Testing a Method to Estimate CO_2 Levels in Deep Time

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INTRODUCTION

Paleoclimate proxies have been developed using various biological and geochemical signatures present in the environment to reconstruct past climates and inform our understanding of modern climate change. Among these proxies are methods to target ancient concentrations of atmospheric carbon dioxide (CO_2), a gas crucial to our analysis of climate trends but difficult to trace in the geological record.

A great source of data for paleo-CO_2 proxies is plant material. Plant leaves engage in carbon assimilation, or photosynthesis, as one of their main life processes. The stomatal complexes responsible for leaf gas exchange are extremely sensitive to shifts in CO_2 {Fig. 1}. Among plants used for paleo-CO_2 proxies, Ginkgo trees (*Ginkgo biloba*) have a record of being very accessible and applicable sources of data. The species has predecessors dating back over 200 million years and is also structurally quite similar to these relatives {Fig. 2}. It is likely that modern *Ginkgo biloba* function similarly to their fossilized ancestors and current models of leaf gas exchange can be accurately applied to the ancient specimens.

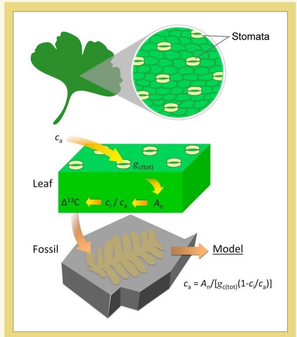


Figure 1. Illustrated Franks model and equation, edited from Franks et al. (2014)

Figure 2. *Ginkgo biloba* on fossilized relative of similar morphology.

A model proposed by Franks et al. (2014) uses the stomatal morphology of *Ginkgo biloba* and other related values to calculate paleo-CO_2 levels {Fig. 1}. This project aims to test the applicability of the Franks method by using a range of elevated CO_2 conditions, a larger sample size, and other varied growth conditions of *Ginkgo biloba* plants to recalculate the input CO_2.

METHODS

- A total of 63 plants, including 15 trees, 20 saplings, and 28 seedlings were grown under varied concentrations of 425 (ambient), 450, 600, 800, and 1000 ppm CO_2 {Fig. 3,4}.
- One leaf was taken from each plant and chemically processed in preparation for SEM imaging {Fig. 5} 5 images of the plants' cells were made per leaf sample.

Figure 3. Layout of plant chambers and CO_2 inputs.

Figure 4. *Ginkgo biloba* tree and younger plants in chamber.

Figure 5. Dried *Ginkgo biloba* leaf and contained samples.

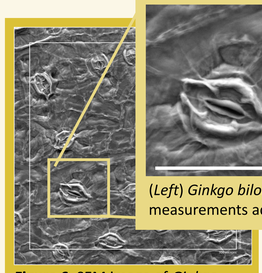
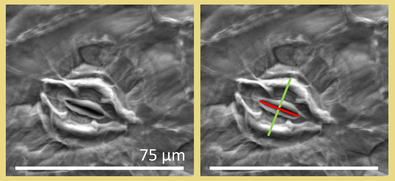


Figure 6. SEM image of *Ginkgo biloba* cells with 300 µm wide box.



(*Left*) *Ginkgo biloba* stoma. (*Right*) Stoma pore and width measurements added in red and green, respectively.

- Stomatal density, pore length, and guard cell width were measured using the Zooniverse platform {Fig. 6}.
- Leaf and air carbon isotope data were also collected at this time.
- Measured data values are input directly into the Franks model along with provided constants and scaling factors to reconstruct CO_2 values {Table 1}.

RESULTS

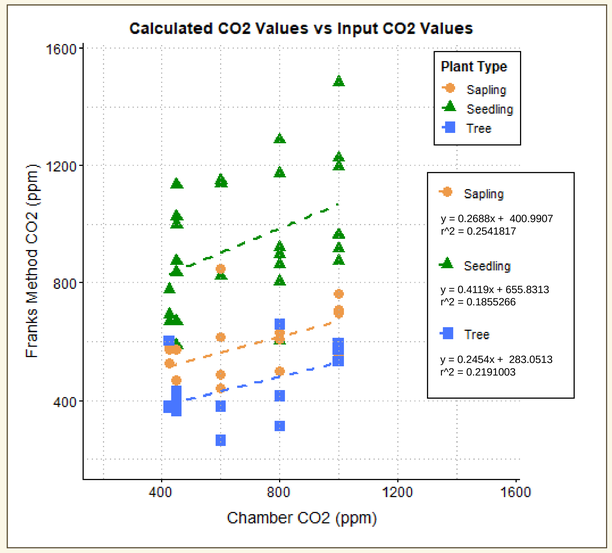


Figure 7. Nominal CO_2 values (ppm) input into chambers plotted versus CO_2 values (ppm) calculated using the Franks method. The data is categorized by plant age group, including trees (blue square), saplings (yellow circle), and seedlings (green triangle).

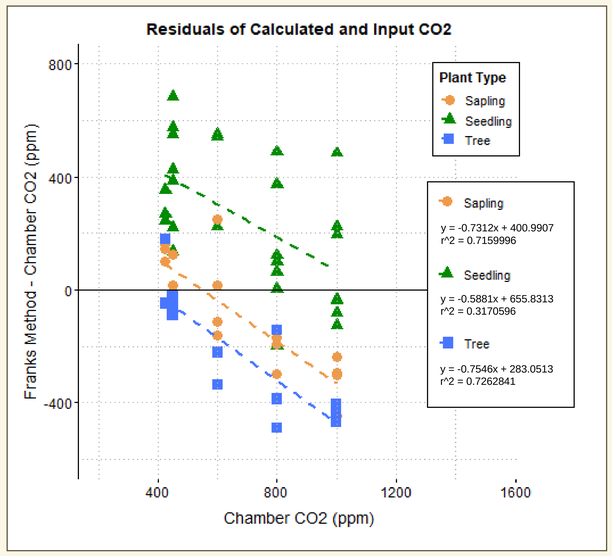


Figure 8. The difference between the calculated and chamber [CO_2] (ppm) plotted to show residual values. A trendline closer to the y = 0 axis displays more similar values to the input chamber [CO_2].

Table 1. Example of values input into the Franks model with added plant type, input [CO_2], and model output [CO_2]. The first 5 samples are included for each plant age: seedlings (green), saplings (yellow), and trees (blue). Standard error was calculated for each model input value.

Image ID	Plant Age	Input [CO_2]	Output [CO_2]	Mean Stomatal Density	Mean Pore Length	Mean Guard Cell Width	Δ13Cleaf	Δ13Cair	$(CO_2)_0$	A_0	g_0	s1	s2	s3	s4	s5
FA21_101L_L1	Seedling	425	822	5.1.E+07	1.6.E-05	2.6.E-05	-35.616	-18.293	418	5.9	2	1	1	0.6	0.21	0.013
FA21_111J_L1	Seedling	450	1484	5.6.E+07	1.2.E-05	2.5.E-05	-45.855	-26.841	418	5.9	2	1	1	0.6	0.21	0.013
FA21_112J_L1	Seedling	600	918	6.4.E+07	1.6.E-05	2.2.E-05	-47.016	-26.841	418	5.9	2	1	1	0.6	0.21	0.013
FA21_113J_L2	Seedling	800	965	6.9.E+07	1.5.E-05	2.6.E-05	-46.884	-26.841	418	5.9	2	1	1	0.6	0.21	0.013
FA21_115J_L1	Seedling	1000	963	6.0.E+07	1.4.E-05	2.3.E-05	-45.710	-26.841	418	5.9	2	1	1	0.6	0.21	0.013
FA21_1901_L1	Sapling	425	849	1.7.E+08	1.0.E-05	2.5.E-05	-38.918	-18.293	418	5.9	2	1	1	0.6	0.21	0.013
FA21_1902_L1	Sapling	450	501	1.5.E+08	1.4.E-05	2.4.E-05	-42.491	-23.636	418	5.9	2	1	1	0.6	0.21	0.013
FA21_1903_L1	Sapling	600	572	1.2.E+08	1.3.E-05	2.3.E-05	-29.694	-11.169	418	5.9	2	1	1	0.6	0.21	0.013
FA21_1904_L1	Sapling	800	699	1.7.E+08	1.3.E-05	2.4.E-05	-48.113	-26.841	418	5.9	2	1	1	0.6	0.21	0.013
FA21_1905_L1	Sapling	1000	764	1.6.E+08	1.2.E-05	2.3.E-05	-48.106	-26.841	418	5.9	2	1	1	0.6	0.21	0.013
FA21_T10_L1	Tree	425	379	2.0.E+08	1.0.E-05	2.0.E-05	-33.945	-18.293	418	5.9	2	1	1	0.6	0.21	0.013
FA21_T12_L1	Tree	450	414	1.6.E+08	1.3.E-05	2.5.E-05	-39.819	-23.636	418	5.9	2	1	1	0.6	0.21	0.013
FA21_T13_L1	Tree	600	376	1.8.E+08	1.6.E-05	2.4.E-05	-28.491	-9.607	418	5.9	2	1	1	0.6	0.21	0.013
FA21_T14_L1	Tree	800	604	1.2.E+08	1.6.E-05	2.4.E-05	-30.229	-9.607	418	5.9	2	1	1	0.6	0.21	0.013
FA21_T15_L1	Tree	1000	380	1.3.E+08	1.6.E-05	2.3.E-05	-26.860	-9.607	418	5.9	2	1	1	0.6	0.21	0.013

CONCLUSIONS & DISCUSSION

- **Our data does not fit the linear regression of the Franks model well, but certain groups such as the saplings have a relatively better fit.**
- **The residual values confirm that accuracy of [CO_2] prediction decreases as the concentration increases.**
- **Grouping by plant age group reveals offset In predicted [CO_2], reaching higher values as plant age decreases.**

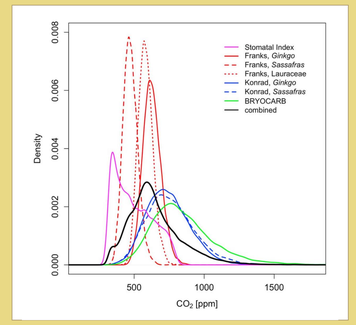


Figure 9. Probability distributions from proxy estimates. The solid black line shows the combined distribution (median = 616 ppm). (Kowalczyk et al. 2018)

The distributions and relationships among the data raise a few questions and potential reasons for why there is not a significant linear fit:

- The base methods of the Franks model are generalized to suit several groups of plants {Fig. 9} and may not be as accurate for ginkgoes.
- Several values in the model are provided constants and scaling factors {Table 1} with presumed rather than measured numerical sources {Fig. 10}.
- The over-predicted CO_2 for the seedlings may in part be caused by differences in cellular morphology {Fig. 11}, such as less stomata, bigger epidermal cells, etc.

Future work and applications:

- Investigating the relationship between age, CO_2 growth conditions, and model effectiveness
- Modifying generic scaling factors using data collected from living plants in the experiment
- Mitigating the differences in stomatal density by using stomatal index, which is less sensitive to cell size increase due to water availability

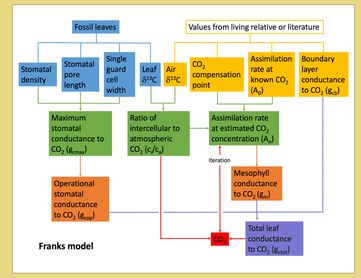


Figure 10. Sources of values for the Franks method with equations summarized and the final end-member being carbon dioxide (CO_2) concentration. (Paleo-CO_2)

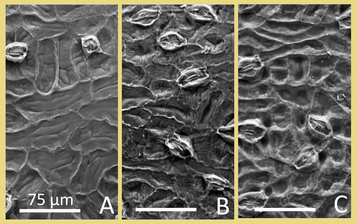


Figure 11. SEM images of *Ginkgo biloba* cells from (A) a seedling, (B) a sapling, and (C) a tree.

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ACKNOWLEDGEMENTS

This research would not have been possible without the generous support of the NHRE program and funding from the NSF (REU Site OCE-1560088). Thank you to Gene, Liz, and Virginia for overseeing the program and my fellow interns for making it a wonderful summer, despite the challenges of a virtual experience. Thank you so much to Matt, Mason, Ben, and Allie for orchestrating much of the data necessary for this project and for aiding my understanding of the topics involved. Lastly, thank you to my wonderful family and friends who endured my poster-making antics, offered creative tips, and gave me emotional support (I'm looking at you, Laika).