation patterns.

Sample analysis, calculations and statistics

Both labeling experiments were conducted with five replicates. Total Carbon (TC) and Inorganic Carbon (IC) content of the solution were measured on a multi N/C 2100 TOC/TN analyzer (Analytik Jena, Germany). The Organic Carbon (OC) was obtained as the difference between TC and IC measured directly. To account for the dilution of the nutrient solution and label by frequent sampling, we used as a reference the OC and

IC concentration changes in pots without plants. For this purpose we quantified the dilution level (% in respect to the initial OC and IC levels measured at the first sampling, $\%dil_t$) at each sampling time in non-planted pots:

$$\%\mathrm{di}l_t = 100 - \frac{solC_t}{solC_1} \mathrm{x}100$$

where $solC_t$ is the measured value of OC and IC in the pots without plants at time t (mg L^{-1}) and $solC_1$ is the measured OC and IC content in the solution without plants at the first sampling.

The obtained percentages were then applied to the treatment plants to recover the expected levels of OC and IC in the solution at each sampling time with the following equation:

$$Ct_{exp} = \frac{C_t \times 250}{1000} + \left(\frac{C_1 \times 250}{1000} \times \%\mathrm{di}l_t/100\right)$$

where Ct_{exp} is an expected value of OC or IC in the solution at time t (mg pot^{-1}) after accounting for the dilution; C_t is the measured value of OC or IC at time t (mg L^{-1}); C_1 is the measured value of OC and IC at the first sampling (mg L^{-1}); 250 is the pot volume.

An aliquot of the solution in *Solution Labeling* was analyzed for $\delta^{13}C$ in OC after precipitating the carbonates with 0.5 M $SrCl_2$. Measurements were done on freeze-dried samples using isotope ratio mass spectrometry (IRMS, DELTAplus, Finnigan). The solution, sampled during *Shoot Labeling,* was analyzed for ^{14}C after the decay of chemiluminescence using a liquid scintillation counter (MicroBeta, TriLux).

NaOH traps from both labeling experiments were analyzed for total trapped CO_2, ^{14}C (solely from *Shoot Labeling*) and $\delta^{13}C$ (solely from *Solution Labeling*). The total CO_2 efflux was estimated by precipitating CO_2 trapped in the NaOH solution with a 0.5 M barium chloride ($BaCl_2$) solution. NaOH was then titrated with 0.1 M hydrochloric acid (HCl) until the threshold pH was reached (Zibilske 1994). The ^{14}C was measured in 1 mL aliquots after the decay of chemiluminescence using a liquid scintillation counter (MicroBeta, TriLux). ^{14}C activity in respired CO_2 is presented as the percentage of ^{14}C recovered during two days of the chase period. The $^{13}C/^{12}C$ isotope ratio of traps was analyzed using isotope ratio mass spectrometry as previously described.

Aboveground and belowground plant biomass was analyzed for ^{14}C activity (solely from *Shoot Labeling*) and ^{13}C (solely for *Solution Labeling*). ^{14}C activity in ground, solid samples was analyzed after combustion in a Biological Oxidizer (OX 4000) and trapping the released $^{14}CO_2$ using a scintillation counter Beckmann 6500. The $^{13}C/^{12}C$ isotope ratio was analyzed on IRMS (DELTAplus, Finnigan). Stable isotope values are expressed in δ notation (‰) in relation to the Vienna Pee Dee Belemnite standard (VPDB).

All statistical analyses were performed using the software STATISTICA 7 (StatSoft). The significance of differences of ^{13}C enrichments and ^{14}C activities measured in various pools and fluxes between the treatments was estimated by ANOVA. Repeated measurement ANOVA was used to estimate the significance of changes in time and between treatments in solution chemistry and respiration in the *Solution Labeling* experiment. One-way ANOVA was used to compare changes in isotope signatures of biomass in *Solution Labeling*. We calculated the least significant difference (LSD 0.05) in a post hoc Fisher test to identify the significant differences between treatments.

Results

Shoot Labeling: time lag due to direct phloem transport

After labeling the maize shoots in a $^{14}CO_2$ atmosphere, 12 h was needed for ^{14}C label to be delivered belowground and to peak in both root-derived CO_2 and nutrient solution (Fig. 1). No ^{14}C was detected in the solution in the first 2 h after labeling (Fig. 1a). The first ^{14}C activity, although very low, was detected in both components at the 4 h sampling. The ^{14}C decreased in the solution during the night and remained close to its maximum during the second sampling day. A small peak of ^{14}C recovery was measured in CO_2 during the second day (Fig. 1b). CO_2 efflux from the nutrient solution had a clear diurnal periodicity. Day-time maxima in CO_2 efflux were measured approximately 9 h after switching on the light (see 2nd day in Fig. 1b).

Solution Labeling: time lag by fast aboveground-belowground coupling

Response of CO_2 efflux to light changes

CO_2 efflux from nutrient solution strongly increased within 2 h after the start of the photoperiod, relative to

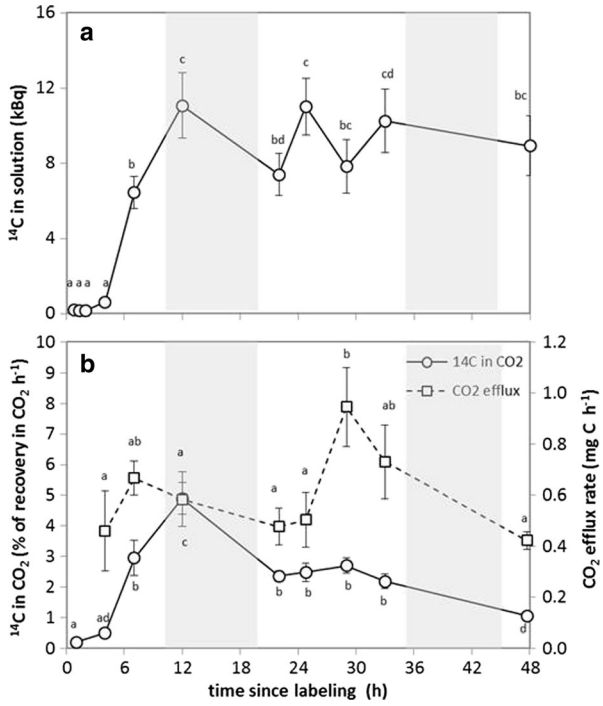

Fig. 1 Dynamics of (**a**) ^{14}C in solution and (**b**) $^{14}CO_2$ and CO_2 dynamics of root-derived respiration after labeling maize plants in $^{14}CO_2$ atmosphere. Grey areas represent night time. Error bars show ±SE of the mean. Letters denote the significance of the difference between timepoints at $p < 0.05$

plants kept constantly in the dark (Fig. 2, Table 1). The respiration increase was 117 % ($p < 0.01$) and 88 % ($p < 0.05$), respectively, for Day and Day/Night plants, compared to the values measured in the dark., Therefore, the time lag between photoassimilation and appearance in root-derived respiration was less than 2 h in the *Solution Labeling* experiment.

During the second 1.5 h darkening of plants in the Day/Night treatment, the CO_2 efflux rates returned to those in the dark period (Table 1). Nonetheless, the respiration rates were not significantly different from those measured in the light at the previous sampling ($p = 0.23$, Table 1). Although the respiration patterns were similar also for Day treatment, respiration remained higher than that measured in the dark ($p < 0.05$). After returning plants to light for the Day/Night treatment, the CO_2 efflux rates increased. At this last sampling, however, the rates significantly increased both in the Day and the Night treatment (Table 1, and Fig. 2).

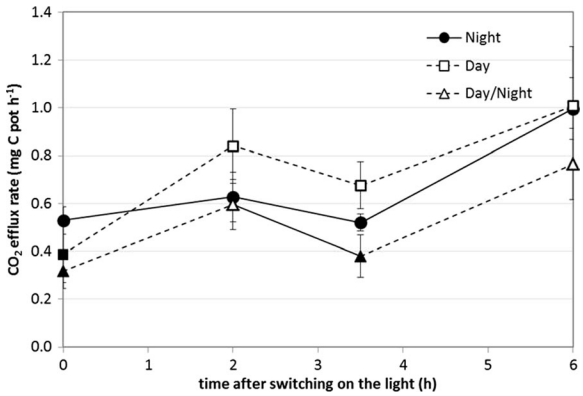

Fig. 2 CO_2 efflux from solution (root respiration and microbial decomposition of glucose) measured at the end of each light/dark phase. Closed symbols and solid line: periods when the respective treatment is darkened; open symbols and dashed line: light periods. Error bars show ±SE of the mean

Response of root exudation to light

OC variations in the nutrient solution were similar among the dark and light-exposed treatments. A daytime increase in OC content (~1.5 h and 6 h after switching on the light) was also measured in plants held under constant darkness (Fig. 3a). Added [^{13}C] glucose was rapidly consumed from the solution, as indicated by a rapid decrease of solution $\delta^{13}C$ (Fig. 4a) during the night period and by concomitant decrease in OC content (Fig. 3a). The label was not metabolized by microorganisms in this period: ^{13}C enrichment in CO_2 remained small in the dark (Fig. 4b). After switching on the light, the solution $\delta^{13}C$ continued to decrease similarly to the

Table 1 Increase of CO_2 efflux expressed as percentage of previous sample and first sample. The reference values are taken as 100 %. "*" significance level at $p < 0.05$, "**" - at $p < 0.01$, "^{ns}" – non significant

Percentage Increase						
Treatment	Night		Day		Day/Night	
Time since switching on the light	% to previsous	% to 1st	% to previsous	% to 1st	% to previsous	% to 1st
−1.5 - 0 h	100	100	100	100	100	100
0–2 h	119^{ns}	119^{ns}	217**	217**	188*	188*
2–3.5 h	83^{ns}	98^{ns}	80^{ns}	174*	64^{ns}	119^{ns}
3.5–6 h	191**	188*	149^{ns}	260**	202*	241*

other treatments up to the end of the first light period (2 h after switching on the light) (Fig. 4a insert). Afterwards, solution $\delta^{13}C$ of light-exposed plants increased and remained more ^{13}C enriched relative to the darkened plants to the end of the experiment (although the differences were not significant). Microorganisms metabolized [^{13}C] glucose during the day period: $\delta^{13}C$ of CO_2 increased substantially in all treatments (light-exposed and dark plants, Fig. 4b). $\delta^{13}C$ of CO_2 continued to increase in the subsequent sampling in the *Night* treatment and remained stable for plants subjected to light. Significant $\delta^{13}C$ differences between *Day* and *Night* treatments were recorded only in the last sampling ($p < 0.05$).

A proportion of the [^{13}C] glucose added to the nutrient solution was taken up by plants and incorporated into plant biomass. The $\delta^{13}C$ increase in roots was 1.5–2.0 ‰ and did not differ significantly between the treatments (Fig. 5). In contrast, aboveground biomass $\delta^{13}C$ did not change.

The IC dynamics, used to quantify OC in solution, differed between the treatments. The IC content increased in all treatments between 1.5 and 2 h after switching on the light (Fig. 3b). This increase, however, was significant only in light-exposed plants ($p < 0.05$), where a new steady state in IC solution content was stabilized.

Discussion

Slow coupling of above- and belowground activity: direct phloem transport

The velocity of assimilate translocation through the phloem could be unequivocally determined by ^{14}C or ^{13}C pulse labeling the shoots and tracing the labeled C in the growth media and respired CO_2 (Subke et al. 2009; Bahn et al. 2009; Shibistova et al. 2012; Epron et al.

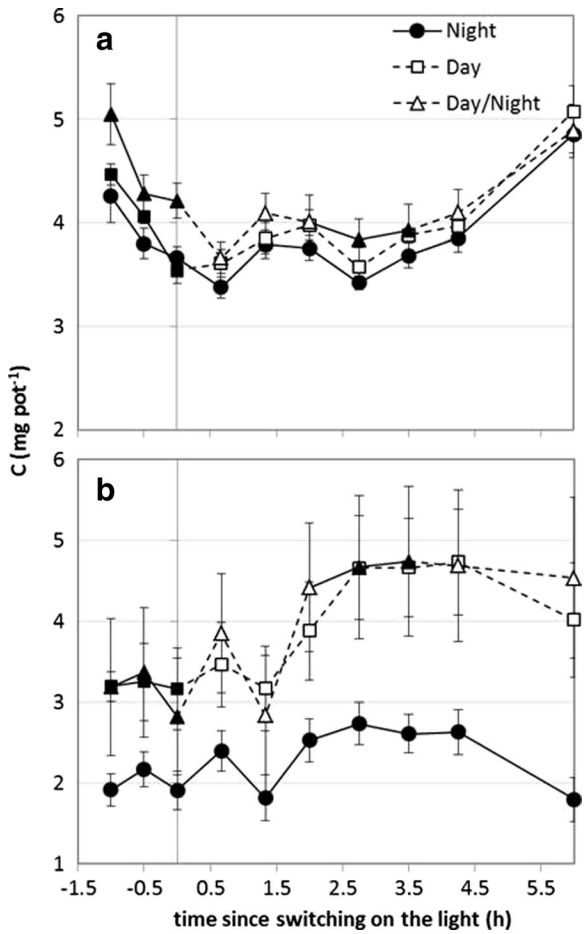

Fig. 3 Dynamics of OC (**a**) and IC (**b**) in the nutrient solution. Closed symbols and solid line: the periods when the respective treatment is darkened; open symbols and dashed line: light periods. Error bars show ±SE of the mean

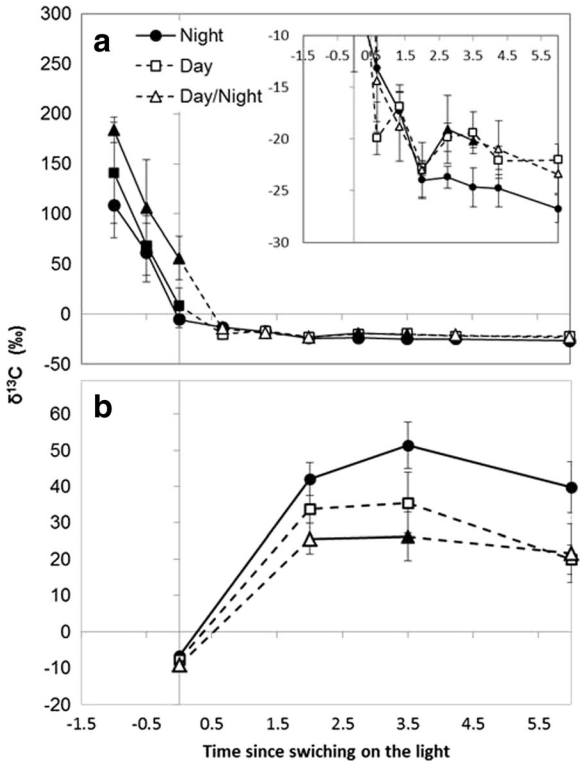

Fig. 4 **a** Variation of $\delta^{13}C$ in the solution during the chasing period; insert: the same data but with shorted y-axis; **b** variation of $\delta^{13}C$ in root-derived respiration measured at the end of each light/dark phase. Closed symbols and solid line: the periods when the respective treatment is darkened; open symbols and dashed line: light periods. Error bars show ±SE of the mean

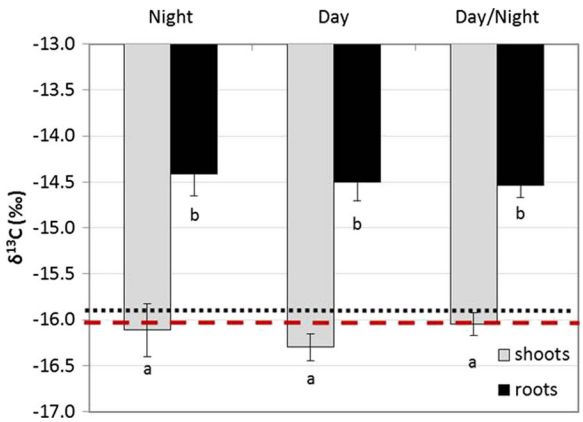

Fig. 5 $\delta^{13}C$ signature of shoots and roots reflecting the uptake of [^{13}C] glucose from the nutrient solution. Dashed line: $\delta^{13}C$ signature of shoots; dotted line: $\delta^{13}C$ of roots in a controlled unlabeled group of plants (n = 3). Letters indicate significant differences between organs and light treatments at $p < 0.05$. Error bars show ±SE of the mean

2012). The effect of maize photosynthesis on the root-derived respiratory activity was highest 12 h after labeling, which we interpret as the time lag between two fluxes (assimilation and root-derived respiration). We are aware that the sampling frequency in *Shoot Labeling* was too low to estimate exactly the time lag, which may take place between 7 and 12 h as well as between 12 and 20 h after labeling. The dynamics of total CO_2 efflux, which was monitored during the *Shoot Labeling* experiment, suggests that the respiration peak occurred slightly earlier than 12 h.

The obtained time lag of ~12 h is congruent with the estimates reported by Kuzyakov and Gavrichkova (2010) for grasses. The presently measured translocation velocity of 0.03 m h^{-1} fits into the upper part of the range 0.003–0.03 m h^{-1}as calculated for grasses from the literature (Kuzyakov et al. 1999; Kuzyakov and Domanski 2002). Unfortunately, only few of the numerous labeling studies with herbaceous vegetation report plant height. Efficient comparison of phloem transfer velocities between plant species, depending on their height and development stages, would require these data.

Fast coupling of above- and belowground activity: pressure/concentration waves

Isotope dilution is the only possible approach to trace the transport and release of unlabeled exudates from roots (Cheng et al. 1993). The accelerated response of respiration and exudation to light variation compared to the translocation velocity of labeled molecules measured by *Shoot Labeling* points to indirect coupling between aboveground and belowgroud activity by means of pressure/concentration wave propagation.

Fast coupling: effect on respiration

The observed increase in CO_2 efflux rates after 2 h of light in the *Solution Labeling* experiment (Fig. 2) suggests a more immediate coupling of aboveground and belowground activities than by direct phloem transport. The recorded translocation velocity (> 0.15 m h^{-1}) is five times higher than the phloem transport of the labeled molecules (0.03 m h^{-1}) as estimated by *Shoot Labeling*. CO_2 efflux doubled during the first 2 h of light in *Solution Labeling*, indicating that indirect coupling has a substantial and fast effect on belowground processes. We therefore confirm hypothesis 1. The OC

supply into the rhizosphere did not increase during the first 2 h in respect to the Night treatment (Fig. 3a). We conclude that additional CO_2 was released directly from roots within root-derived respiration rather than from microbial mineralization of exudates, so that we can't completely confirm hypothesis 4 (see also discussion below). For the same timeframe in the *Shoot Labeling* experiment, no ^{14}C was detected in the solution or CO_2 efflux. Although respiration in the Day treatment remained stable for up to 3.5 h after the start of the photoperiod, the frequency of respiration measurements was insufficient to support or reject hypothesis 2. We did not consider the effect of root growth on the amount of emitted CO_2 during one day. We assume that root mass remained nearly unchanged during a one-day period and, consequently, the C investments into root growth are small compared to the root biomass.

Beyond the increase of total CO_2 efflux, the IC content in the solution also substantially increased in all *Solution Labeling* treatments 1.5–2 h into the photoperiod (Fig. 3b). The CO_2 increase was significant in light-exposed plants, where a new steady state in IC content was reached. We attribute this IC increase to the establishment of a new equilibrium between the CO_2 from root respiration and HCO_3^- in solution. Therefore, the IC content is driven by higher root respiration after photosynthesis. The time resolution of IC measurements was higher than for respiration flux, so that we can exclude a short-lived pulse-like effect of pressure/concertation wave propagation on belowground functioning (hypothesis 2).

Root respiration showed a small short-term response to the transient darkening of plants in the Day/Night treatment (Fig. 2, and Table 1). The duration of shading was probably insufficient to induce considerable changes in phloem loading rates after photosynthesis cessation. Accordingly, the CO_2 efflux rates oscillated between values measured in the dark and in the light (Table 1). Remobilization of the starch pool helps maintain phloem loading for some period in the dark (Göttlicher et al. 2006; Gessler et al. 2007; Gavrichkova et al. 2011). Longer dark periods would be required to deplete this pool. Therefore, hypothesis 3 is only partially confirmed and calls for further experiments to evaluate how long plants can maintain constant phloem loading rates without light.

In *Solution Labeling*, the increased respiration rate in all treatments at the last sampling event (6 h into the photoperiod, Fig. 2) was associated with a higher OC concentration (Fig. 3a). This time point in *Shoot Labeling* was associated with the first ^{14}C appearance in solution and in the CO_2 efflux (4–7 h, Fig. 1). Therefore, additional CO_2 and C in solution in *Solution Labeling* is represented by morning photosynthates that are delivered to roots by the phloem flow after 4–6 h of light. Unexpectedly, increased CO_2 efflux and OC content were measured in all treatments including "Night". Such observations are, however, not new. Plants are able to maintain their diurnal exudation patterns unchanged even in the absence of active photosynthesis (Kuzyakov and Cheng 2001, 2004). Endogenous regulation was proposed to explain diurnal periodicity of root-derived CO_2 in shaded plants. In fact, exudation is related to the C supply to roots in general (recent assimilates and remobilized reserves) rather than solely to photosynthesis (Dilkes et al. 2004). Adjustments of the root metabolism and exudation rates when the C supply from leaves is insufficient may involve a relative increase in the consumption of reserves (Gessler et al. 2002, 2007). Roots can successfully control exudation by opening the membrane pores, thus increasing molecule diffusion and compensating for lower sap concentrations during shading (Jones et al. 2004). Indeed, sink activity was demonstrated to affect C allocation patterns (Farrar and Jones 2000; Andersen 2003). Increased respiration flux in plants from the Night treatment at the last sampling can be interpreted as the microbial metabolisation of the organics exuded from roots stimulated by inherent circadian patterns.

Fast coupling: effect on exudation

Contrary to our expectations the OC concentration in the nutrient solution was similar in light- and dark-exposed plants (Fig. 3a). OC dynamics in solution are driven by the release of root exudates and by their microbial consumption (Jones et al. 2004). The imbalance between OC input and output creates oscillations in the OC concentration. Four reasons could explain the absence of the difference in OC between light- and dark-exposed plants. Firstly, exudation is affected by changes in the solute gradient between root cells and growth media. Lowering the concentration gradient between the solution and the root cells will affect the passive diffusion and thus dampen the exudation rates (Jones and Darrah 1993; Jones et al. 2004). After adding the

[^{13}C] glucose, its concentration in solution was 0.08 mM. The solute concentration in the cell cytoplasm may range from 0.5–10 mM, i.e. 10 to 100 times higher than in solution (Jones et al. 2004). Although the recorded gradient is high, we tested experimentally whether glucose addition affected OC patterns. In order to further increase the concentration gradient between the cytoplasm and the environment (Neumann and R mheld 2007), the design of the *Solution Labeling* experiment was repeated without glucose addition to the solution. The obtained patterns of the OC dynamics were similar (Fig. S2): no differences between dark- and light-exposed plants were measured up to 4.5 h after the light onset. Indeed, the growth media may affect the exudation patterns qualitatively and quantitatively. Nonetheless, a recent comparison of exudate sampling techniques revealed no particular discrepancies in measured exudation patterns between nutrient solution and soil media and different sampling techniques (Oburger et al. 2013).

Secondly, additional organics exuded in response to propagation of pressure/concentration waves might be consumed by microorganisms and respired back to the atmosphere. This would maintain OC levels unchanged. However, this was not the case starting from 3.5 h into the photoperiod, where the organics exuded from roots triggered a gradual increase in the OC concentration of the solution (Fig. 3a). Accordingly, there was no or very low additional supply of root exudates in the timeframe between 0 to 3.5 h in all treatments. The ^{13}C isotope dilution in the solution and in the released CO_2 supports this: δ^{13}C solution was very similar between treatments in the first two hours after the light onset (Fig. 4a). Microbial respiration δ^{13}C is therefore expected to be similar for this period. Light treatments, however, demonstrated trends for lower ^{13}C enrichment of CO_2 during the first 2 h of the photoperiod. This could be explained by a major contribution of roots to CO_2 efflux after propagation of pressure/concentration waves, which deliver unlabeled C (Fig. 4b). Exudation of unlabeled organics from C4 maize roots subsequently increased the δ^{13}C of the solution in light treatments, whereas in the dark the δ^{13}C of the solution was more ^{13}C depleted (Fig. 4a insert). In the same timeframe, δ^{13}C of CO_2 from the light-exposed plants started to deviate from those in the dark, reaching maximum differences at the last sampling; this confirms the major contribution of unlabeled exudates to flux of microbial CO_2 in light-exposed treatments.

Thirdly, roots can uptake low molecular weight organics from solution (Jones and Darrah 1992; Näsholm et al. 1998; Macek et al. 2000; Biernath et al. 2008). If maize took up part of the [^{13}C] glucose together with the exuded organics, this should be reflected in the plant biomass ^{13}C signature. Glucose uptake was confirmed by an increase in δ^{13}C of root biomass in all treatments (Fig. 5). In contrast to the results of Biernath et al. (2008) and Jones et al. (2013) obtained on soil-grown maize and wheat, we did not detect any significant ^{13}C amount in shoots. The duration of the *Solution Labeling* experiment could also be too short to ensure sufficient upward transfer (Jones et al. 2013). Root enrichment and the amount of taken up organics were similar between light and dark plants, so that these factors cannot explain the OC patterns in the solution.

The remaining, fourth, explanation of the observed OC patterns in solution is related to the mechanism involved in linking aboveground-belowground activities. The propagation of pressure/concentration waves or any other mechanism of fast coupling of aboveground and belowground C fluxes did not affect the exudation activity to a detectable level. Consequently, microbial activity in the rhizosphere remained constant in the absence of additional C supply, and hypothesis 4 was not confirmed. As discussed above, the OC in the solution started to increase later, 3.5–6 h into the photoperiod, when the newly assimilated C was delivered belowground with the phloem flow (as demonstrated by the *Shoot Labeling* experiment). The δ^{13}C increase in solution and decrease in CO_2 at the last sampling supports this conclusion, indicating a major availability of easily decomposable subtstrates in the solution under the light-exposed plants. Diurnal exudation dynamics have been confirmed for maize (Kuzyakov et al. 2003) and wheat (Oburger et al. 2014). The absence of an exudation response to carbohydrate delivery induced by wave propagation is quite surprising and could be explained by endogenous regulation of root exudation activity (see above). This lack of a response may also raise questions about the nature of fast aboveground-belowground coupling. We did not measure direct changes of pressure or concentration in the phloem, but focused on the expected consequences of the wave propagation on belowground processes. One of those consequences, an increase of exudation, was not confirmed. These results call for further experiments designed to identify the nature of fast mechanisms of above-belowground coupling.

The *Solution Labeling* experiment was too short to verify how long and to what extent respiration and exudation will increase in response to photoassimilates allocation belowground. $^{14}CO_2$ release kinetics in *Shoot Labeling* was used to quantify the C amount respired in *Solution Labeling* during the first hours not covered by sampling. The results of *Shoot Labeling* demonstrated that it takes 12 h for $^{14}CO_2$ label to reach solution and to peak in root-derived respiration. Total CO_2 efflux also steadily increased in this experiment over 9 h of light exposure (Fig. 1b, dashed line). Accordingly, we assume that CO_2 efflux from the nutrient solution in S*olution Labeling* will to continue to increase after the last sampling (Fig. 6). Root-derived ^{14}C respiration reached 46 % of its maximum 6 h after assimilation in *Shoot Labeling*, assuming linear increase in ^{14}C between the successive samplings. This time corresponds to the last sampling in S*olution Labeling*. Accordingly, the expected day-time respiration maximum in *Solution Labeling,* 12 h into the photoperiod, could be obtained based on a simple proportional approach (Fig. 6). The respiration minimum in the following night is assumed to be equal to the flux rate measured at the end of the common Night period. This approach enables us to estimate the amount of C evolved by respiration flux during the whole day until the next considerable perturbation in water potential equilibrium (i.e., capable of inducing another transmission of pressure/concentration wave). This estimation is preliminary and subject to potential errors because there were no direct measurements performed after 6 h of light exposure in the *Solution Labeling* experiment. The contribution of the fast transport, whose effect is seen 2 h after switching on the light, corresponds to ~5 % of the total C evolved in 24 h and, as discussed above, this wave-delivered C is mainly of root origin. The other 95 % is respired after the start of the delivery of the first assimilates with the phloem flow. Microbial respiration, being driven by root exudation stimulation, contributes to respiration increase at this stage.

We caution that our estimation of the pressure/concentration wave contribution to the root-derived CO_2 is preliminary. Pressure waves and mass transfer of assimilates both function through the same path (phloem) and involve the same substrate (photoassimilates) to affect root metabolism. There are probably no sharp time boundaries between when the pressure/concentration wave stops and when the release of phloem-transported assimilates starts and ends. Therefore a 5 % contribution may be a minimum estimation of pressure wave contribution, if effects persist after delivery of new photoassimilates to roots.

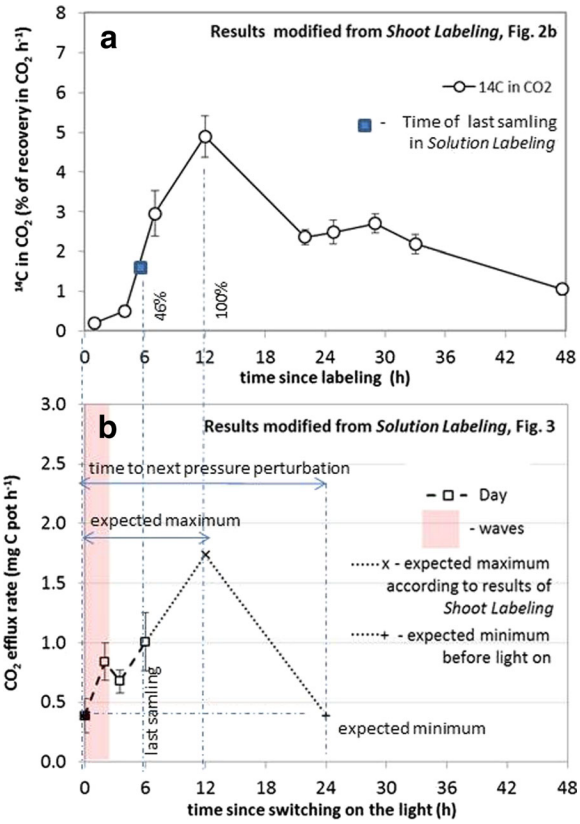

Fig. 6 Conceptual model and calculation of the contribution of pressure/concentration wave to the total light-induced CO_2 increase. Figure 6a is modified after Figs. 1b and 6b is modified after Fig. 2 (Day treatment results). Red area shows the CO_2 efflux increase induced by propagation of pressure/concentration waves (~ 5 % of total CO_2). The time of the expected respiration maximum in *Solution Labeling* (Fig. 6b) was determined by the time of the respiration peak in *Shoot Labeling* (Fig. 6a). The absolute value of the respiration maximum was quantified taking into account that 46 % of the peak is reached by the time of the last sampling (Fig. 6b). The respiration minimum was fixed at the end of the second experimental night and assumed to be equal to the respiration rates measured during the night period in *Solution Labeling* (Fig. 6b)

Conclusions

We conclude that photosynthesis is one of the main factors driving belowground C turnover and CO_2 efflux from soil, affecting belowground processes by distinct mechanisms with different time-scales. The time lag between photosynthesis and belowground C fluxes varies from minutes to hours (and days), depending on

the mechanism involved in aboveground-belowground coupling. Fast mechanisms of C translocation exist and affect root activity in addition to the slower mechanism (direct phloem transfer of assimilates). The fast translocation (pressure/concentration waves) is reflected by a rapid response of root respiration to photosynthesis changes. The effect of this fast mechanism on the initial respiration intensity is relevant, but amounts to no more than 5 % of the overall diurnal respiration efflux. Accordingly, analyses of drivers of CO_2 efflux from soil, especially on a very short time scales, should include light effects. Root exudation, however, was not (or much less) affected by the fast above-belowground coupling and may be regulated by internal circadian clocks governing the release of photoassimilates, rather than directly by photoperiod. This would imply that rhizosphere microorganisms are less dependent on the dynamics of photoassimilation. The nature of the mechanism of fast aboveground-belowground coupling remains a major uncertainty in the short-term C cycle and calls for further creative experiments.

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