

# Tracing spatial and temporal pattern in metabolic processes by natural isotope composition and compound specific labelling

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We present the first species comparison of spatio-temporal variations of respired  $\delta^{13}\text{CO}_2$  ( $\delta^{13}\text{C}_{\text{res}}$ ) and its putative substrate (water-soluble organic matter, WSOM) of leaves and roots along the plant axis and over the diurnal course using a rapid in-tube incubation technique. Pronounced spatial differences in  $\delta^{13}\text{C}_{\text{res}}$  (up to 10.2‰) between the most enriched sun leaves at the top canopy and the most depleted roots tips were found along the plant axis. Additionally, a diurnal enrichment up to 15.9‰ of leaf respiration above WSOM was found. Furthermore, we found very rapid post-illumination changes in  $\delta^{13}\text{C}_{\text{res}}$ , which generally exhibited a 2 to 5‰ decrease within 30 min of darkness. Interestingly, the magnitude of this decrease exhibited a diurnal cycle. Compound specific labelling with positional labelled pyruvate and theoretical calculations were used to explore different hypotheses for the observed variations in  $\delta^{13}\text{C}_{\text{res}}$  i) changes in signature and pool-size of the putative respiratory substrate, ii) apparent fractionation in the dark respiratory pathways, iii) potential effects of a transient decarboxylation of an enriched malate pool and, iv) Rayleigh fractionation processes of enzymatic reactions in the respiratory pathways on  $\delta^{13}\text{C}_{\text{res}}$ . The marked dynamics in leaf respired  $\delta^{13}\text{C}_{\text{res}}$  both on the spatial and the diurnal scale were independent from the putative respiratory substrates. Positional labelling experiments showed that the observed diurnal enrichment is due to an increase in C flux through pyruvate dehydrogenase PDH over the light period, probably into the secondary metabolism (e.g. isoprene or aromatic compounds), relative to a constant Krebs cycle (KC) activity. In contrast to foliage respiration none of the investigated species displayed distinct diurnal pattern in  $\delta^{13}\text{C}_{\text{res}}$  of roots. However, neither shifts in PDH and KC activity nor decarboxylation of an enriched malate pool can fully explain the diurnal  $\delta^{13}\text{C}_{\text{res}}$  enrichment or post-illumination changes. A theoretical approach indicates that a Rayleigh fractionation process against  $^{13}\text{C}$  of any respiratory enzyme may explain the observed pattern. Further research on the fractionation mechanisms is required, where compound specific labelling provides an efficient method to identify the processes involved in  $\delta^{13}\text{C}$  variation of dark respiration.