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Experimental evidence for diel variations of the carbon isotope composition in leaf, stem and phloem sap organic matter in *Ricinus communis*

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ABSTRACT

Carbon isotope fractionation in metabolic processes following carboxylation of ribulose-1,5-bisphosphate (RuBP) is not as well described as the discrimination during photosynthetic CO_2 fixation. However, post-carboxylation fractionation can influence the diel variation of $\delta^{13}C$ of leaf-exported organic matter and can cause inter-organ differences in $\delta^{13}C$. To obtain a more mechanistic understanding of post-carboxylation modification of the isotopic signal as governed by physiological and environmental controls, we combined the modelling approach of Tcherkez *et al*., which describes the isotopic fractionation in primary metabolism with the experimental determination of $\delta^{13}C$ in leaf and phloem sap and root carbon pools during a full diel course. There was a strong diel variation of leaf water-soluble organic matter and phloem sap sugars with relatively ^{13}C depleted carbon produced and exported during the day and enriched carbon during the night. The isotopic modelling approach reproduces the experimentally determined day–night differences in $\delta^{13}C$ of leaf-exported carbon in *Ricinus communis*. These findings support the idea that patterns of transitory starch accumulation and remobilization govern the diel rhythm of $\delta^{13}C$ in organic matter exported by leaves. Integrated over the whole 24 h day, leaf-exported carbon was enriched in ^{13}C as compared with the primary assimilates. This may contribute to the well-known – yet poorly explained – relative ^{13}C depletion of autotrophic organs compared with other plant parts. We thus emphasize the need to consider post-carboxylation fractionations for studies that use $\delta^{13}C$ for assessing environmental effects like water availability on ratio of mole fractions of CO_2 inside and outside the leaf (e.g. tree ring studies), or for partitioning of CO_2 fluxes at the ecosystem level.

Key-words: isotope modelling; post-carboxylation fractionation; starch; transport.

INTRODUCTION

Whereas carbon isotope discrimination during photosynthetic CO_2 fixation is a comparatively well-described and understood phenomenon (Farquhar, O'Leary & Berry 1982; Farquhar, Ehleringer & Hubick 1989), much less is known about the isotopic fractionation associated with the metabolic processes following carboxylation in leaf tissues (Hobbie & Werner 2004; Badeck *et al.* 2005; Brandes *et al.* 2006). However, fractionations because of equilibrium, kinetic and fragmentation (Tcherkez *et al.* 2004) isotope effects beyond CO_2 diffusion and fixation by ribulose 1·5-bisphosphate carboxylase/oxygenase (Rubisco) are of importance because they result in differences in isotopic signatures among metabolites and in non-statistical intramolecular isotope distributions (Schmidt & Gleixner 1998; Schmidt 2003; Tcherkez & Farquhar 2005).

Among the most obvious consequences of these effects is that the carbon isotope composition of organic matter may differ between plant organs depending on the $\delta^{13}C$ of exported and non-exported compounds. Badeck *et al.* (2005) reviewed more than 80 publications for differences in $\delta^{13}C$ between organs and showed that heterotrophic tissues are generally enriched in ^{13}C compared wirht autotrophic organs. As temporal variations in photosynthetic discrimination were excluded as an explanation of inter-organ differences, there must be either post-carboxylation fractionation in autotrophic tissues and export of ^{13}C-enriched metabolites across organ boundaries (Hobbie & Werner 2004) or fractionation during heterotrophic metabolism (Helle & Schleser 2004), or both (Brandes *et al.* 2006).

Post-carboxylation carbon isotope fractionation might account for diel variations in the isotopic composition of carbon exported from the leaves to heterotrophic tissues

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