**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 ($\delta^{13}C_{AIR}$) 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 $\delta^{13}C_{air}$ 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 $\delta^{13}C_{air}$ 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 $\delta^{13}C_{AIR}$, 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 $\delta^{13}C_{AIR,}$ 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, $\delta^{13}C_{SR}$ was stable during the diurnal cycle of the pre-label day (data not shown) with only small variations between replicates. However, $\delta^{13}C_{SR}$ 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 $\delta^{13}C_{SR}$ indicates a very fast utilization of recent photo-assimilates for shoot respiration and demonstrates that $\delta^{13}C_{SR}$ is not suitable to assess, for instance, water-use efficiency at the whole-season field scale, as originally proposed by Barbour *et al*. (2011)[i].

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.

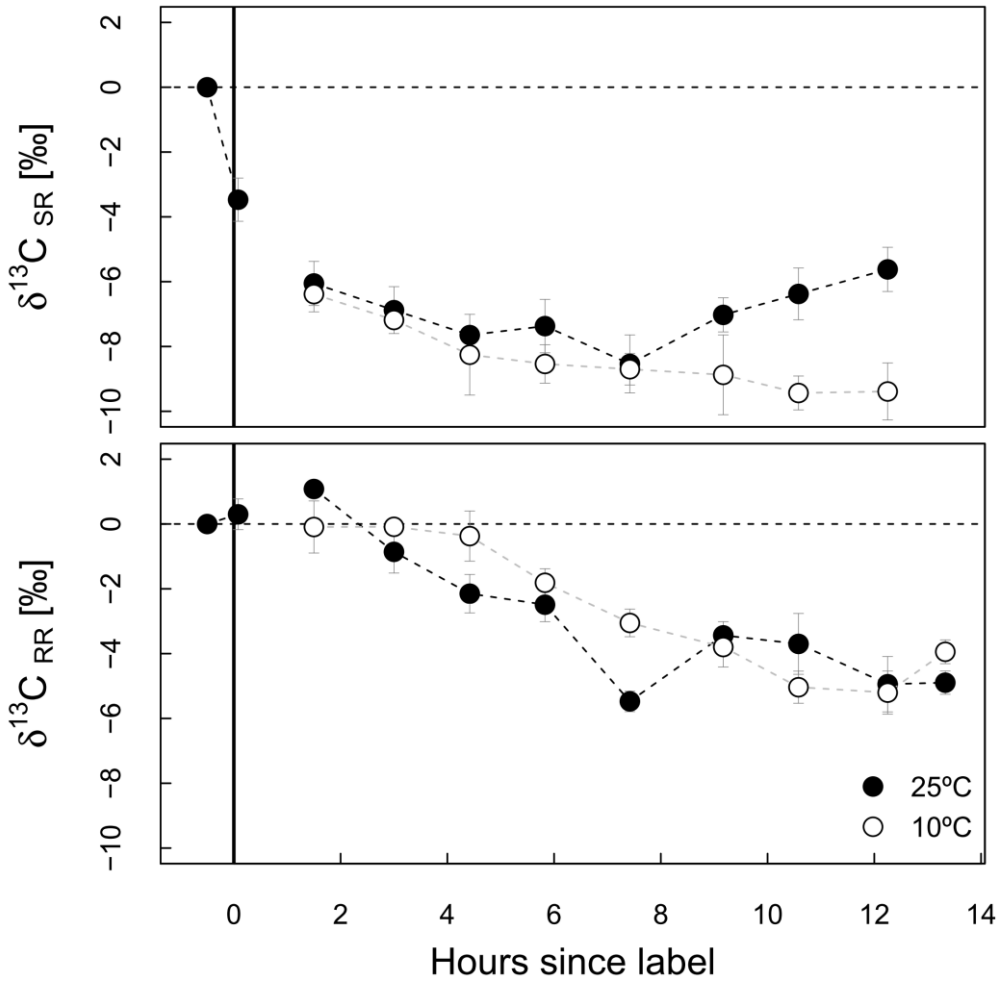


Figure S1: Time course of relative change of $\delta^{13}C$ in shoot ($\delta^{13}C_{SR}$; *upper panel*) and root ($\delta^{13}C_{RR}$; *lower panel*) respired $CO_2$ during continuous label; control treatment (25 ºC, *closed symbols*); cold treatment (10 ºC; *open symbols*); sample allocation to warm 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)[1].

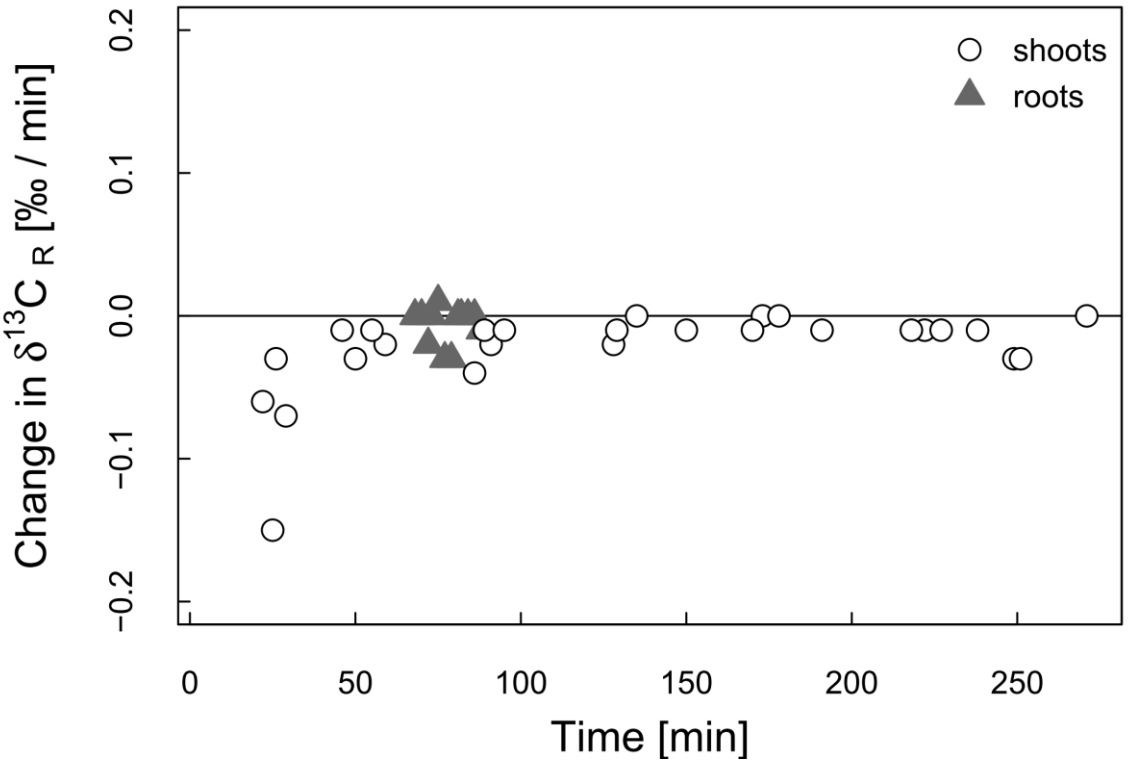


Figure S2: Rate of change of $\delta^{13}C$ in respiration of shoots and roots ($\delta^{13}C_R$) after harvest. $\delta^{13}C_{SR}$ is highly variable after initial placement in the dark, but is stable after 2 - 2.5 hours. Based on these results, shoot samples were left in the dark for 2 hours, before 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‰).

[1i] Barbour, M.M., Tcherkez, G., Bickford, C., Mauve, C., Lamothe, M., Sinton, S., and Brown, H.: $\delta^{13}C$ of leaf-respired $CO_2$ reflects intrinsic water-use efficiency in barley. Plant Cell Environ., 34, 792-799, 2011.