

# **Dynamics in respiratory $\delta^{13}\text{CO}_2$ of ecosystem components (soil, roots, and leaves) reveal insights into the regulation of respiratory metabolism and carbon allocation**

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A thorough understanding of processes driving respiration and the metabolism of respiratory compounds is nowadays of major interest. Pronounced short-term dynamics in isotopic composition of respired  $\text{CO}_2$  ( $\delta^{13}\text{C}_{\text{res}}$ ) of ecosystems, soils, roots and foliage have been observed and a number of hypotheses were proposed, such as changes in photosynthetic discrimination, respiratory substrates, and post-photosynthetic discrimination processes due to the interplay of different respiratory pathways, carbon deviation into secondary metabolism and carbon translocation between plant organs. We used a straight-forward Intube-incubation approach, enabling high-time resolved measurements of respiratory  $\delta^{13}\text{C}$  of all major ecosystem components (roots, soils, foliage) to disentangle drought-induced variation in respiratory metabolism. Soil respiration exhibited a depleted isotopic signature and no marked seasonal variations, similar to ecosystem respired  $\text{CO}_2$  ( $\delta^{13}\text{C}_{\text{R}}$ ), providing evidence for a stable carbon-source and minor influence of recent photosynthate from plants. By contrast, we found large short-term variations in  $\delta^{13}\text{C}_{\text{res}}$  from foliage and roots in response to decreasing water availability. While foliage respiration exhibited diurnal enrichment (up to 6‰) and depletion during night,  $\delta^{13}\text{C}_{\text{res}}$  from roots exhibited an opposite pattern with enrichment at nighttime (up to 5.5‰). This effect became more pronounced with increasing drought. These new findings are in accordance with recent theories regarding post-photosynthetic fractionation in the dark respiratory pathways and during phloem loading, which can largely affect  $\delta^{13}\text{C}_{\text{R}}$ . Isotopic composition of root respired  $\text{CO}_2$  further indicated a drought-induced relocation of fresh assimilates to roots. Controlled experiments on the mechanism of short-term variations in  $\delta^{13}\text{C}_{\text{res}}$ , by combining real-time gas exchange measurements, mass spectrometry and positional labeling (pyruvate), revealed the origin behind these rapid dynamics. The diurnal enrichment in foliage respiration (up to 10‰) depended on species, growth status and leaf structure, while roots showed rather constant  $\delta^{13}\text{C}_{\text{res}}$ . We found that the isotopic pattern in foliage and root respired  $\text{CO}_2$  are governed by photosynthetic discrimination and temporal changes in carbon deviation into secondary metabolism. Under drought however, these patterns change substantially, probably involving fractionation during carbon transport and substrate changes. Our results contribute to a process-based understanding of the regulation of respiratory metabolism and isotopic variation in ecosystem respired  $\text{CO}_2$ .