

Carbon starvation in glacial trees recovered from the La Brea tar pits, southern California

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The Rancho La Brea tar pit fossil collection includes *Juniperus* (C_3) wood specimens that ^{14}C date between 7.7 and 55 thousand years (kyr) B.P., providing a constrained record of plant response for southern California during the last glacial period. Atmospheric CO_2 concentration ($[CO_2]$) ranged between 180 and 220 ppm during glacial periods, rose to ≈ 280 ppm before the industrial period, and is currently approaching 380 ppm in the modern atmosphere. Here we report on $\delta^{13}C$ of *Juniperus* wood cellulose, and show that glacial and modern trees were operating at similar leaf-intercellular $[CO_2]$ (c_i)/atmospheric $[CO_2]$ (c_a) values. As a result, glacial trees were operating at c_i values much closer to the CO_2 -compensation point for C_3 photosynthesis than modern trees, indicating that glacial trees were undergoing carbon starvation. In addition, we modeled relative humidity by using $\delta^{18}O$ of cellulose from the same *Juniperus* specimens and found that glacial humidity was $\approx 10\%$ higher than that in modern times, indicating that differences in vapor-pressure deficits did not impose additional constrictions on c_i/c_a in the past. By scaling ancient c_i values to plant growth by using modern relationships, we found evidence that C_3 primary productivity was greatly diminished in southern California during the last glacial period.

low CO_2 | paleoclimate | *Juniperus* | c_i/c_a | ancient wood | ancient NPP

The climate of the late Pleistocene involved a series of pronounced glacial/interglacial cycles, with glacial periods characterized by low temperatures and reduced atmospheric CO_2 concentration ($[CO_2]$) (1). During the last glacial period, minimum $[CO_2]$ occurred between 18 and 15 thousand years (kyr) B.P. (radiocarbon age) at values of 180–220 ppm, and modeling efforts suggest that such glacial values were among the lowest that occurred during the evolution of higher land plants (2). Modern plants with the C_3 photosynthetic pathway exhibit major reductions in photosynthesis [by 50–75% (3)] and growth [by 52–92% (4, 5)] at glacial versus modern $[CO_2]$, and may fail to reproduce as a result of carbon limitations (6). These stress responses are due to limiting CO_2 availability, which decreases net photosynthetic rates as a result of reduced CO_2 substrate and increased rates of photorespiration (3). At higher spatial scales, Francois *et al.* (7) modeled global net primary productivity (NPP) between the last glacial maximum and the recent preindustrial period. The authors estimated that NPP values were only 38 gigatonnes (Gt) of C per year during the glacial maximum and increased to 53 Gt of C per year during the preindustrial period. Francois and coworkers attributed much of the reduction in NPP for the last glacial period to the effects of low $[CO_2]$ on vegetation. Furthermore, Harrison and Prentice (8) modeled changes (BIOME4) in global vegetation between the last glacial period and modern times and found that, when climate change only (temperature and precipitation) was considered, the extent of forest cover in temperate, boreal, and, especially, tropical regions was greatly overestimated without the inclusion of low- $[CO_2]$ effects on plant physiology.

It is critical that we understand what effects the low $[CO_2]$ that occurred during the last glacial period had on the physiological responses of actual terrestrial vegetation samples, which will then improve our estimates of ancient primary productivity and biospheric carbon stocks (7–9). If glacial C_3 plants responded to low $[CO_2]$ in a manner similar to modern plants, wide-scale reductions in productivity would have occurred during the last glacial period, particularly in regions that were too cold to support C_4 species, which are highly tolerant of low $[CO_2]$ (6). Therefore, physiological studies of ancient plants are necessary to determine how vegetation responded to stressful periods of low $[CO_2]$ and whether these responses influenced other aspects of ecosystem functioning during the last glacial period.

The Rancho La Brea tar pit fossil collection [George C. Page Museum of La Brea Discoveries, Los Angeles (10)] contains one of the finest bone collections of late Pleistocene mammals of North America. It also includes a very rare series of preserved *Juniperus* (C_3) wood specimens that span a large portion of the last glacial period (7.7 to 55 kyr B.P.). This wood collection provides a spatially constrained record of plant response for the Los Angeles basin and allows comparisons between glacial and modern trees in identifying the effects of low $[CO_2]$ on plant physiology. Recent stable carbon isotope studies with bone collagen extracted from ancient La Brea mammals (11) indicate that C_3 plant species were dominant in animal diets in southern California during the last glacial period. Although low $[CO_2]$ reduces photosynthetic CO_2 uptake in C_3 plants, quantum yield and photorespiration models for CO_2 uptake predict that C_4 plants were still not favored in southern California during the last glacial period because of low growing-season temperatures (12). Thus, we are focusing on the effects of low $[CO_2]$ on C_3 plants (such as woody tree species) for glacial southern California.

Here we compare the physiological (stomatal-regulation) and modeled-growth responses of glacial and modern *Juniperus* trees (C_3) at La Brea by using stable carbon isotope methodologies. We also reconstruct ancient relative humidity levels for southern California by using oxygen isotope measurements (13) with the same wood specimens, which allowed us to predict whether possible changes in vapor-pressure deficit (VPD) may have influenced stomatal regulation during the last glacial period.

Materials and Methods

^{14}C Dating and Wood Identification. The ^{14}C ages of 18 La Brea tar pit wood specimens were determined by dating extracted α -cellulose that was free of asphalt contamination (see purity levels below). One additional sample (not from the tar pits) dated at

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Abbreviations: kyr, thousand years; $[CO_2]$, CO_2 concentration; c_i , leaf-intercellular $[CO_2]$; c_a , atmospheric $[CO_2]$; VPD, vapor-pressure deficit.

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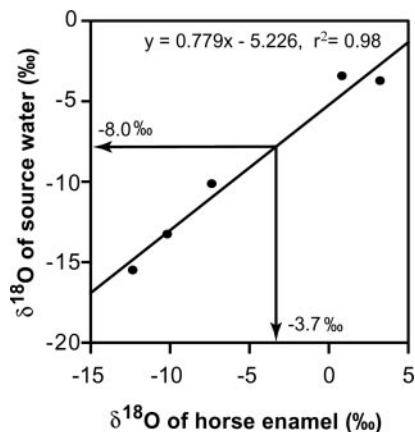


Fig. 1. The modern linear relationship between $\delta^{18}\text{O}$ of source water and horse tooth enamel from a range of locations. This relationship was used to determine the $\delta^{18}\text{O}$ of ancient source water at La Brea from horse tooth enamel recovered from the tar pits (see *Materials and Methods* for details) for purposes of modeling ancient relative humidity. Glacial values for $\delta^{18}\text{O}$ of source water and horse tooth enamel at La Brea are indicated with arrows.

atmospheric humidity was determined from known fractionation factors that occur between liquid and vapor phases of water at the site of evaporation (nearby Pacific Ocean) assuming equilibrium (see ref. 25 for equation). The calculated $\delta^{18}\text{O}$ of atmospheric humidity was determined to be -10.5‰ for modern times and -5.6‰ for the last glacial period. These values are based on sea-surface temperatures of 10°C for modern times and 6°C for the last glacial period (from CLIMAP, National Oceanic and Atmospheric Administration Paleoclimatology Program, Boulder, CO) at 45°N latitude in the vicinity of the Gulf of Alaska, where the majority of storm fronts occurring in southern California originate during the growing season (February values were used). Relative humidity was modeled by using an air temperature of 16°C for modern times and the $7.665\text{ }^{14}\text{C}$ kyr B.P. time point; relative humidity was modeled through the range of $5\text{--}11^\circ\text{C}$ for glacial time points because of variation in temperature estimates for this period. Leaf temperature was assumed to equal air temperature for both ancient and modern samples because of the small size of *Juniperus* leaves, which minimizes potential differences in leaf–air temperatures; barometric pressure was 100 kPa (at sea level), and boundary-layer conductance was $2\text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for both ancient and modern samples (windy coastal conditions).

For validation purposes, we found that modeled estimates of modern relative humidity based on the ^{18}O of modern α -cellulose and the above-mentioned conditions gave a value of 52% .

This value is in close agreement with the long-term average of $52\text{--}64\%$ (varies by location in the city) provided by the National Climatic Data Center (Comparative Climatic Data, National Climatic Data Center, National Oceanic and Atmospheric Administration, 2001, www.ggweather.com/ccd/avgrh.htm) for afternoon conditions in Los Angeles during the month of February between 1960 and 2002.

Results and Discussion

We compared the physiological and modeled growth responses of glacial and modern *Juniperus* trees (C_3) from southern California and reconstructed ancient relative humidity levels to predict whether changes in VPD may have influenced stomatal regulation during the last glacial period.

We found that all sample wood specimens were *Juniperus* sp. based on anatomical features that are unique to the genus (see Fig. 2 for these defining features). Consistent with these identifications, *J. californica* has been shown to be abundant in the La Brea tar pits based on preserved leaf and seed specimens (10). In addition, an abundance of *J. californica* leaves were recovered in wood-rat middens (17.47 kyr B.P.) from nearby northern Baja California (26), and peak levels of juniper/cypress pollen occur in glacial sediment cores from coastal southern California (27). The presence of *J. californica*, in conjunction with other open-woodland conifers, indicates a relatively cooler climate during the last glacial period in comparison with the modern climate of southern California that is dominated by oak woodland and shrub vegetation (27).

We found evidence for severe and sustained carbon starvation in glacial *Juniperus* trees at La Brea. Both Δ and c_i/c_a (Fig. 3) were similar in both modern and full-glacial trees ($P = 0.60$ for Δ , $P = 0.50$ for c_i/c_a), even though atmospheric $[\text{CO}_2]$ reached minimum values during the last glacial period (2). As a result, leaves of full-glacial trees had extremely low calculated c_i values (averaging 113 ppm) that were 25% lower than in leaves of postglacial trees (c_i of 150 ppm between 7.665 and 12.450 kyr B.P.), and 40% lower than in leaves of modern trees (average c_i of 187 ppm , Fig. 4). Glacial c_i values of 113 ppm are unprecedented in modern vegetation and are much closer to the CO_2 -compensation point for C_3 photosynthesis ($\text{ca. } 40\text{--}70\text{ ppm}$ for C_3 plants; c_i where carbon uptake from photosynthesis is equal to carbon lost from respiration). This level is critical when considering that plants must operate well above compensating c_i to achieve sufficient photosynthetic rates for adequate growth and reproduction and for maintaining long-term survival (6). These low c_i values were not unique to southern California, because glacial leaves of *Pinus flexilis* from the Great Basin exhibited c_i values of 110 ppm (19), supporting the notion that trees in nearby regions were also carbon-starved during the last glacial period.

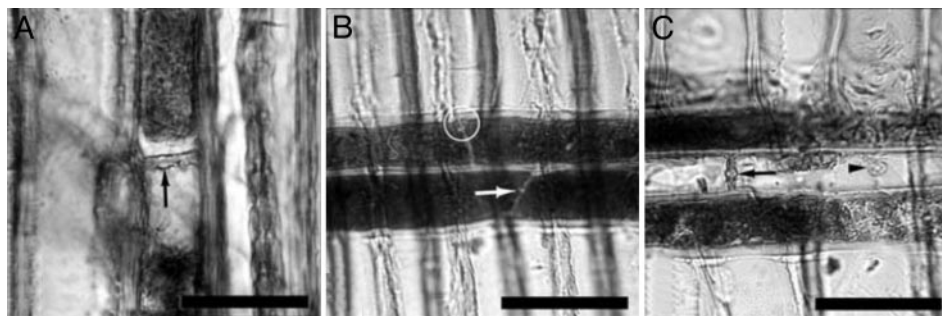


Fig. 2. Representative wood images from tar pit *Juniperus* showing anatomical features that define the genus. (A) Tangential section showing axial parenchyma cells with nodular end walls (arrow). (B and C) Radial section showing ray parenchyma cells with nodular end walls (arrows) and indenture (circle). Note the cupressoid pit (arrowhead) that is characteristic of *Juniperus*. (Scale bars, $50\text{ }\mu\text{m}$.)

environmental factors occurring in southern California such as higher humidity, greater precipitation (27), and lower $[\text{CO}_2]$ (1, 20, 21) would have favored increased c_i/c_a in glacial plants relative to modern plants, although this was not observed at La Brea or in other regions of North America (19, 32).

It is interesting to reflect on the near-constant c_i/c_a values within *Juniperus* between the last glacial period and modern times, particularly because severe carbon limitations would have imposed strong selective pressure for increased c_i . Polley and coworkers (5) found near-constant c_i/c_a within modern mustard, oats, and wheat grown across a $[\text{CO}_2]$ gradient ranging from 150 to 350 ppm for one generation. Our results indicate that c_i/c_a may be conserved over longer, evolutionary time scales (31), whereby reductions in c_i may have been compensated for by leaf anatomical and physiological adaptations [e.g., changes in stomatal density (33)], and/or delayed initiation of reproduction (34). This prediction suggests that plants are capable of adjusting c_i in response to indirect cues from changing c_a and opens the possibility of sensing through direct cues as well (35). Furthermore, Ehleringer and Cerling (31) have suggested that observations of constrained c_i/c_a values may represent a physiological setpoint that has been conserved within species throughout

evolutionary time scales ranging over the last several million years.

The patterns of low c_i observed in this regionally constrained study and in other nonconstrained studies (19, 32) show direct evidence for wide-scale reductions in C_3 primary productivity during the last glacial period in regions such as southern California. As a result of low $[\text{CO}_2]$ effects on photosynthesis, many aspects of the terrestrial carbon cycle are likely to have been impacted, including ecosystem distribution, resource availability to herbivores, and the abundance of C_3 versus C_4 plants.

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