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## Linking carbon isotope signatures of nighttime leaf-respiratory and daytime assimilatory CO<sub>2</sub> fluxes observed with laser spectrometry under field conditions

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The  $^{13}\text{C}/^{12}\text{C}$  ratio ( $\delta^{13}\text{C}$ ) of atmospheric CO<sub>2</sub> is a valuable tool for constraining the impact of the terrestrial biosphere on atmospheric CO<sub>2</sub> dynamics. Alterations of the  $^{13}\text{C}$  signal of terrestrial net CO<sub>2</sub> fluxes are generally attributed to variations in photosynthetic  $^{13}\text{C}$  discrimination. Yet, over the past decade, evidence has emerged that plant metabolism and respiration modify the initial  $\delta^{13}\text{C}$  signature of recent photosynthetic assimilates. Such post-photosynthetic  $\delta^{13}\text{C}$  modifications were reported for all plant organs, but leaf respiratory metabolism may play a central role as it impacts carbon turnover in other plant tissues. Leaf-respired CO<sub>2</sub> is frequently  $^{13}\text{C}$  enriched with respect to leaf organic matter. Mechanisms potentially explaining this enrichment include the differential use of carbon sources, metabolite fragmentation or the expression of kinetic isotope effects of respiratory enzymes. For global and ecosystem-scale applications of  $\delta^{13}\text{C}$ , it is now important to study, under field conditions, the variability of  $\delta^{13}\text{C}$  in leaf-respired CO<sub>2</sub> ( $\delta^{13}\text{C}_{RES}$ ) and the deviation of the latter from  $\delta^{13}\text{C}$  of recent assimilates ( $\delta^{13}\text{C}_{AS}$ ). Here, we present 74 days of hourly  $\delta^{13}\text{C}$  measurements for daytime assimilatory and nighttime respiratory CO<sub>2</sub> fluxes on leafy branches of three mature *Fagus sylvatica* trees in a temperate forest. Measurements were conducted with a laser spectrometer (QCLAS-ISO, Aerodyne Research Inc.) measuring CO<sub>2</sub> isotopologue mixing ratios in ambient and sampling air from photosynthetic gas exchange chambers. We used daytime measurements of photosynthetic  $^{13}\text{C}$  discrimination for diurnally flux-weighted estimates of  $\delta^{13}\text{C}_{AS}$ , and found that flux-weighted  $\delta^{13}\text{C}_{RES}$  roughly tracked previous-day shifts in  $\delta^{13}\text{C}_{AS}$ . Deviations between flux-weighted  $\delta^{13}\text{C}_{AS}$  and  $\delta^{13}\text{C}_{RES}$  were further robustly predicted by previous-day assimilation, with  $\delta^{13}\text{C}_{RES}$  displaying  $^{13}\text{C}$  enrichment on low and  $^{13}\text{C}$  depletion on high assimilation days. On the hourly timescale,  $\delta^{13}\text{C}_{RES}$  either significantly decreased by more than 0.2‰ hr<sup>-1</sup> or remained relatively stable during one third of all nights each. For the other third of all nights, observed  $\delta^{13}\text{C}_{RES}$  patterns were highly variable. These nighttime trends were not related to any monitored environmental (e.g. leaf temperature) or physiological (e.g. previous-day assimilation) variable, nor to trend measures of the respective variables. Given that nighttime leaf respiration is fully fuelled by starch accumulated during the previous day, we simulated daytime synthesis and nighttime degradation of layered starch granules, in which  $^{13}\text{C}$  signal and layer thickness depended on daytime-measured  $\delta^{13}\text{C}_{AS}$  and assimilation strength. Albeit disregarding any potential metabolic and respiratory modification of the  $\delta^{13}\text{C}_{AS}$  signature, the simulation frequently produced  $\delta^{13}\text{C}_{RES}$  patterns similar to the ones measured. In conclusion, the results indicate that the observed  $\delta^{13}\text{C}_{RES}$  variability on hourly timescales probably originated in leaf catabolic processes, or it could also reflect hourly variability of previous-day photosynthetic  $^{13}\text{C}$  discrimination. The relationship between flux-weighted means of  $\delta^{13}\text{C}_{AS}$  and  $\delta^{13}\text{C}_{RES}$  asserted the strong link between assimilatory and respiratory  $^{13}\text{C}$  signals reported in several ecosystem studies.