Starch Carbon Isotope Ratios for Comparing the Strength of CAM Photosynthesis

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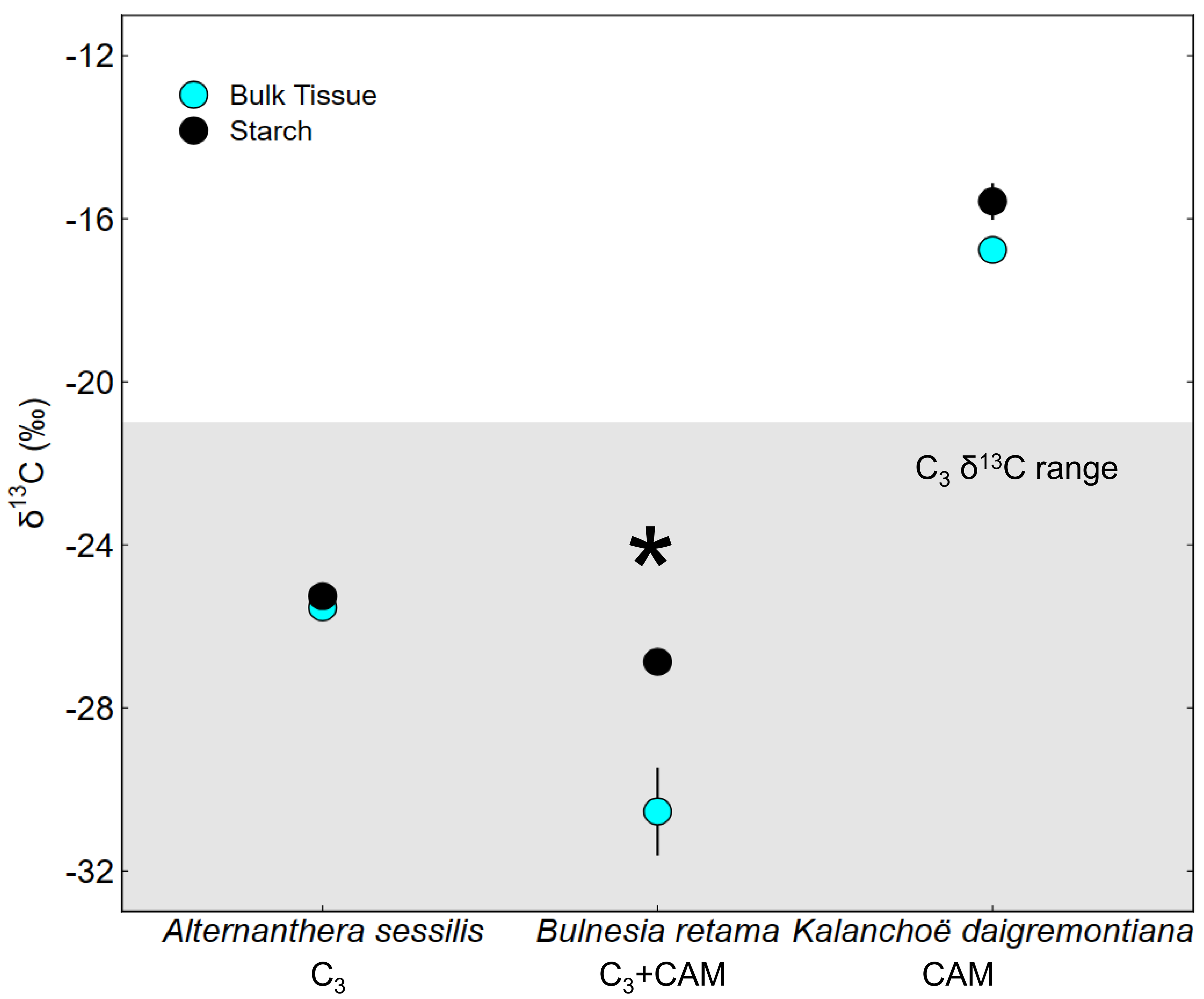
Introduction

- Climate warming and atmospheric CO_2 enrichment provide a competitive advantage to plants that do not invest resources in a carbon concentrating mechanism (CCM) to reduce photosynthetic inhibition [1].
- Crassulacean acid metabolism (CAM) plants can deactivate their CCMs under high CO_2 concentrations, reducing their fitness in arid environments relative to C_3 photosynthetic plants [1].
- CAM plants' response to global change can be evaluated through comparative analysis of CCM strength via carbon isotope ratios ($\delta^{13}C$) of photosynthetic tissue [2]. Starch pool $\delta^{13}C$ include only recently assimilated carbon and thus more accurately represent CAM activity than bulk tissue $\delta^{13}C$, especially in intermediate C_3+CAM plants [2].
- We assess the efficacy of starch extraction for $\delta^{13}C$ analysis through study of a C_3 plant, an obligate CAM plant, and an intermediate C_3+CAM plant.

Methods

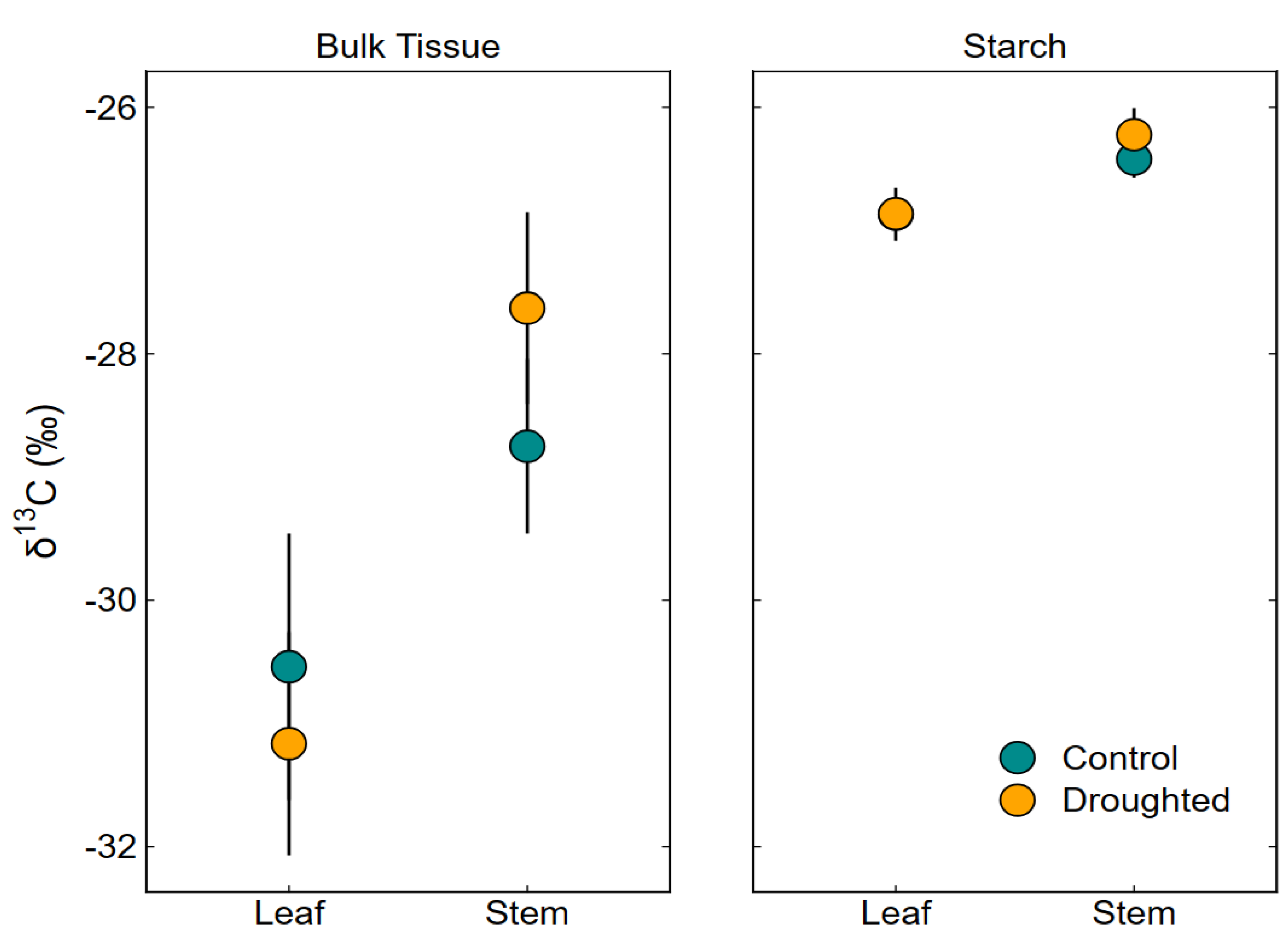
Leaves of the C_3 plant *Alternanthera sessilis*, leaves of the obligate CAM plant *Kalanchoë daigremontiana*, and leaves and stems of the C_3+CAM plant *Bulnesia retama* were sampled in the late afternoon of sunny days [2,3,4]. Samples were dried then treated with methanol and chloroform to isolate soluble starch [2]. Starch was boiled in solution with diH_2O until gelatinized, then treated with α-amylase to convert the starch to sugars [2]. $\delta^{13}C$ of the isolated sugars and of dried bulk tissue from all three species were determined at the Washington State University Stable Isotope Core Laboratory.

Leaf starch and bulk tissue carbon isotope discrimination in C_3, C_3+CAM, and obligate CAM plants



*p-value <0.002 with independent t-test

Bulnesia retama (C_3+CAM) carbon isotope discrimination



Results

- Starch $\delta^{13}C$ are significantly less negative than bulk tissue $\delta^{13}C$ in *Bulnesia retama* (C_3+CAM) leaves and stems.
- There is no significant difference between starch and bulk tissue $\delta^{13}C$ in the leaves of *Alternanthera sessilis* (C_3) and *Kalanchoë daigremontiana* (CAM).

Discussion

- CCMs discriminate less against $^{13}CO_2$ than the C_3 cycle, making $\delta^{13}C$ useful in differentiating between C_3 and CAM plants [4].
  - Strong CAM $\delta^{13}C$ range: [-10‰, -20‰]
  - C_3+CAM $\delta^{13}C$ range: [-20‰, -25‰]
  - C_3 $\delta^{13}C$ range: [-21‰, -32‰]
- The $\delta^{13}C$ difference between bulk leaf and starch samples in *Bulnesia retama* (C_3+CAM) is due to CCM activity in addition to exclusion of post-fixation isotope discrimination in the starch pool [4].
- The small difference between bulk leaf and starch $\delta^{13}C$ in *Kalanchoë daigremontiana* (CAM) can be attributed in part to C_3 cycle activity in CAM photosynthesis phases II and IV [1,unpublished data].
- Starch extraction for $\delta^{13}C$ analysis is a more accurate method for assessing relative CAM strength in C_3+CAM plants than use of bulk tissue, which will be useful in assessing both CAM loss under elevated atmospheric CO_2 and CAM evolutionary intermediacy.

Literature Cited

1. **Ehleringer JR and Osmond CB** (1989) Stable Isotopes. In Pearcy RW, Ehleringer JR, Mooney HA, Runde PW (eds) *Plant Physiological Ecology: Field methods and instrumentation*, pp 281-300. Chapman and Hall, London
2. **Adachi S, Stata M, Martin DG, Cheng S, Liu H, Zhu X-G, Sage RF** (2023) The evolution of C_4 photosynthesis in *Flaveria* (Asteraceae): insights from the *Flaveria linearis* complex. Plant Physiol 191: 233-251
3. **Stata M, Sage TL, Rennie TD, Khoshravesh R, Sultmanis S, Khaikin Y, Ludwig M, Sage RF** (2014) Mesophyll cells of C_4 plants have fewer chloroplasts than those of closely related C_3 plants. Plant Cell Environ 37: 2587-2600
4. **Mok D, Leung A, Searles P, Sage TL, Sage RF** (In Press) CAM photosynthesis in Bulnesia retama (Zygophyllaceae), a non-succulent desert shrub from South America. Ann Bot

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