Introduction
Paleoclimate proxies are used to reconstruct climates of the past, giving us a window into the relationships between the atmosphere, oceans, land, and 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 fossils 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 13C/12C in the sample divided by the ratio in a standard material, expressed as a percentage. δ13C is reported as a standard deviation of 0.82‰.

Methods
Ginkgo biloba trees have been growing under ambient (both in chambers and outside) and elevated CO2 levels (in chambers) for the last 2 years. Concentration is analyzed every 15 minutes using a LICON 7000 gas analyzer. We sampled the CO2 supply and air near outdoor and chamber trees weekly from leaf break to the end of June (11 weeks).

These air samples were analyzed for δ13C and isotopic composition at the SIRFER Lab at the University of Utah. Analytical error of gas measurements is ±0.5‰. 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.

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 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 Confo IV Analytical error of the leaf mass measurements is ±0.06‰. All calculations of raw isotope values are performed with laboratory 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 δ13Cleaf for the week. All trees showed declining leaf δ13C values from week 1 to week 8 (Figure 5), so we calculated leaf discrimination (D), the difference between leaf and air composition 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.

Results
In theory, as the concentration of CO2 in the air increases, the concentration of CO2 inside the leaves will also increase. 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 CO2 concentration on discrimination was reported in controlled growth chamber experiments (Figure 10). Others have found no effect on concentration discrimination and field studies have tended to show the opposite.

Our study has an intermediate level of control. The ginkgo leaves are subject to natural variations in temperature, humidity, and precipitation, but CO2 levels are fixed. The ginkgo trees are subject to control. The ginkgo trees are subject to control. The ginkgo trees are subject to control. The ginkgo trees are subject to control. The ginkgo trees are subject to control.

Discussion
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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 throughout the season.

The relationship between discrimination and CO2 concentration is complex. Trees at the same treatment level exhibit 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.

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