Rapid report

Glacial trees from the La Brea tar pits show physiological constraints of low CO_2

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Summary

- While studies of modern plants indicate negative responses to low [CO_2] that occurred during the last glacial period, studies with glacial plant material that incorporate evolutionary responses are rare. In this study, physiological responses to changing [CO_2] were compared between glacial (La Brea tar pits) and modern *Juniperus* trees from southern California.
- Carbon isotopes were measured on annual rings of glacial and modern *Juniperus*. The intercellular : atmospheric [CO_2] ratio (c_i/c_a) and intercellular [CO_2] (c_i) were then calculated on an annual basis and compared through geologic time.
- *Juniperus* showed constant mean c_i/c_a between the last glacial period and modern times, spanning 50 000 yr. Interannual variation in physiology was greatly dampened during the last glacial period relative to the present, indicating constraints of low [CO_2] that reduced responses to other climatic factors. Furthermore, glacial *Juniperus* exhibited low c_i that rarely occurs in modern trees, further suggesting limiting [CO_2] in glacial plants.
- This study provides some of the first direct evidence that glacial plants remained near their lower carbon limit until the beginning of the glacial–interglacial transition. Our results also suggest that environmental factors that dominate carbon-uptake physiology vary across geologic time, resulting in major alterations in physiological response patterns through time.

Introduction

The last glacial period began *c.* 110 000 yr ago and reached a maximum for global ice volume at 18 000–20 000 yr ago. Glacial conditions persisted (except for brief interstadials) until the abrupt transition to the current interglacial period, beginning *c.* 14 000 yr ago. At the peak of the last glacial period, atmospheric CO_2 concentrations ([CO_2]) ranged between 180 and 200 ppm, which are among the lowest concentrations that occurred during the evolution of land plants (Berner, 2006; also see Pagani *et al.*, 2009 for an account of similarly low concentrations *c.* 15 million yr ago). When grown at glacial vs modern [CO_2], modern C_3 plants show 40–70% reductions in photosynthesis and biomass production (Polley *et al.*, 1993; Sage & Coleman, 2001), 20–30% lower survival (Ward & Kelly, 2004), and may even fail to reproduce (Dippery *et al.*, 1995). This is a result of reduced CO_2 substrate concentrations at carboxylation sites, as well as higher photorespiration rates. However, even at reduced paleo-temperatures where photorespiration is decreased, plants are still unable to overcome the severe, negative effects of low [CO_2] (Ward *et al.*, 2008). Such pronounced effects originating at the level of autotrophic physiology have been modeled at the ecosystem scale, and have been predicted to greatly reduce net primary production and carbon storage during glacial periods (Turcq *et al.*, 2002; François *et al.*, 2006). Admittedly, however, modern plants are often grown in glacial conditions for only a single generation, and therefore do not reflect evolutionary responses to low [CO_2]. This realization prompted our recent studies of glacial *Juniperus* (juniper) trees that were fully preserved within the La Brea tar pits in southern California (Los Angeles), and that had tens of thousands of years to adapt to low [CO_2]. Analysis of stable carbon isotope ratios of complete wood samples (that integrate all tree rings from a given individual) show severely reduced internal [CO_2] (c_i) during the last glacial period that are unprecedented in modern equivalents, strongly suggesting the existence of major carbon limitations on tree physiology (Ward *et al.*, 2005). Thus, the low [CO_2] of glacial periods likely produced a bottleneck on carbon exchange through reduced transfer of CO_2 from the atmosphere to the biosphere. However, it is still unknown if low [CO_2] presented an overriding limitation on plant physiology relative to other climatic factors (e.g. water, temperature).

The last glacial period represents an ideal time period for addressing this issue, since climate was more variable on an interannual basis and [CO_2] was exceptionally low relative to modern times (Mayewski *et al.*, 2004). More specifically, ice cores from Greenland indicate extreme stability of Holocene climate compared with that of the last glacial period (Dansgaard *et al.*, 1993). In addition, Dansgaard–Oeschger (D–O) cycles, which are periods of rapid and abrupt changes in temperature, dust content, ice accumulation and greenhouse gas concentrations, were more prominent during the last glacial period relative to the Holocene (Broecker, 1994; Roy *et al.*, 1996). These patterns recorded in Greenland ice are also documented in ocean sediment cores from the Santa Barbara Basin (Behl & Kennett, 1996; Heusser, 1998; Hendy & Kennett, 1999; Hendy *et al.*, 2002), *c.* 100 km northwest of La Brea, our primary research site. Analyses of these cores show a strong teleconnection between atmospheric trends over Greenland and ocean dynamics off the California coast, identifying synchronous climatic events between the two records over the last 60 000 yr (Behl & Kennett, 1996; Heusser, 1998; Hendy & Kennett, 1999; Hendy *et al.*, 2002).

In previous work, we did not have wood specimens that allowed for carbon isotope analysis of individual tree rings. Recent excavations at the La Brea tar pits have now yielded higher-quality *Juniperus* specimens, allowing for discernment of individual tree rings. Thus, these wood specimens make an excellent model system for testing the constraints of low [CO_2] on tree physiology relative to the effects of other climatic factors during the last glacial period. Here we compare long-term responses of tree physiology, as well as interannual variation within individuals, between the last glacial period and modern times. In doing so, we find the first evidence that low [CO_2] constrained the physiology of glacial trees, as evidenced by a dampened response to interannual climate variability.

Materials and Methods

Site selection

For this study, glacial trees from the Rancho La Brea tar pits (Los Angeles) were sampled and ^{14}C dated to 14.5–47.6 kyr before present (BP), with the majority of specimens dating to the last glacial period. *Juniperus* samples from Rancho La Brea cannot be identified to the species level, although analysis by a wood anatomy expert (Ward *et al.*, 2005) and species distributions indicate these samples are either *J. californica* or *J. occidentalis*. Cores of modern trees were collected from three low elevation sites in the Angeles National Forest (*J. californica*, two trees per site, one core per tree) and three high elevation sites in the San Bernardino National Forest (*J. occidentalis*, three trees per site, one core per tree), which are close in proximity to La Brea. Only modern trees from natural areas with well-drained, nonirrigated soils were sampled. Low-elevation sites provided a same-site control for glacial La Brea (with the full suite of environmental changes through time), whereas high-elevation sites controlled for lower temperatures and higher precipitation of the last glacial period (see Table 1, Heusser, 1998; Daly *et al.*, 2008), allowing for isolation of CO_2 effects. Note that conditions at glacial La Brea were wetter than modern times, which differs from most regions that were drier during the last glacial period. While [CO_2] does not vary with elevation, CO_2 partial pressure decreases in proportion to total atmospheric pressure. Under modern conditions, partial pressures of CO_2 at high-elevation sites are 10–30% lower than

Table 1 Climate data for glacial and modern *Juniperus* sampling sites

Site category	Site name and coordinates	Elevation (m)	Mean annual precipitation (mm)	Mean annual temperature (°C)
Glacial	La Brea Tar Pits + 34°3′48″, – 118°21′22″	80	*c.* 600	*c.* 7.5–9.5
Modern high elevation (SBNF)	Big Bear Lake + 34°16′12″, – 116°55′29″	2830	696	7.3
	Hwy 38 Bend + 34°11′35″, – 116°47′6″	2300	452	11.1
	Wildhorse Springs + 34°9′52″, – 116°43′14″	2300	572	8.7
Modern low elevation (ANF)	Mt. Emma Rd + 34°28′55″, – 118°4′2″	1340	242	14.7
	Littlerock Reservoir + 34°29′42″, – 118°1′36″	1045	230	14.9
	Lyttle Creek + 34°11′22″, – 117°26′11″	630	390	18.2

Climate data for glacial La Brea (Heusser, 1998), and modern sampling sites (Daly *et al.*, 2008). Modern samples were collected from San Bernardino National Forest (SBNF) and Angeles National Forest (ANF).

at low-elevation sites, producing an even more conservative comparison between glacial and modern conditions.

Stable isotope measurement

We measured stable carbon isotope ratios on alpha-cellulose from individual tree rings of glacial and modern *Juniperus*. Whole tree rings were analyzed in order to provide an integrated measure of the full annual response. Ring wood was separated under a dissecting microscope and alpha-cellulose was extracted from each ring using the method described by Ward *et al.* (2005). Previous work using this method has documented high purity levels of alpha-cellulose with no indication of asphalt contamination from the tar pits. Purity was based on theoretical O : H ratios (weight percent oxygen : weight percent hydrogen) of 7.79–8.08 for alpha-cellulose, with actual values falling well within this range (8.01 ± 0.02 and 7.97 ± 0.04 for modern and glacial samples, respectively, Ward *et al.*, 2005). Because our specific compound reflected high purity levels, we do not believe that diagenetic processes would have influenced our results.

Of the five glacial wood specimens that were available with an adequate number of tree rings, three are trunk specimens while two may be either portions of the trunk or large branch sections. For modern trees, the 10 rings nearest the center were excluded, as is common on dendrochronological work, as the juvenile stage often exhibits altered physiological patterns. Apart from this exception, all available rings in all samples were analyzed. Isotope measurements were performed at the Keck Paleoenvironmental and Environmental Stable Isotope Laboratory (KPESIL) at the University of Kansas. δ^{13}C values were calculated using the following formula:

$$\delta = R_{\text{sample}}/R_{\text{standard}} - 1$$

where R is the ratio of ^{13}C : ^{12}C, using belemnite carbonate from the Pee Dee Formation, Hemingway, SC (PDB) as the standard. Data were converted to 'per mil' (‰) notation by multiplying δ values by 1000. $\delta^{13}\text{C}_{\text{cell}}$ was converted to $\delta^{13}\text{C}_{\text{leaf}}$ using a constant offset of –3.2‰ (Leavitt & Long, 1982; Ward *et al.*, 2005). Carbon isotope discrimination was calculated as:

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

Conversion to carbon discrimination is necessary as it incorporates changes in $\delta^{13}\text{C}_{\text{air}}$ through time. $\delta^{13}\text{C}_{\text{air}}$ was −0.0066 (−6.6‰) during glacial times, but has decreased in modern times to −0.008 (−8.0‰; Leuenberger *et al.*, 1992). From Δ, c_i/c_a was calculated as

$$\frac{c_i}{c_a} = \frac{\Delta - a}{b - a}$$

where a is the fractionation against ^{13}C as a result of slower diffusion across the stomata (4.4‰) and b is the fractionation against ^{13}C as a result of Rubisco (27‰).

For each ring, c_i was also calculated from the c_i/c_a ratio using c_a values. For modern samples, c_a values were obtained from direct atmospheric measurements (Keeling *et al.*, 2009) and the Taylor Law Dome ice core (Etheridge *et al.*, 1996). For glacial trees, c_a values were obtained from the Vostok and EPICA Dome C ice cores (Lüthi *et al.*, 2008). To obtain the appropriate c_a values, ^{14}C ages of glacial trees were first converted to calendar ages in order to coincide with ice core data (Beck *et al.*, 2001). Since atmospheric [CO_2] showed only minimal changes throughout the latter portion of the last glacial period that is encompassed in our study, we are confident that [CO_2] values corresponding to converted ages are accurate to the actual conditions experienced by glacial trees.

Statistical analyses

Mean c_i/c_a values for high- and low-elevation modern *Juniperus* were not significantly different despite environmental differences between these locations (0.53 ± 0.05 and 0.53 ± 0.06, $P = 0.1$; ANOVA), and therefore, the two modern sets were grouped together for comparison to glacial values. Since the variance in c_i/c_a was significantly different between modern and glacial *Juniperus* ($P < 0.0001$), a Welch's ANOVA was used to compare modern and glacial c_i/c_a values that account for lack of equivalence of variance.

The coefficient of variation (CV) was calculated for c_i/c_a in both modern and glacial samples. CV provides a measure of dispersion of data around the mean, allowing us to compare variation between groups. CV was calculated as:

$$\text{CV} = \frac{s}{\bar{x}}$$

where s is the standard deviation, and $\bar{x}$ is the mean. Data are shown in percentage notation by multiplying CV by 100. In order to account for differences in chronology length between glacial (shorter) and modern (longer) samples, the following correction (Sokal & Rohlf, 1995) was applied to CV:

$$\text{CV}_{\text{corr}} = \left(1 + \frac{1}{4n}\right)\text{CV}$$

Correlation of modern c_i/c_a with climate

To determine correlations of modern c_i/c_a values with climate, monthly temperature and precipitation data were obtained for each site from PRISM (Daly *et al.*, 2008). The PRISM model is ideal for this comparison as it accurately reflects climatic conditions in mountainous coastal regions with large elevational gradients and complex topography (Daly *et al.*, 2008). Measures of temperature and precipitation alone provided only weak correlations with c_i/c_a, so vapor pressure deficit (VPD) was used for this correlation. VPD is a more integrative climatic parameter that combines water and temperature relationships and is closely linked to evapotranspiration, making this measure more directly related to plant physiology than temperature or precipitation alone. VPD was calculated from monthly average maximum ($T_{\max}$), minimum ($T_{\min}$) and dewpoint (T_{dew}) temperatures using:

$$\text{VPD} = \frac{e_s(T_{\max}) + e_s(T_{\min})}{2} - e_s(T_{\text{dew}})$$

where $e_s(T)$ is the saturation vapor pressure at temperature T, calculated as:

$$e_s(T) = 0.6112 \exp\left(\frac{17.67\,T}{T + 243.5}\right)$$

In order to correlate ring isotopic composition with VPD, rings of modern trees were associated with specific calendar years. Ring width patterns from trees within the same site were correlated and aligned using marker years of high precipitation and growth.

Results and Discussion

The c_i/c_a ratio is driven by two fundamental processes: stomatal conductance, which controls the rate of CO_2 diffusion from the atmosphere into the intercellular spaces of leaves; and chloroplast demand for CO_2, which is determined by internal CO_2 diffusion rates to carboxylation sites and photosynthetic biochemistry. Long-term trends in c_i/c_a over evolutionary timescales reflect the degree of coordination between processes affecting CO_2 supply and demand within the leaf. In addition, shorter-term trends in c_i/c_a (e.g. annual rings) reflect integrated shifts in tree physiology in response to changing environmental conditions within the lifespan of a single individual.

We found that mean c_i/c_a of *Juniperus* was similar between glacial and modern trees (Fig. 1a; glacial average, 0.52 ± 0.02; modern average, 0.53 ± 0.05; $P > 0.2$). One possible explanation for this, although one not supported by the literature, is that both stomatal conductance and chloroplast demand for CO_2 remained constant across this expansive period of [CO_2] and climatic change. On the other hand, if only one of these factors predominantly changed through time, there would have been shifts in c_i/c_a, which were not observed here. It is therefore most likely that both stomatal conductance and chloroplast demand for CO_2 were higher during the last glacial period, which would have enhanced CO_2 uptake under limiting carbon conditions. When supply and internal demand for CO_2 covary in the same direction, as has been observed even in highly disparate taxa (Franks & Beerling, 2009a), there are opposing effects on c_i/c_a, likely producing the stabilization effect observed here. When moving into the interglacial period, both stomatal conductance and chloroplast CO_2 demand likely decreased, with the effect of saving water and nitrogen as CO_2 became less limiting. In support of this idea, Ehleringer & Cerling (1995) hypothesized that c_i/c_a represents a metabolic set point that is maintained within species across time. In addition, increases in stomatal conductance are almost always observed in modern C_3 plants grown at low [CO_2] (Gerhart & Ward, 2010), and studies with glacial leaves show evidence for increased stomatal density and decreased stomatal size, which would have increased maximum stomatal conductance in the past (Beerling *et al.*, 1993; Franks & Beerling, 2009b; but also see Malone *et al.*, 1993 for responses of modern plants grown at low [CO_2]). The wetter conditions of the last glacial period may have also provided increased nitrogen availability to support higher leaf nitrogen contents, which may have enhanced photosynthetic capacity.

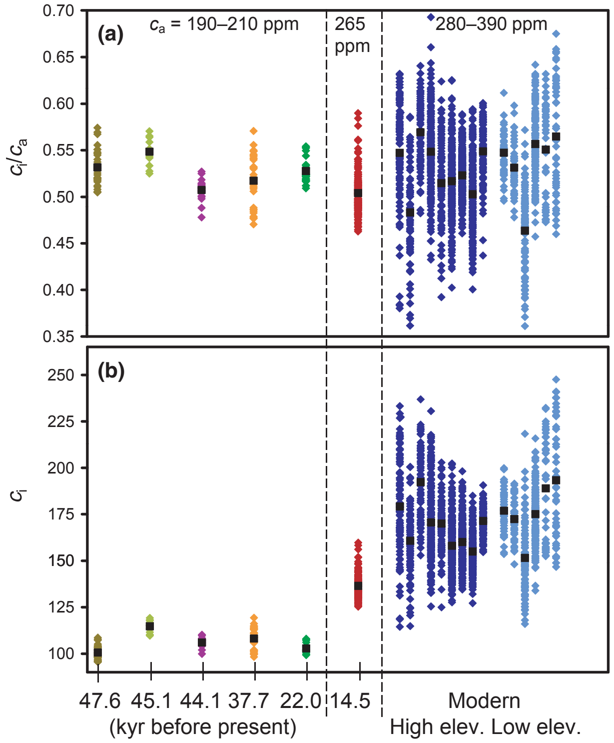

Fig. 1 Results of stable carbon isotope measurements for glacial and modern *Juniperus* tree rings. (a) Intercellular : atmospheric [CO_2] ratio (c_i/c_a values); (b) corresponding intercellular [CO_2] (c_i values). Each point represents an individual tree ring, and vertical groups represent results from all available tree rings for an individual tree, with values stacked from highest to lowest. Glacial samples are shown in different colors, while modern samples are grouped by elevation (to distinguish two different control groups), with means labeled as black boxes. Atmospheric [CO_2] values (c_a) are provided for each group. The range provided for modern samples reflects a temporal gradient experienced by each tree over its lifetime as a result of rapid changes in atmospheric [CO_2] in the modern period.

Despite any physiological adjustments, c_i values remained extremely low in glacial trees relative to modern trees as a result of consistently low c_a throughout the last glacial period (Fig 1b; glacial average, 106 ± 6; modern average, 168 ± 20; $P < 0.0001$). Past studies have reported similarly low c_i values in glacial needles of *Pinus flexilis* preserved in packrat middens (Van de Water *et al.*, 1993; Beerling, 1994). When considering all available rings, the vast majority of glacial c_i values fell outside the range of modern values. In fact, no modern trees experienced c_i values below 114 ppm, and no glacial trees experienced values > 120 ppm, leaving only a narrow overlapping range. It is also interesting to note that c_i values of glacial trees never fell below 90 ppm over an integrated annual period. This suggests that this may be a limiting concentration below which juniper trees may not maintain a positive carbon budget for basic physiological functions for survival (Campbell *et al.*, 2005).

Interannual variation in c_i/c_a, represented by CV$_{corr}$, was significantly lower in glacial vs modern trees ($P < 0.0002$; Fig. 2). More specifically, low- and high-elevation modern trees showed CV$_{corr}$ values of 8 ± 2% and 8 ± 3%, respectively. The only available Holocene specimen (14.5 kyr BP) showed an intermediate CV$_{corr}$ value of 5%, while glacial specimens showed the lowest values, averaging 3 ± 1%. Furthermore, although modern trees show occasional, short-term periods of low interannual variation, these periods are rare. Glacial trees show consistently low variation in c_i/c_a in all cases. In fact, the two oldest glacial *Juniperus* samples (45.1 and 47.6 kyr old) correspond to the timing of D–O events recorded in Greenland glaciers (Blunier & Brook, 2001). The maintenance of low interannual variation in c_i/c_a, even during time periods of rapid and drastic environmental change that are characteristic of D–O cycles, suggests that the maintenance of low variation in glacial *Juniperus* physiology was consistent throughout the last glacial period.

In a plethora of past studies, modern *Juniperus* in southern California and the southwestern US exhibits high interannual variation in c_i/c_a, mainly as a result of changes in soil water availability from year to year (Leavitt & Long, 1989; Feng & Epstein, 1995; Moore *et al.*, 1999; Leffler *et al.*, 2002). In our study, the c_i/c_a of modern trees showed the strongest correlations with monthly or seasonal VPD (R^2 = 0.06–0.25; $P < 0.05$–0.0001), whereby the months showing the strongest correlations were offset between elevations. Although these correlations were relatively low, similar correlations have been reported for modern *Juniperus*

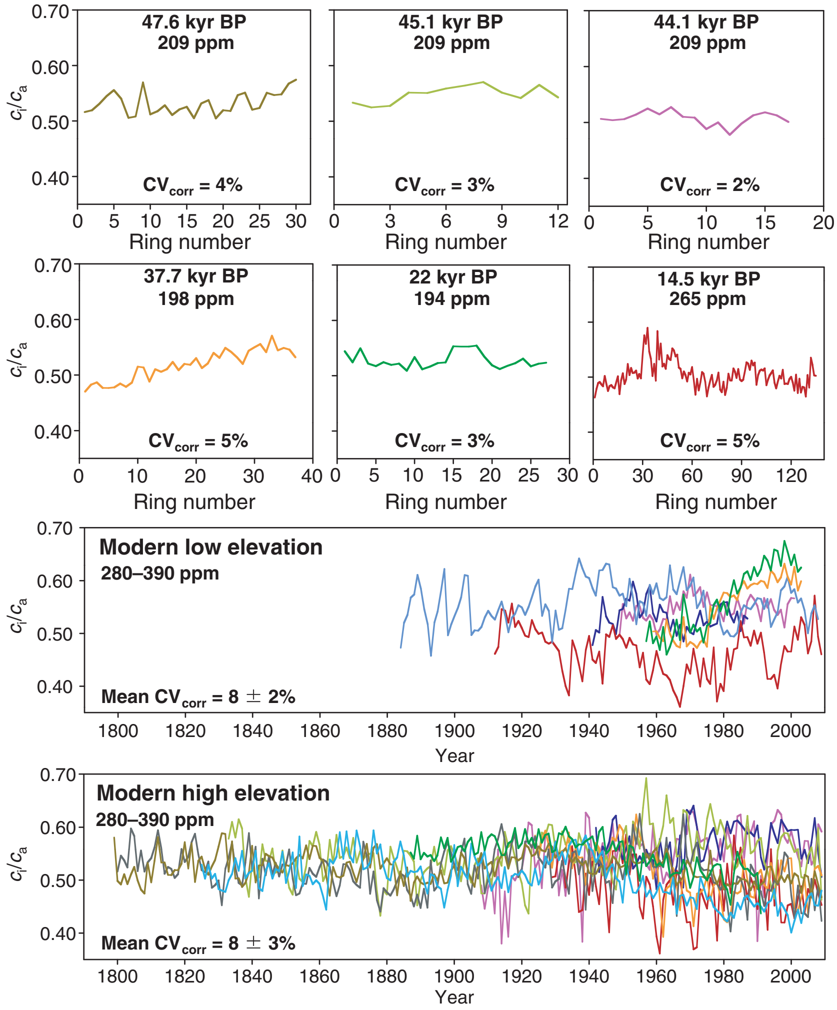

Fig. 2 Annual responses of intercellular : atmospheric [CO$_2$] ratio (c_i/c_a) for modern and glacial *Juniperus*. These are the same data as in Fig. 1, although in this case, data are placed in chronological order throughout the development of each tree. Full chronologies are not available for glacial trees, and therefore data are arranged from youngest (ring number 1) to oldest. Glacial samples are shown in the same colors as in Fig. 1, although modern samples are given different colors in order to distinguish their responses. ^{14}C age (thousands of years before present, kyr BP) atmospheric [CO$_2$], and CV$_{corr}$ (interannual variation in c_i/c_a, see the 'Materials and Methods' section for details) are provided for each sample and/or control group for the sake of comparison. The atmospheric [CO$_2$] range provided for modern samples reflects a temporal gradient experienced by each tree over its lifetime as a result of rapid changes in atmospheric [CO$_2$] in the modern period. [Correction added after online publication 20 December 2012: a corrected version of Fig. 2 is now published here, where the following corrections have been applied: Row 1, panel three: the text which previously read as 'CV$_{corr}$ = 3%' now reads as 'CV$_{corr}$ = 2%'; row 2, panel three: the text which previously read as 'CV$_{corr}$ = 59%' now reads as 'CV$_{corr}$ = 5%'].

in other studies (Leavitt & Long, 1989), and in all cases, soil water parameters and/or VPD correlate most closely with tree physiology (Leavitt & Long, 1989; Feng & Epstein, 1995; Moore *et al.*, 1999; Leffler *et al.*, 2002).

Since glacial climate was much more variable than in modern times, one would expect glacial c_i/c_a to also show higher variation if trees were responding to similar climatic factors. To the contrary, we found reduced amounts of interannual variation in c_i/c_a during the last glacial period (Fig. 2), indicating that a stable environmental factor dominated tree physiology. During the last glacial period, [CO_2] was extremely stable from year to year (EPICA, 2004), while water availability and temperature were predicted to have been highly variable (Behl & Kennett, 1996; Heusser, 1998; Hendy & Kennett, 1999; Hendy *et al.*, 2002). In our study, extremely low c_i values coupled with reduced variation in $c_i/c_{a,}$ even under a highly fluctuating glacial climate, point strongly to low [CO_2] constraints on tree physiology. While short-term studies with modern plants grown at glacial [CO_2] show major carbon limitations on physiology, our findings highlight the strength and consistency of low CO_2 constraints over evolutionary timescales.

In conclusion, this study has demonstrated that mean c_i/c_a has been maintained in *Juniperus* between the last glacial period and modern times, despite changes in temperature, precipitation and [CO_2]; that glacial c_i values were extremely low on an annual basis and occur only rarely in modern trees; that a limiting level for *Juniperus* physiology may exist at or near 90 ppm; and that interannual variation in c_i/c_a was greatly reduced in glacial *Juniperus*, likely as a result of the constraints of low [CO_2] that overrode responses to other climatic factors. This is the first direct evidence from trees that actually lived and evolved under low [CO_2] that carbon limitation persisted on an annual basis during the last glacial period. Moreover, our results suggest that the environmental factors that dominate carbon-uptake physiology can vary across geologic timescales, resulting in major alterations in physiological response patterns through time.

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