

Ginkgo-ing into the Geologic Past: Testing a Proxy for Deep Time CO₂ Levels

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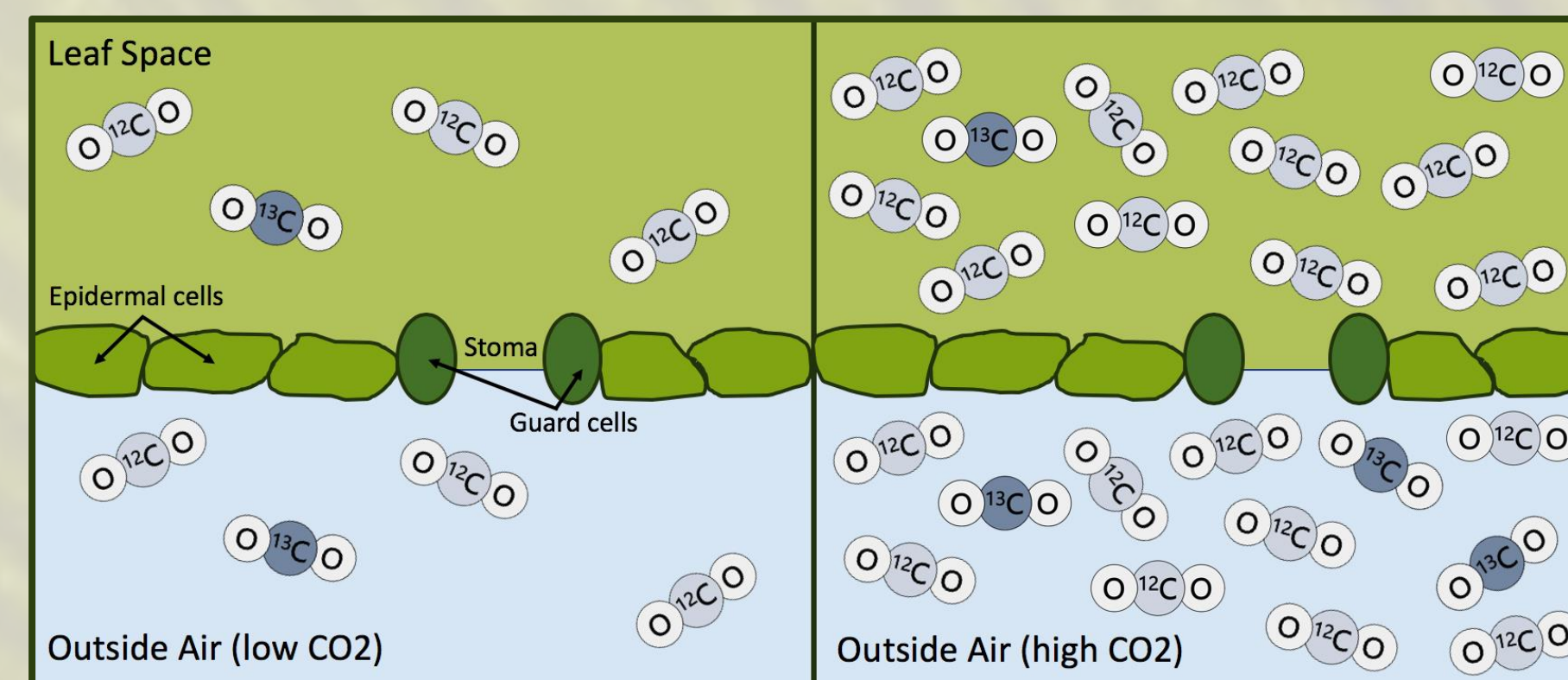
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Introduction

Paleoclimate proxies are used to reconstruct climates of the past, giving us a window into the relationships between the atmosphere, oceans, land, and life through geologic time. Of particular interest, given the current anthropogenic influences on our planet, are CO₂ concentrations. *Ginkgo* trees are a widely used proxy for ancient CO₂ reconstructions. This genus has been living for millions of years, and because they're still alive today, we can carry out experiments to see how elevated levels of CO₂ will change the anatomy and chemistry of living leaves, and then apply these relationships to fossil leaves in order to reconstruct CO₂ levels.

Plants preferentially incorporate ¹²C over ¹³C due to the lighter mass of ¹²C, resulting in the isotopic fractionation of carbon in plant material. Fractionation is reported in delta notation; δ = the ratio of ¹²C/¹³C in the sample divided by the ratio in a standard minus 1. It is expected that leaf carbon fractionation will increase under elevated CO₂ conditions. If so, the difference between leaf δ¹³C and air δ¹³C (termed discrimination, Δ) should increase with increasing concentration of CO₂ (referred to as [CO₂]). In this study, *Ginkgo biloba* trees grown under elevated CO₂ conditions (600, 800, and 1000 ppm CO₂) were used to constrain the relationship between Δ¹³C and [CO₂].

Figure 1. Under low CO₂ conditions, it is expected that discrimination is low. Under high conditions, higher discrimination is expected, leading to a lower internal ratio of ¹³C to ¹²C.



Methods

Ginkgo biloba trees have been grown under ambient (both in chambers and outside) and elevated CO₂ levels (in chambers) for the past 2 years. CO₂ concentration is analyzed every 15 minutes using a LiCOR 7000 gas analyzer. We sampled the CO₂ supply and air near outdoor and chambered trees weekly from leaf break to the end of June (11 weeks). These air samples were analyzed for [CO₂] and isotopic composition at the SIRFER Lab at the University of Utah. Analytical error of gas measurements is 0.2‰. Using the isotopic composition of supplied CO₂ and of ambient air as end-members, we constructed mixing lines to predict the isotopic composition of CO₂ in each of the chambers (Figure 2). (Separate mixing lines were necessary for each week as the isotopic composition of supplied CO₂ is not constant.) We calculated the mean daytime isotopic composition of CO₂ for each chamber from concentration data taken every 15 minutes.

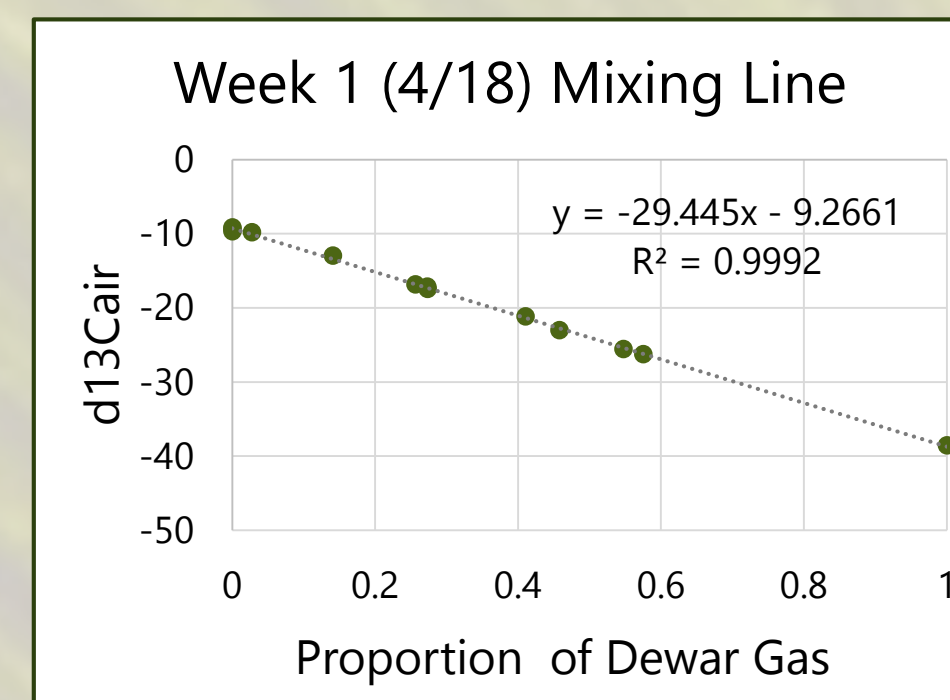


Figure 2. Sample mixing line

Leaves collected weekly through 11 weeks of leaf flush on the North and South sides of the trees were dried, homogenized using a mortar and pestle, and analyzed for δ¹³C at the Smithsonian's Museum Conservation Institute (MCI). All samples were run on a Thermo Delta V Advantage mass spectrometer in continuous flow mode coupled to an Elementar vario ISOTOPE Cube Elemental Analyzer (EA) via a Thermo ConFlo IV. Analytical error of the leaf measurements is ±0.2‰. All calculations of raw isotope values are performed with Isodat 3.0 software. At least one leaf was analyzed from each tree each week. When more than one leaf was analyzed, the average of these values was used as the d13Cleaf for the week. All trees showed declining leaf δ¹³C values from week 1 to week 8 (Figure 5), so we calculated leaf level discrimination from only weeks 8-11 using Equation 1, (Farquhar et al.) We scored the health of trees semi-quantitatively on a three-point scale using leaf color, leaf size, and growth of new shoots.



Figure 3. Week 1 and 5 *Ginkgo* leaves

$$\Delta^{13}C = \frac{\delta^{13}C_{air} - \delta^{13}C_{leaf}}{1 + \delta^{13}C_{leaf}}$$

Equation 1. Leaf discrimination

Results

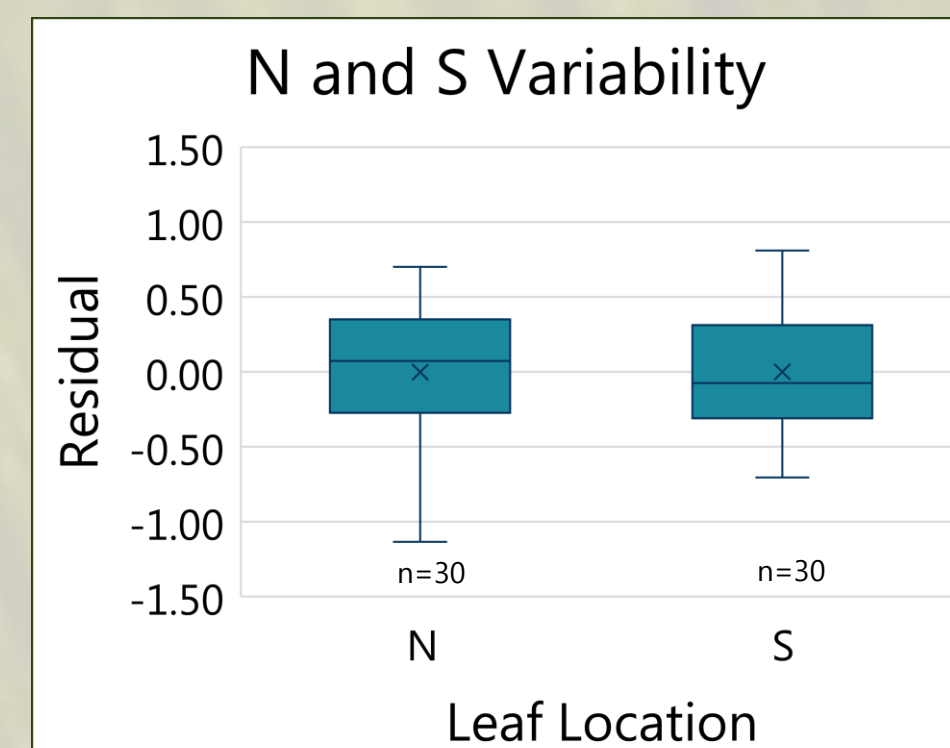
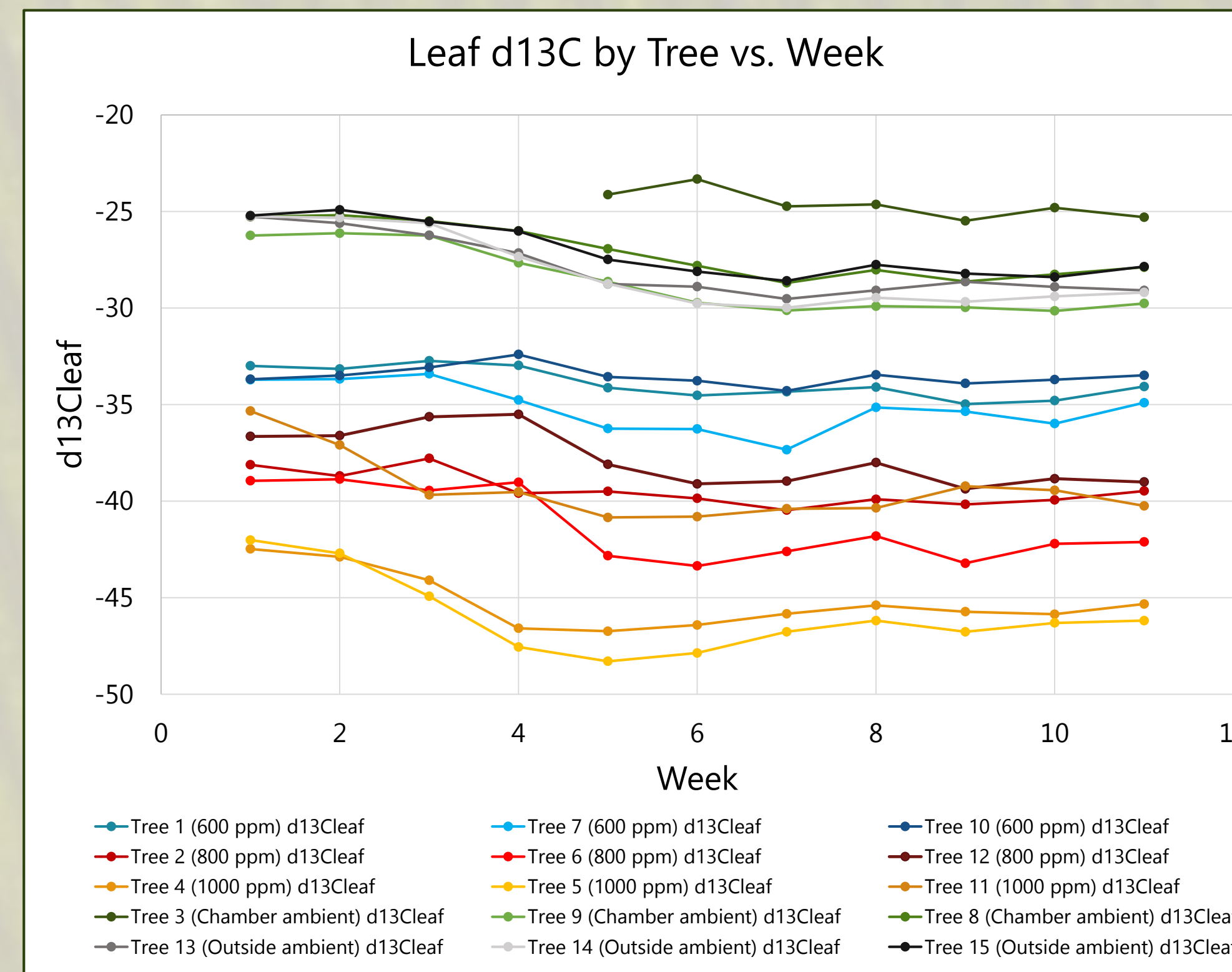
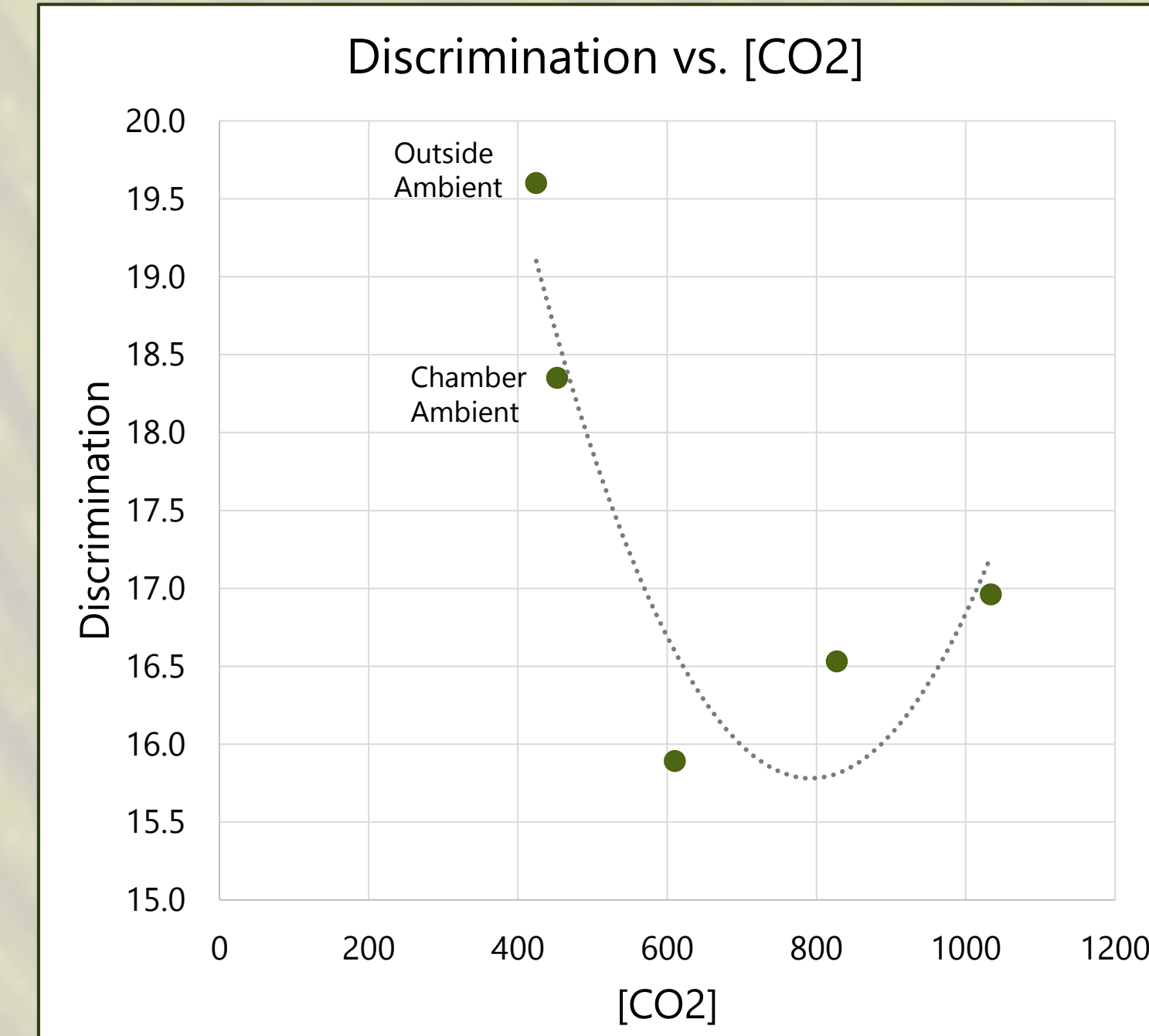


Figure 4. Residuals of North and South leaves taken from the same tree on the same day near the end of leaf flush

Leaf δ¹³C data showed little variability among leaves taken from the same tree, with a maximum standard deviation of 0.82‰ and a minimum of 0.16‰. North and South sides of trees do not have significantly different carbon isotope values (Figure 4), so one leaf adequately represents its tree.



Discrimination showed an interesting relationship with [CO₂] (Figure 6,7). From outdoor ambient ([CO₂]=425 ppm) to chamber ambient trees ([CO₂]=453 ppm), there was about a 1.5‰ decrease in discrimination and a 2.5‰ decrease from chamber ambient trees to 600 ppm trees. This trend then reverses from 600 to 800 ppm trees, where we observed a .5‰ increase, followed by another .5‰ increase from 800 to 1000 ppm trees. The non-monotonic nature of this curve leads us to believe that there is some effect of elevated treatment on discrimination that we haven't accounted for. Likely, the effect is the result of the starch source including carbon fixed from before the trees entered the elevated CO₂ conditions, meaning the elevated CO₂ trees are not yet in equilibrium with the δ¹³C of the elevated CO₂ air.

Figure 6. Average leaf discrimination for each treatment level plotted against actual [CO₂] during the sampling period (11 weeks)

Discussion

In theory, as the concentration of CO₂ in the air increases, the concentration of CO₂ inside the leaf increases if the stomata do not respond by closing. The higher concentration of CO₂ inside the leaf should lead to increased discrimination. Some prior studies have supported this idea; the best evidence for an effect of concentration on discrimination comes from well-controlled growth chamber experiments (Figure 10)². Others have found no effect of concentration on discrimination^{3,4}, and field studies have tended not to show an effect.

Our study has an intermediate level of control. The ginkgo trees are subject to natural variations in temperature, humidity, and precipitation, but CO₂ levels are controlled. It appears in our study that the effect of tree health is more important than that of CO₂ level. The effect of water stress possibly completely masks the effects of CO₂ concentration on discrimination.

Figure 10. Modified from Figure 2, Schubert and Jahren (2012). Shows positive relationship between discrimination and [CO₂]

increases experimentally. The trees in this study were 1.5m tall when they entered the experimental conditions, and already had stored starch reserves from ambient air. Under ambient conditions, this starch would have a δ¹³C in the range of -25‰ to -30‰, which is a heavier value than the carbon fixed under elevated CO₂ conditions. The admixture of this isotopically heavier stored carbon may explain the unexpected sharp decline in Δ¹³C from the ambient chamber trees to the elevated CO₂ trees. Future studies should investigate changes in starch sources and tree health since entering the experiment. Wood samples can be analyzed for starch sources, and past year's leaf δ¹³C values for correlations with health.

Figure 5. d13Cleaf values plotted against time for each tree. Trees under the lowest CO₂ conditions exhibit the heaviest d13Cleaf values, and those under the highest CO₂ conditions exhibit the lightest δ¹³Cleaf values.

Through leaf flush, the δ¹³C of leaves declined by about 2.9‰, on average, before plateauing at about week 6 and becoming quite stable by week 11 (Figure 5). This pattern was found in each treatment level and points to stored starches being mobilized to grow the leaf before it begins to photosynthesize and incorporate atmospheric carbon. Ambient (chamber and outdoor) trees had the heaviest δ¹³C values, followed by 600, 800, and 1000 ppm treatment levels (Figure 5). This is largely the effect of the light CO₂ supplied to the chambers, but may also be affected by the composition of stored starches in the tree.

Figure 8. Box and whisker plot of discrimination against tree health, 3 being the healthiest and 1 the sickest

Tree health also has a large effect on discrimination. The healthiest trees, on average, had a discrimination value about 4‰ above the sickest trees, and 1 ‰ above medium-health trees (Figure 8). Stressed tree values could therefore also be influencing the Discrimination vs. [CO₂] curve (Figure 6).

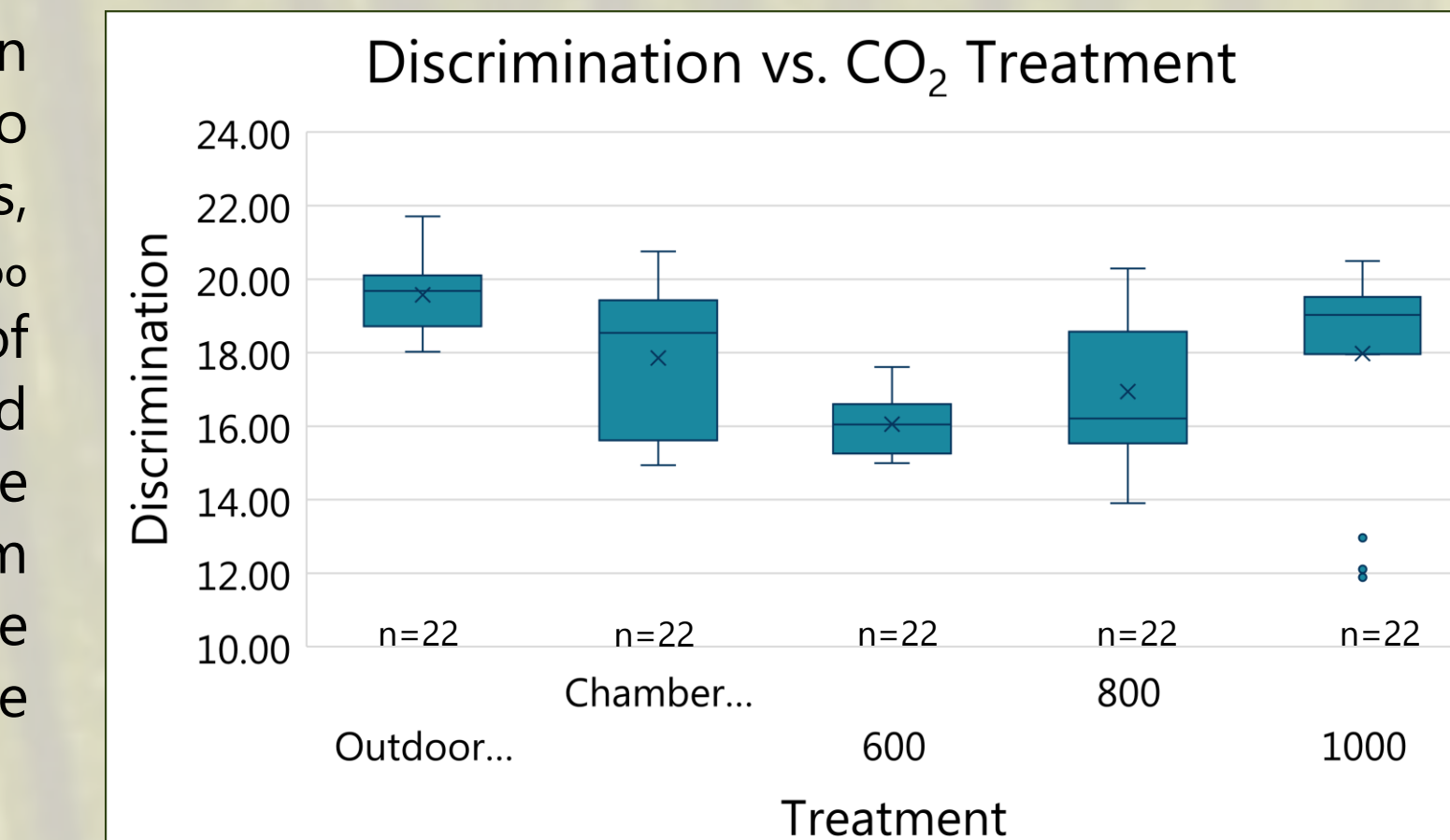
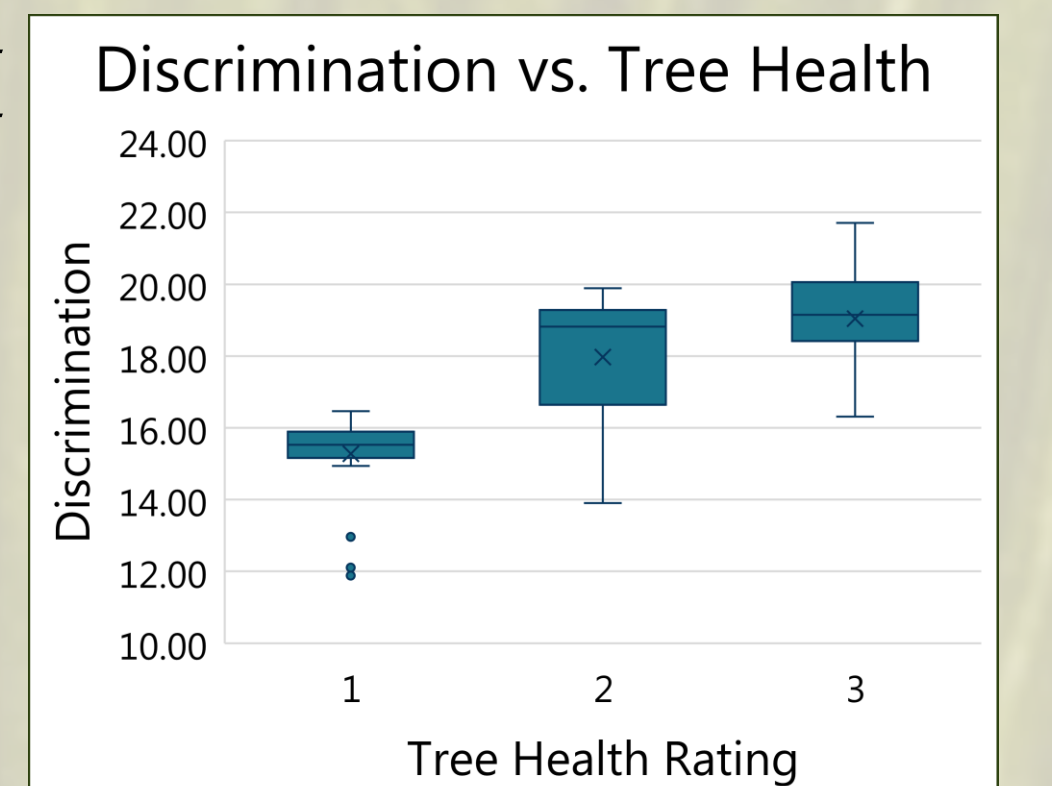


Figure 7. Box and whisker plot of discrimination against treatment level



Figure 9. Leaves at week two. Branches of new growth are tagged with tape on each tree, and sampling locations with aluminum tags.

Conclusions

- Variability in δ¹³C among leaves from the same tree is very low, making one leaf representative of the tree.
- Leaves start out growing from stored carbon sources (starches) that continue to influence the δ¹³C of the leaf through flush.
- The relationship between discrimination and [CO₂] is complicated. Trees at the same treatment level show very different results, correlating with tree health.
- Clarity of the results can be obscured by the composition of stored starches and a lack of equilibrium with the atmospheric δ¹³C. This is an area that requires further research.

Acknowledgements

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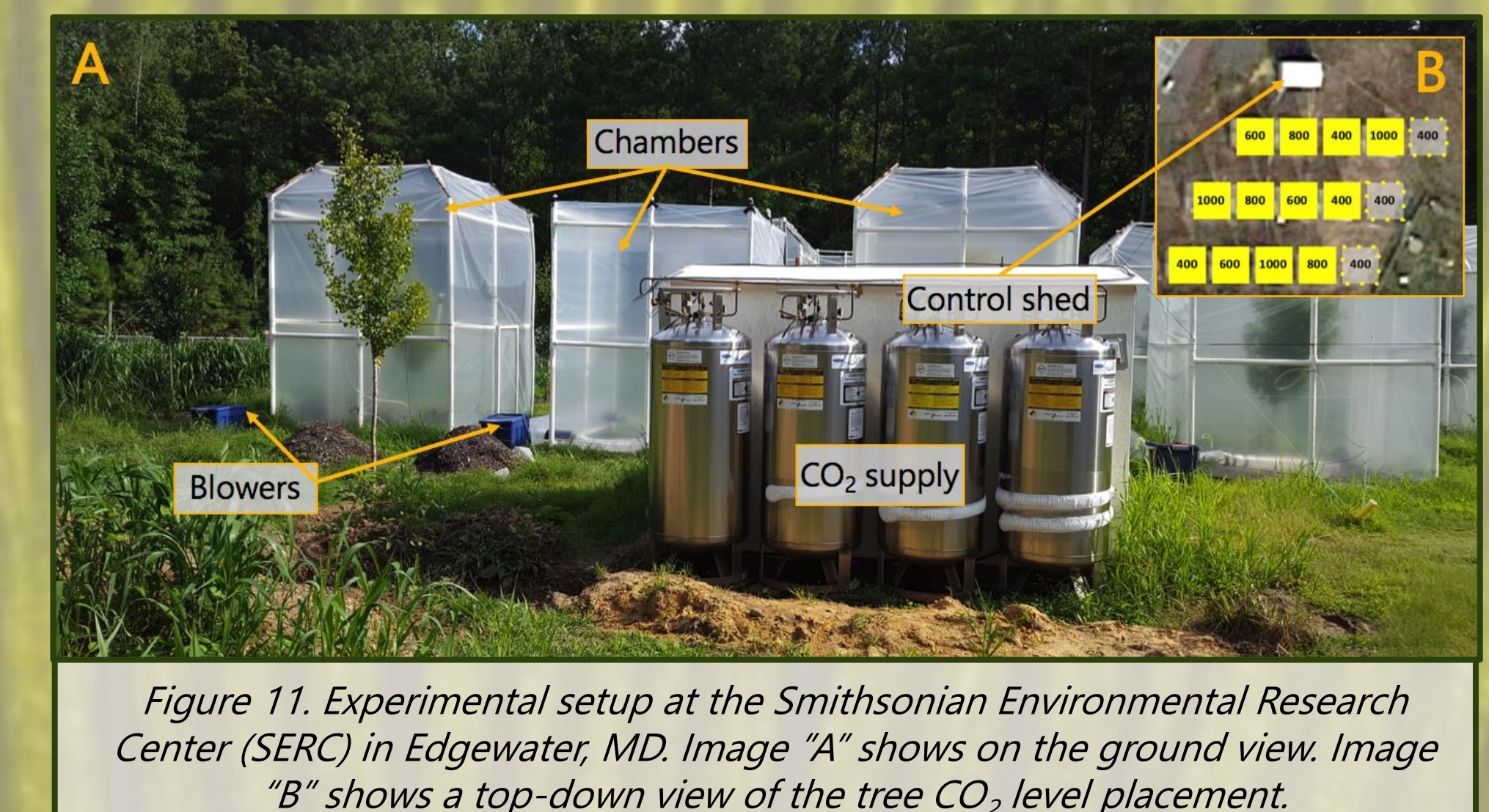


Figure 11. Experimental setup at the Smithsonian Environmental Research Center (SERC) in Edgewater, MD. Image "A" shows on the ground view. Image "B" shows a top-down view of the tree CO₂ level placement.