## Does *p*CO<sub>2</sub> affect carbon isotope discrimination on evolutionary timescales?

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Annual tree-ring datasets and growth chamber experiments suggest that the concentration of carbon dioxide in the atmosphere  $(pCO_2)$  have an important effect on the carbon isotope fractionation between plant tissue and the atmosphere ( $\Delta^{13}$ C). This effect has been identified in Quaternary-aged speleothems and bulk organic matter and within carbon isotope excursion events such as the Paleocene-Eocene Thermal Maximum. Identification of this effect, however, on longer timescales has proven difficult. In order to test whether changes in  $pCO_2$  affect carbon isotope discrimination over evolutionary timescales, we compiled >2000  $\delta^{13}$ C values measured on plant tissues, bulk organic matter, and specific organic compounds (e.g., n-alkanes and n-alkanoic acids) spanning the last 65 million years. We derive  $\Delta^{13}C$  using the Cenozoic atmospheric  $\delta^{13}C$  values and our compiled dataset, and show that  $\Delta^{13}C$  follows pattern of  $pCO_2$  based on several other proxies. This is consistent with the fundamental photorespiration effect on  $\Delta^{13}C$  observed in field experiments and growth chambers. Therefore, we reconstruct  $pCO_2$  for the last 65 million years using the new C<sub>3</sub> plant pCO<sub>2</sub> proxy approach and assess the uncertainties using Monte Carlo error propagation. The data suggest elevated pCO<sub>2</sub> during the early Eocene climate optimum (~52-50 Ma) and the Miocene Climate Optimum (~17-15 Ma), and a decline in  $pCO_2$  from early Pliocene into the onset of Pleistocene glacial-interglacial cycles (5-1 Ma). The reconstruction also resolves the  $pCO_2$  oscillation between ~150 and 300 ppmv during the late Pleistocene (1 - 0 Ma), consistent with the icecore  $pCO_2$  records. These results suggest the need to account for the underlying effect of  $pCO_2$ when intereting  $\Delta^{13}$ C across evolutionary timescales.