Nitrogen nutrition effects on $\delta^{13}C$ of plant respired CO_2 are mostly caused by concurrent changes in organic acid utilization and remobilization

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Nitrogen (N) nutrition impacts on primary carbon metabolism and can lead to changes in $\delta^{13}C$ of respired CO_2. However, uncertainty remains as to whether (i) the effect of N nutrition is observed in all species, (ii) N source also impacts on respired CO_2 in roots and (iii) a metabolic model can be constructed to predict $\delta^{13}C$ of respired CO_2 under different N sources. Here, we carried out isotopic measurements of respired CO_2 and various metabolites using two species (spinach, French bean) grown under different NH_4^+:NO_3^- ratios. Both species showed a similar pattern, with a progressive ^{13}C-depletion in leaf-respired CO_2 as the ammonium proportion increased, while $\delta^{13}C$ in root-respired CO_2 showed little change. Supervised multivariate analysis showed that $\delta^{13}C$ in respired CO_2 was mostly determined by organic acid (malate, citrate) metabolism, in both leaves and roots. We then took advantage of non-stationary, two-pool modelling that explained 73% of variance in $\delta^{13}C$ in respired CO_2. It demonstrates the critical role of the balance between the utilization of respiratory intermediates and the remobilization of stored organic acids, regardless of anaplerotic bicarbonate fixation by phosphoenolpyruvate carboxylase and the organ considered.

Looking ahead, we also recognize that our modelling exercise disregarded intramolecular isotope compositions. There are important $\delta^{13}C$ differences between C-atom positions, including those in organic acids. Unfortunately, there is presently no method implementable routinely to analyze $\delta^{13}C$ of organic acids. Typically, using quantitative ^{13}C-NMR would require sample preparation to convert COOH groups to their reduced forms (-CH_2OH), break molecular symmetry (for example, in the case of citrate) and block configuration changes and equilibria. To our knowledge, no such method has been published yet. We are trying to deal with this technical challenge which will be the focus of future research aimed at refining our understanding of the isotope signature of CO_2 generated by plant respiration.