SUPPLEMENT

Natural abundance labelling

The experiment described in the main article is based on a preliminary study, which used natural abundance isotopic variations of $\delta^{13}$CO$_2$ in air ($\delta^{13}$C$_{\text{AIR}}$) to assess the effect of a sudden decrease in air temperature on C transport from above- to belowground in rye-grass.

In the preliminary experiment, the continuous introduction of fresh air into the growth cabinets was suppressed over the entire growing period resulting in a $^{13}$C enrichment of cabinet CO$_2$ due to the highly productive plants growing within them, discriminating against $^{13}$C. High photosynthetic rates changed cabinet air conditions to c. 100 ppm [CO$_2$] and $\delta^{13}$C$_{\text{air}}$ to c. -1‰ which subsequently also changed $\delta^{13}$C values of respiration. Labelling was achieved by re-starting fresh air supply resulting in near-ambient air conditions inside the cabinets with [CO$_2$] of 400 ppm and $\delta^{13}$C$_{\text{air}}$ of c. -9‰.

Fresh air supply started at the same time as the random allocation of plants to one of two cabinets – one with a warm treatment (25°C; equal to the daytime growing conditions; control) and one with a cold treatment (10°C). However, we found that the $\delta^{13}$C signal of shoot respiration was very sensitive to changes in $\delta^{13}$C$_{\text{AIR}}$, impeding the interpretation of the results. In order to overcome the sensitivity of the $\delta^{13}$C signal of shoot respiration to small changes in $\delta^{13}$C$_{\text{AIR}}$, we followed up on those initial results using a $^{13}$C pulse-labelling approach which is described in the main article.

Results

The $\delta^{13}$C of pre-label leaf-respired CO$_2$ was around -11‰ which is much less depleted than usual C$_3$ plant respiration (of around -27‰). Further, $\delta^{13}$C$_{\text{SR}}$ was stable during the diurnal cycle of the pre-label day (data not shown) with only small variations between replicates. However, $\delta^{13}$C$_{\text{SR}}$ was very responsive to the labelling. Immediately after introducing ambient fresh air, shoot respiration was already much more depleted in $^{13}$C (change of c. 3.5‰) compared to the average values during pre-labelling (Fig S1, upper panel). Such short-term high sensitivity of $\delta^{13}$C$_{\text{SR}}$ indicates a very fast utilization of recent photo-assimilates for shoot respiration and demonstrates that $\delta^{13}$C$_{\text{SR}}$ is not suitable to assess, for instance, water-use efficiency at the whole-season field scale, as originally proposed by Barbour et al. (2011).
Within about 7 h, $\delta^{13}$C$_{SR}$ had changed by the full magnitude of the treatment (c. 8‰). There was no difference in the response of $\delta^{13}$C$_{SR}$ between warm and cold treatments. However, after 8 h post-labelling, $\delta^{13}$C$_{SR}$ of the warm treatment increased again by c. 3‰, whereas the cold treatment continued to decline, levelling out at 10.5 h post-labelling. The change of $\delta^{13}$C$_{SR}$ by the full magnitude of the label indicates that the pool of recent photo-assimilates in leaves is quickly turned over.

![Figure S1](image)

Figure S1: Time course of relative change of $\delta^{13}$C in shoot ($\delta^{13}$C$_{SR}$; upper panel) and root ($\delta^{13}$C$_{RR}$; lower panel) respired CO$_2$ during continuous label; control treatment (25 ºC, closed symbols); cold treatment (10 ºC; open symbols); sample allocation to warm and cold cabinets and label start (vertical line). The data shown are means ± standard error (n = 3)

The $\delta^{13}$C signal of root respiration ($\delta^{13}$C$_{RR}$) on the other hand, showed a time-lagged response to the label application with a faster response in the warm (after c. 2 h) compared to the cold
treatment (after c. 5 h; Fig S1, lower panel). Unlike $\delta^{13}C_{SR}$, $\delta^{13}C_{RR}$ of both treatments did not change by the full magnitude of the label, shifting only by 5‰.

**Dark incubation time**

Selection of the dark incubation time was based on diurnal measurements, which showed that $\delta^{13}C_{SR}$ is highly variable after initial placement in the dark, but is stable after 2 h (Fig S2), which was consistent with Barbour et al. (2011)\textsuperscript{1}.

![Figure S2: Rate of change of $\delta^{13}C$ in respiration of shoots and roots ($\delta^{13}C_R$) after harvest. $\delta^{13}C_{SR}$ is highly variable after initial placement in the dark, but is stable after 2 - 2.5 hours. Based on these results, shoot samples were left in the dark for 2 hours, before being incubated (in CO$_2$-free air, also in the dark) for approximately 20 minutes, prior to measurement of $\delta^{13}C_{SR}$. Roots were measured approximately 1 h after harvesting, and showed little variation in $\delta^{13}C_{RR}$ around this time. Note, nearly all values fall within the 1σ standard deviation of the instrument’s target calibration standard (0.12‰).](image)