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## Evolutionary history underlies plant physiological responses to global change since the last glacial maximum

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### Abstract

Assessing family- and species-level variation in physiological responses to global change across geologic time is critical for understanding factors that underlie changes in species distributions and community composition. Here, we used stable carbon isotopes, leaf nitrogen content and stomatal measurements to assess changes in leaf-level physiology in a mixed conifer community that underwent significant changes in composition since the last glacial maximum (LGM) (21 kyr BP). Our results indicate that most plant taxa decreased stomatal conductance and/or maximum photosynthetic capacity in response to changing conditions since the LGM. However, plant families and species differed in the timing and magnitude of these physiological responses, and responses were more similar within families than within co-occurring species assemblages. This suggests that adaptation at the level of leaf physiology may not be the main determinant of shifts in community composition, and that plant evolutionary history may drive physiological adaptation to global change over recent geologic time.

### Keywords

Atmospheric [CO<sub>2</sub>]; climate change; last glacial maximum; leaf nitrogen content; maximum photosynthetic capacity; packrat middens; stomatal conductance; stomatal index

## INTRODUCTION

Changes in atmospheric CO<sub>2</sub> concentration ( $c_a$ ), climate and nutrient availability have likely impacted plant physiology since the last glacial maximum (LGM, ~21 kyr BP). However, even within C<sub>3</sub> plant assemblages, taxonomic groups may have varied in their responses to

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### AUTHORSHIP

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these changes. Such variation may have played a critical role in driving temporal shifts in plant species distributions and community composition. Evaluating the relationship between plant community dynamics and physiological responses to global change since the LGM requires analysis of co-occurring plant taxa at a single site through time. Ancient plant specimens preserved within packrat middens are invaluable in this context since they allow for comparisons between co-occurring plant lineages that exhibit different ecological, physiological and life-history traits. Here, we used modern plants and ancient plants preserved within packrat middens from the Snake Range, NV (Fig. 1) to investigate the physiological responses of a mixed montane conifer community that underwent known shifts in composition since the LGM (Wells 1983). In doing so, we provide new insights into fundamental mechanisms that underlie species-, family- and community-level responses to long-term global change.

During the LGM,  $c_a$  was among the lowest levels that occurred during the evolution of vascular plants, reaching minimal values of 180 p.p.m. (IPCC 2007). Prior to the Industrial Revolution,  $c_a$  rose to 270 p.p.m. and has continued to rise to 400 p.p.m. in modern times due to fossil fuel emissions (IPCC 2007); this is a level that has not occurred in the atmosphere for possibly 15 million years (Tripathi *et al.* 2009). Studies with modern plants grown under glacial and modern conditions indicate that glacial  $c_a$  negatively impacts leaf-level physiology leading to reductions in growth, survival and reproductive output (*reviewed in* Gerhart & Ward 2010). Studies of actual glacial plants further support these conclusions, indicating that  $c_a$  likely affected leaf-level physiology since the LGM (Van de Water *et al.* 1994; Beerling 2005; Ward *et al.* 2005; Gerhart *et al.* 2012).

Two physiological mechanisms may have alleviated the negative effects of low  $c_a$  on leaf-level physiology and growth during the LGM. The first strategy involves stomatal regulation of CO<sub>2</sub> diffusion into leaves. More specifically, when CO<sub>2</sub> availability is limiting, increased stomatal conductance ( $g_s$ ) via reduced stomatal sensitivity to vapour pressure deficit (Maherali *et al.* 2003), increased pore size and/or increased stomatal index (SI) or density (Franks & Beeling 2009) may have enhanced CO<sub>2</sub> diffusion into leaves (Fig. 2). In support of this idea, elevated  $g_s$  has been observed for a wide variety of modern plants grown under low  $c_a$  (*reviewed in* Gerhart & Ward 2010). Isotopic and morphological evidence from some ancient plants also indicates that  $g_s$  was likely higher under low  $c_a$  (Beerling 2005; Franks & Beeling 2009; Gagen *et al.* 2011; Gerhart *et al.* 2012).

The second physiological strategy for enhancing carbon (C) gain at low  $c_a$  involves maximising leaf photosynthetic capacity. Higher maximum photosynthetic capacity ( $A_{max}$ , Fig. 2) may have increased net photosynthetic rates and growth under limiting CO<sub>2</sub> availability (Sage & Coleman 2001; Smith *et al.* 2012). There is empirical evidence for photosynthetic acclimation to low  $c_a$  via increased  $A_{max}$  in modern plants (Crous *et al.* 2010; Smith *et al.* 2012; Ripley *et al.* 2013), and previous work with ancient *Juniperus* suggests that  $A_{max}$  may have been elevated during the LGM (Gerhart *et al.* 2012). Despite evidence for both increased  $g_s$  and  $A_{max}$  under low  $c_a$  in some ancient plants as well as modern plants grown under glacial conditions, these types of responses have not been assessed in a mixed, intact plant community as far back as the LGM.

The net benefit of high  $g_s$  and  $A_{max}$  for leaf-level physiology and growth would have been affected by the relative strength of water and nutrient limitations as well as  $c_a$ . In particular, maintaining high  $g_s$  under low  $c_a$  during the LGM could have increased the risk of xylem embolism due to greater water loss through stomata (Quirk *et al.* 2013). Consequently, variation in water limitation as well as drought tolerance likely impacted the ability of plants to respond to low  $c_a$  via changes in  $g_s$ . In the Snake Range, palaeoclimate models indicate that conditions were wetter during the LGM compared to today (Braconnot *et al.* 2007). Higher water availability may have provided the ideal conditions under which glacial plants could have increased  $g_s$  to overcome C limitations imposed by low  $c_a$ . This strategy may have allowed certain plant species to survive and even expand their range under glacial conditions. In contrast, decreases in water availability from glacial to modern times may have constrained  $g_s$  and contributed to shifts in community composition by favouring more drought-tolerant species.

Increased  $A_{max}$  under low  $c_a$  could have enhanced C gain without negatively impacting drought tolerance. However, increased  $A_{max}$  would have required greater investment in the production of Rubisco (Ribulose-1,5-bisphosphate carboxylase oxygenase). Given that Rubisco accounts for as much as 30% of total leaf nitrogen (N), increasing Rubisco content would have greatly increased plant demand for N (Sage & Coleman 2001; Ripley *et al.* 2013). Thus, the strength of N limitation and the ability of plants to compete for N likely impacted the degree to which plants could increase  $A_{max}$  under low  $c_a$ . Recent evidence from stable N isotopes indicates that global N availability decreased from ~15 to 7 kyr BP, after which N availability remained relatively constant (McLauchlan *et al.* 2013). Assuming that this trend continued back to the LGM, higher N availability in the past may have provided the ideal conditions under which glacial plants could have increased  $A_{max}$  to compensate for low  $c_a$ . In contrast, decreased N availability over more recent geologic history may have strengthened the trade-off between C gain and N demand, and potentially contributed to shifts in plant community composition by favouring species that were more competitive or efficient in their use of N.

Absolute values for  $g_s$  and  $A_{max}$  cannot be measured directly in ancient samples. In this study, we present a conceptual model for inferring the likelihood of changes in  $g_s$  and  $A_{max}$  since the LGM using leaf stable carbon isotope ratios ( $\delta^{13}\text{C}$ ), which reflect processes that influence  $\text{CO}_2$  supply and demand (Ehleringer & Cerling 1995), coupled with measures of stomatal characteristics and leaf %N. By measuring these leaf traits in a mixed, intact plant community spanning glacial through modern times we assessed (1) the potential for higher  $g_s$  and  $A_{max}$  in ancient plants that grew under low  $c_a$  increased for many generations, (2) variation among plant families and species in the magnitude and timing of leaf-level responses to global change, and (3) potential links between leaf-level physiology, species distributions and community composition. We hypothesised that  $g_s$  and  $A_{max}$  decreased in response to rising  $c_a$  combined with decreasing water and N availability since the LGM (Fig. 2). However, because plant species differ in drought tolerance and/or their ability to compete for nutrients (Saurer *et al.* 2004; Waterhouse *et al.* 2004; Shuxia *et al.* 2006; Liu *et al.* 2007; Crous *et al.* 2010), we also hypothesised that taxa would differ in their ability to alter  $g_s$  and

$A_{max}$  as  $c_a$  increased, and that these physiological differences would correspond with temporal shifts in species distributions and community composition.

The results of our study provide strong evidence that some ancient plants increased  $g_s$  and  $A_{max}$  under low  $c_a$ , and that plant families and species differed in the relative magnitude and timing of leaf-level responses to changing conditions since the LGM. Our results also suggest that leaf-level physiology contributed to the dominance of *Pinus longaeva* during the LGM. More broadly, however, it appears that changes in leaf-level physiology *per se* did not drive shifts in overall community composition from glacial to modern times. Instead, similar responses within plant families (i.e. within evolutionary lineages in the Snake Range) indicate that evolutionary history is an important determinant of physiological adaptation to global change.

## METHODS

### Site description and sample collection

The Snake Range, NV is located in the central Great Basin, U.S. (Fig. 1). Mean annual temperature in this region has risen from  $\sim 2.5^\circ\text{C}$  during the LGM (Braconnot *et al.* 2007) to  $8.6^\circ\text{C}$  in modern times (NOAA 2011). Simultaneously, mean annual precipitation has decreased from  $\sim 511 \text{ mm year}^{-1}$  during the LGM (Braconnot *et al.* 2007) to  $353 \text{ mm year}^{-1}$  during modern times (NOAA 2011). Over this time period,  $c_a$  has risen from  $\sim 180 \text{ p.p.m.}$  during the LGM to  $\sim 400 \text{ p.p.m.}$  today (IPCC 2007).

We sampled leaves from seven conifer species representing the dominant woodland community at mid-to-high elevations in the Snake Range, including five Pinaceae species: *Abies concolor* (ABCO), *Pinus flexilis* (PIFL), *Pinus longaeva* (PILO), *Pinus monophylla* (PIMO) and *Pinus ponderosa* (PIPO); and two Cupressaceae species: *Juniperus communis* (JUCO) and *Juniperus osteosperma* (JUOS). In addition, we sampled leaves from representatives of three other plant families, Asteraceae (ASTER, primarily *Artemisia* and *Chrysothamnus* spp.), Ephedraceae (EPHED, *Ephedra* sp.) and Poaceae (POA, likely *Oryzopsis*, *Pseudoroegneria* and *Stipa* spp.). Previous surveys of plant specimens preserved in Snake Range middens indicate that vast stands of PILO dominated much of this region during the LGM, while PIFL and JUCO became more abundant during the glacial–interglacial transition (Wells 1983). Other species, including ABCO, PIMO, PIPO and JUOS were not well represented in the Snake Range until the Holocene (Wells 1983).

We examined leaf samples from five time periods: (1) the LGM (radiocarbon age 21–31 kyr BP), (2) the glacial–interglacial transition (11–12 kyr BP), (3) the mid-Holocene (4.3–4.7 kyr BP), (4) the historic contemporary period (13–76 year BP) and (5) the modern period (present day). Glacial, Transition and Holocene samples were extracted from packrat middens in the Philip Wells Memorial Packrat Midden Collection (curated by Ward). All midden samples used in this study were previously extracted from a thin layer of the midden as described in Wells (1983). Samples were then cleaned to remove any remaining amberat (crystallised packrat urine), dust, or pollen (see Supporting Information). Visual inspection of a subset of leaves under high magnification showed that our cleaning protocol successfully removed contaminants from the leaf surface. Additional tests further showed

that prior exposure to packrat urine did not alter leaf  $\delta^{13}\text{C}$  or leaf %N (see Supporting Information). Midden samples were radiocarbon dated at the University of California, Irvine KECK-CCAMS facility (Irvine, CA) and the Woods Hole NOSAMS facility (Woods Hole, MA; see Table S2 for the average age of each midden). For comparison, historic samples were obtained from herbaria. These samples originated primarily from the Snake Range, although some were collected from nearby locations in the Western U.S. (Table S2). Finally, modern samples were collected from living plants in the Snake Range in the fall of 2011 (Fig. 2).

### Isotopic model of leaf physiology

We used a modelling approach that combines multiple lines of evidence to infer changes in  $g_s$  and  $A_{max}$  since the LGM (Table 1a). Specifically, we used leaf  $\delta^{13}\text{C}$  values to calculate several physiological parameters: (1)  $c_i$ , the concentration of  $\text{CO}_2$  in the leaf intercellular space, (2) the slope of the relationship between  $c_i$  and  $c_a$ , which reflects the relative rate of change in each variable since the LGM, (3) the  $c_i/c_a$  ratio, which reflects the balance between leaf-level processes influencing  $\text{CO}_2$  supply and demand at a given  $c_a$  and (4)  $c_a - c_i$ , which reflects the gradient for  $\text{CO}_2$  movement into the leaf (Farguhar *et al.* 1982; Ehleringer & Cerling 1995; McCarroll *et al.* 2009). In combination, patterns in the slope of  $c_i$  vs.  $c_a$ ,  $c_i/c_a$ , and  $c_a - c_i$  can be used to infer changes in  $g_s$  and  $A_{max}$  since the LGM (see Table S1 and Fig. S1 for a hypothetical illustration of this model). It has been suggested that simultaneous changes in both  $g_s$  and  $A_{max}$  are necessary to preserve the balance between stomatal and non-stomatal limitations on leaf-level physiology with rising  $c_a$  (Ehleringer & Cerling 1995; Beerling 1996; Beerling & Rundgren 2000; Gerhart *et al.* 2012). Disproportionate decreases in  $g_s$  and  $A_{max}$  would differentially affect the rate of change in  $c_i$  as well as the magnitude and direction of changes in  $c_i/c_a$  and  $c_a - c_i$ . For example, if  $g_s$  decreased more than  $A_{max}$  since the LGM as predicted based on increasing  $c_a$  combined with decreasing water availability, then  $c_i$  would increase slower than  $c_a$  (Table 1, Hypothesis 2). However, if  $A_{max}$  decreased more than  $g_s$  since the LGM as predicted based on increasing  $c_a$  combined with decreasing N availability, then  $c_i$  would increase faster than  $c_a$  (Table 1, Hypothesis 3). Alternatively, if changes in  $g_s$  perfectly offset simultaneous changes in  $A_{max}$ , or if neither parameter changed over time, then  $c_i$  would increase proportionally to  $c_a$  (Table 1, Hypothesis 1). Observations of stomatal characteristics and leaf %N can provide mechanistic support for potential changes (or lack thereof) in  $g_s$  and  $A_{max}$ .

### Sample analysis

Isotopic analyses were conducted on 2–3.5 mg of crushed leaf tissue at the University of Kansas Keck Paleoenvironmental and Environmental Stable Isotope Laboratory (Lawrence, KS). We calculated  $c_i$ ,  $c_i/c_a$ , and  $c_a - c_i$  from leaf  $\delta^{13}\text{C}$  measurements and published  $c_a$  records (Petit *et al.* 1999; EPICA 2004) using the methods of Gerhart *et al.* (2012) (see Supporting Information for detailed methods and  $c_a$  values). Mass-based leaf %N was analysed in conjunction with  $\delta^{13}\text{C}$  for the seven dominant conifer species.

SI and pore size were measured on the most common glacial (PILO) and Holocene species (JUOS) due to sample availability and the time-intensive nature of these measurements. Leaves were imaged using a scanning electron microscope or epifluorescence light

microscope (see Supporting Information). Images were then analysed in ImageJ to determine SI (number of stomata per epidermal cell) and pore size ( $\mu\text{m}^2$ ).

### Data analysis

We analysed a total of 131 leaf samples (Table S2). For each time period, sample sizes per plant family and species ranged from 2 to 23 and 2 to 7, respectively, depending on sample availability in the herbaria and packrat midden collections (Table S3).

Data were analysed in R (Version 2.15.3, The R Foundation for Statistical Computing, Vienna, Austria) using three statistical approaches (see Tables S3–S6 for full statistical results). First, to assess the rate of change in  $c_i$  relative to  $c_a$  since the LGM, we used linear regression to determine the slope, 95% confidence intervals and  $R^2$  of the relationship between  $c_i$  and  $c_a$  for each plant family and species. Second, for each taxonomic group we used separate one-way analysis of variance (ANOVAS) to assess temporal changes in  $c_i/c_a$ ,  $c_a - c_i$ , %N, SI and pore size (N and stomatal data were assessed at the species level). Third, we used two-way ANOVAS to compare temporal changes in  $c_i/c_a$ ,  $c_a - c_i$ , and %N among co-occurring conifer species using time period, species and their interaction as independent variables. We excluded herbaria samples from the N analyses because some of these samples were collected from locations outside the Snake Range, and spatial variation in N availability could obscure temporal patterns in leaf %N. For the two-way ANOVAS we grouped the species according to those that were dominant during the Transition (JUCO, PIFL, PILO) and those that were dominant during the Holocene (ABCO, JUOS, PIMO, PIPO). Grouping taxa in this manner provided insight into whether co-occurring taxa exhibited similar responses to global change, and whether leaf-level physiology may have contributed to shifts in community composition since the LGM.

## RESULTS

Our results indicate that  $g_s$  and  $A_{max}$  decreased since the LGM, although the timing and magnitude of these physiological responses varied among plant families and species. Below we describe variation in physiological responses among the five plant families, species that were dominant during the Transition and species that were dominant during the Holocene. In general, physiology was more similar within plant families regardless of when species were dominant.

### Variation among plant families

Patterns in the slope of  $c_i$  vs.  $c_a$ ,  $c_i/c_a$ , and  $c_a - c_i$  indicate that relative changes in  $g_s$  and  $A_{max}$  differed among plant families (Table 1b). First,  $c_i$  increased faster than  $c_a$  since the LGM for Asteraceae (slope 95% CI = 1.10–1.32), but slower than  $c_a$  for Cupressaceae (slope 95% CI = 0.65–0.94) and Poaceae (slope 95% CI = 0.59–0.90, Fig. 3a). For both Ephedraceae and Pinaceae,  $c_i$  increased proportionally to rising  $c_a$  since the LGM (slope not different from 1, Fig. 3a). Second,  $c_i/c_a$  increased for Asteraceae, Cupressaceae, Ephedraceae and Pinaceae ( $P < 0.05$  in all cases, Fig. 3b), but the magnitude of these increases varied across time within each family. For Pinaceae, the largest change in  $c_i/c_a$  occurred following the Transition time period, while for Asteraceae, Cupressaceae and

Ephedraceae, the change in  $c_i/c_a$  was greatest following the Holocene time period. In contrast to the other plant families,  $c_i/c_a$  was relatively constant over time for Poaceae ( $P > 0.5$ , Fig. 3b). Third, the direction and timing of changes in  $c_a - c_i$  differed among families (Fig. 3c). Specifically,  $c_a - c_i$  increased since the LGM for Poaceae and Cupressaceae ( $P < 0.05$  for both), but decreased for Asteraceae ( $P < 0.0001$ ). For Asteraceae, the decrease in  $c_a - c_i$  did not occur until after the Holocene time period. Finally,  $c_a - c_i$  remained relatively constant over time for Ephedraceae and Pinaceae ( $P > 0.4$  for both).

The combination of changes in  $c_i$  vs.  $c_a$ ,  $c_i/c_a$  and  $c_a - c_i$  since the LGM indicate that  $A_{max}$  decreased more than  $g_s$  in Asteraceae,  $g_s$  decreased more than  $A_{max}$  in Poaceae and Cupressaceae, and either there was no change in leaf-level physiology or  $g_s$  and  $A_{max}$  decreased equally in Ephedraceae and Pinaceae (Table 1b). For the Pinaceae, species-level responses (see below) support the hypothesis that changes in  $g_s$  offset changes in  $A_{max}$ .

### Variation among the dominant Transition species

Patterns in the slope of  $c_i$  vs.  $c_a$ ,  $c_i/c_a$  and  $c_a - c_i$  indicate that leaf-level responses to global change varied among the dominant Transition species. First,  $c_i$  increased slower than  $c_a$  since the Transition in PIFL (slope 95% CI = 0.55–0.97, Fig. 3d). In contrast,  $c_i$  increased proportionally to  $c_a$  in JUCO and PILO (slope not different from 1, Fig. 3d). Second,  $c_i/c_a$  increased since the Transition in PIFL, PILO and JUCO; however, the timing of these increases differed among species (time  $\times$  species,  $P < 0.05$ , Fig. 3e). For PILO, which was the only conifer species represented in the glacial samples,  $c_i/c_a$  remained constant from the Glacial to the Transition, and then increased steadily from the Transition to the Modern. In contrast,  $c_i/c_a$  increased from the Transition to the Historic for JUCO and PIFL, but then remained constant from the Historic to the Modern. Third, temporal patterns in  $c_a - c_i$  also varied among species (time  $\times$  species,  $P < 0.01$ , Fig. 3f). Specifically,  $c_a - c_i$  increased significantly since the Transition for PIFL ( $P < 0.05$ ), but remained constant for JUCO ( $P > 0.1$ ). In contrast to both PIFL and JUCO,  $c_a - c_i$  first increased and then decreased for PILO resulting in no net difference in  $c_a - c_i$  between Glacial and Modern trees ( $P > 0.4$ ).

Temporal patterns in leaf %N varied among the dominant Transition species (time  $\times$  species,  $P < 0.001$ , Fig. 4a), further indicating that these taxa differed in their leaf-level responses to global change. In particular, leaf %N decreased significantly since the LGM in PILO ( $P < 0.01$ ). In contrast, leaf % N did not change over time in JUCO ( $P > 0.3$ ), and only marginally decreased since the Transition in PIFL ( $P = 0.08$ ).

The combination of temporal patterns in  $c_i$  vs.  $c_a$ ,  $c_i/c_a$ ,  $c_a - c_i$  and %N indicates that neither  $g_s$  nor  $A_{max}$  changed over time in JUCO, but that  $g_s$  decreased more than  $A_{max}$  since the Transition in PIFL. For PILO, isotopic and %N results indicate that both  $g_s$  and  $A_{max}$  decreased since the LGM (Table 1c); however, neither SI nor pore size changed significantly in this species over time ( $P > 0.1$ , Fig 5).

### Variation among the dominant Holocene species

Patterns in the slope of  $c_i$  vs.  $c_a$ ,  $c_i/c_a$  and  $c_a - c_i$  indicate that leaf-level responses to global change varied among the dominant Holocene species. First, although  $c_i$  increased

proportionally to rising  $c_a$  since the Holocene in all four species (slope not different from 1, Fig. 3g), the rate of this change was slightly faster in ABCO. Second,  $c_i/c_a$  increased since the Holocene in ABCO, JUOS, PIMO and PIPO ( $P < 0.05$  in all cases); however, the magnitude of these changes varied significantly among species (time  $\times$  species,  $P < 0.01$ , Fig. 3h). In particular, compared to the other three species, ABCO exhibited a greater increase in  $c_i/c_a$  from Historic to Modern times. Third, temporal patterns in  $c_a - c_i$  varied substantially among species (time  $\times$  species,  $P < 0.01$ , Fig. 3i). Specifically, from Holocene to Modern times,  $c_a - c_i$  decreased significantly in ABCO ( $P < 0.05$ ), but only marginally in PIPO ( $P = 0.07$ ). In contrast,  $c_a - c_i$  exhibited no change since the Holocene in JUOS and PIMO ( $P > 0.1$  for both).

Temporal patterns in leaf %N varied among the dominant Holocene species (time  $\times$  species,  $P < 0.001$ , Fig. 4b), further indicating that these taxa differed in their leaf-level responses to global change. In particular, leaf %N decreased significantly since the Holocene in ABCO, PIMO and PIPO ( $P < 0.01$  in all cases). In contrast, leaf %N did not change over time in JUOS ( $P > 0.9$ ).

The combination of changes in  $c_i$  vs.  $c_a$ ,  $c_i/c_a$ ,  $c_a - c_i$  and %N since the Holocene indicates that  $g_s$  and  $A_{max}$  decreased equally in PIMO and PIPO,  $A_{max}$  decreased more than  $g_s$  in ABCO, and neither  $g_s$  nor  $A_{max}$  changed in JUOS (Table 1d). For JUOS, the lack of a significant change in SI and pore size ( $P > 0.1$ , Fig. 5) further indicates that  $g_s$  did not decrease over time.

## DISCUSSION

Comparisons of leaf-level physiology across co-occurring taxa within intact communities will improve our understanding of physiological responses to global change, and provide insights into changes in physiology that may underlie shifts in species distributions and community composition across geologic time. Until now, analyses of physiological responses in glacial plants have been limited to one or a small number of species per study and site (e.g. Van de Water *et al.* 1994; Beerling & Rundgren 2000; Ward *et al.* 2005; Gerhart *et al.* 2012). Here, we sampled leaves from a mixed, intact plant community in the Snake Range over a ~30 kyr time scale. Using stable carbon isotopes, leaf %N and stomatal characteristics, we inferred temporal shifts in leaf-level physiology, and compared those responses to well-documented shifts in species distributions and community composition since the LGM (Wells 1983). Our results provide new insights into several key aspects of plant and community responses to global change over recent geologic history. We show that (1)  $g_s$  and/or  $A_{max}$  decreased with changing conditions since the LGM in most of the sampled plant taxa, (2) leaf-level physiology combined with unique life-history traits likely influenced the distribution of PILO, (3) changes in measured physiological traits were not associated with shifts in community composition since the LGM and (4) evolutionary history of plant lineages is an important determinant of physiological adaptation to global change.

In most cases, we found evidence that  $g_s$  and/or  $A_{max}$  decreased with global change since the LGM (Table 1); however, the magnitude of these changes often varied across time within a

family or species. For example, isotopic data for the Pinaceae were consistent with a larger decrease in  $g_s$  from the LGM to the Transition, while patterns from the Transition to the Holocene suggest a larger decrease in  $A_{max}$  (Fig. 3a–c; Table 1a). A similar pattern of temporal variation in physiological responses was observed in the Asteraceae. These temporal patterns indicate that  $g_s$  and  $A_{max}$  may differ in the degree or rate at which they respond to global change (Anderson *et al.* 2001; Andreu-Hayles *et al.* 2011), and that shifts in the relative strength of carbon vs. water or nutrient limitations through geologic history can strongly influence leaf-level physiology (Gerhart *et al.* 2012).

In this study, we found evidence that leaf-level physiology combined with unique life-history traits may have influenced the distribution and abundance of PILO in the Snake Range. Similar to the other Pinaceae species,  $g_s$  and  $A_{max}$  likely decreased in PILO since the LGM. However, in contrast to the other species, PILO shifted from a near monoculture during the LGM to small relict populations today (Wells 1983). We suggest that differences in several key life-history traits allowed PILO to take greater advantage of high  $g_s$  and  $A_{max}$  during the LGM, and potentially contributed to the shift in this species' distribution over time. Specifically, low-temperature tolerance (Kral 1993) combined with high  $g_s$  and  $A_{max}$  may have allowed PILO to become the dominant species during the LGM when both temperature and  $c_a$  were low. The extreme longevity of PILO leaves (up to 45 year, Ewers & Schmid 1981) also likely offset the greater investment in leaf N content needed to maintain high  $A_{max}$  by allowing these plants to increase carbohydrate production per unit of N over longer periods of time. Furthermore, the moderate drought tolerance of PILO (Kral 1993) may have allowed this species to maintain the higher rates of  $g_s$  necessary to benefit from increased  $A_{max}$  during the LGM. Interestingly, the lack of temporal changes in SI and pore size for PILO supports the hypothesis that reduced sensitivity to vapour pressure deficit caused higher  $g_s$  under low  $c_a$  (Arneth *et al.* 2002; Maherali *et al.* 2003). During more recent time periods, rising  $c_a$  combined with higher temperatures and greater water limitation likely reduced the time frame in which PILO could take advantage of high  $A_{max}$ . In addition, studies show low levels of ectomycorrhizal inoculum in modern PILO forests (Bidartondo *et al.* 2001). Since ectomycorrhizal fungi (EMF) increase N uptake (Hobbie & Hobbie 2006), low fungal colonisation of PILO roots could reduce the ability of this species to compete for N in a more diverse community. Taken together, the results of these studies suggest that a unique combination of physiological, ecological and life-history traits impacted PILO's distribution and response to global change in the Snake Range.

Although we found evidence of linkages between physiology and shifts in PILO's distribution, temporal changes in leaf-level physiology were not strongly associated with well-documented shifts in overall community composition following the Transition period (Wells 1983). It is possible that other physiological traits beyond the scope of individual leaves, such as whole-plant resource allocation patterns, may have contributed to community shifts. Extreme climate events (e.g. fires, droughts) that are difficult to reconstruct over geologic time may have also played a role in determining community composition.

Interestingly, leaf-level responses were most similar within a family, regardless of when a particular species was dominant. For example, JUCO, PIFL and PILO were codominant during the Transition period while ABCO, JUOS, PIMO and PIPO became more abundant

during the Holocene (Wells 1983). If leaf-level physiology contributed to this shift in composition, then patterns in  $g_s$  and  $A_{max}$  should differ between the dominant Transition and Holocene taxa. Instead, Pinaceae species were generally more responsive to global change than co-occurring Cupressaceae species. This suggests that evolutionary history may be an important driver of family-level adaptation to long-term changes in resource availability in the Snake Range.

The differences between Pinaceae and Cupressaceae taxa were the most striking example of taxonomic variation in leaf-level physiology. These two families, though both conifers, diverged over 200 Ma (Rai *et al.* 2008; Mao *et al.* 2012), and modern species exhibit different physiological, ecological and life-history traits. The comparison of these taxa greatly extends the temporal range of studies describing variation in family- and species-level responses to global change (Waterhouse *et al.* 2004; Shuxia *et al.* 2006; Liu *et al.* 2007; Crous *et al.* 2010). For the Pinaceae, reductions in leaf %N combined with results inferred from isotopic data support the hypothesis that both  $g_s$  and  $A_{max}$  decreased since the LGM. These results are also consistent with the general hypothesis that changes in both leaf parameters are likely required to maintain the balance between stomatal and non-stomatal controls on photosynthesis (Ehleringer & Cerling 1995). In sharp contrast, results for the Cupressaceae suggest that these taxa were unresponsive to changing conditions since the LGM. Although, at the family level, isotopic results for Cupressaceae were consistent with a slight decrease in  $g_s$  over time, individual results for JUCO and JUOS suggest that neither  $g_s$  nor  $A_{max}$  changed in response to shifting resource availability. Since JUOS increased in abundance in the Snake Range after the Holocene, overall lower  $g_s$  for JUOS compared to JUCO could explain the family-level results. These data differ from studies of other ancient *Juniperus* species in southern California (La Brea tar pits) where isotopic results suggest that *Juniperus* was more responsive to global change since the LGM (Gerhart *et al.* 2012). A greater change in water limitation over time for plants at that site compared to the Snake Range may have contributed to the differences between these studies.

Interestingly, temporal patterns in leaf %N differed greatly between Pinaceae and Cupressaceae families in our study. Associations with mycorrhizal fungi could have contributed to these patterns since EMF typically colonise Pinaceae species, whereas arbuscular mycorrhizal fungi (AMF) typically colonise *Juniperus* species (Wang & Qiu 2006; EMF can colonise JUCO in some systems). Since EMF increase N uptake (Hobbie & Hobbie 2006) more so than AMF (Leigh *et al.* 2009), these fungal symbionts may have allowed their Pinaceae hosts to better compete for N, which as suggested by our results, could have enhanced their ability to respond to global change by altering  $A_{max}$  (Table 1).

In conclusion, our assessment of leaf-level physiology in an ancient plant community provides novel insights into physiological responses to global change since the LGM. Through analyses of modern and ancient plant specimens preserved in packrat middens, we show that a combination of unique life-history traits and leaf-level physiology likely influenced the distribution of PILO since the LGM. However, our results indicate that changes in leaf-level physiology cannot fully explain major shifts in community composition. Instead, for the Snake Range, we show that evolutionary history at the level of plant family underlies variation in leaf-level responses to global change since the LGM.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

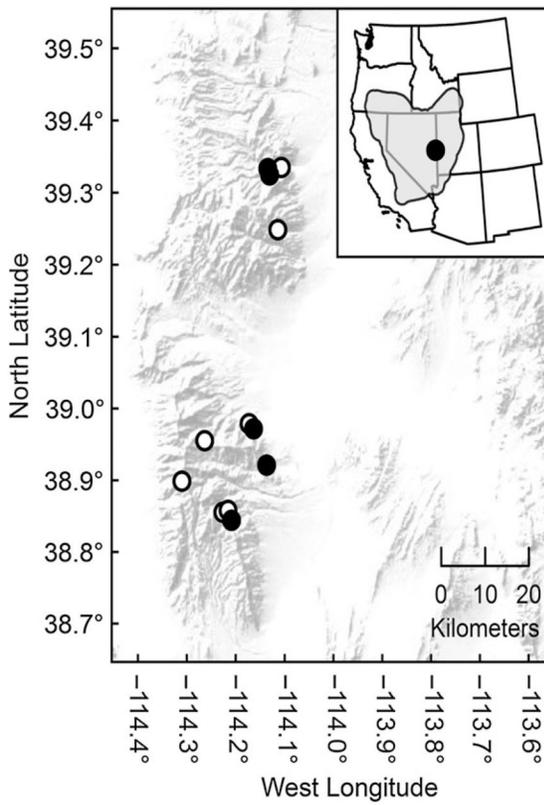
We thank the late Professor Philip Wells for his lifelong efforts to form and maintain the packrat midden collection, and Anke Wells for donating that extensive collection to the University of Kansas. We also thank the R. L. McGregor and University of Nevada-Reno Herbaria for providing historic samples, and Melissa Buzzard (Bureau of Land Management, NV) and Gary Reese (Logan Simpson Design, Inc., Tempe, AZ) for collecting modern leaf samples. Modern samples were collected under permits issued by the U.S. National Park Service – Great Basin National Park and the U.S. National Forest Service – Humboldt Toiyabe National Forest. Finally, we thank Charley Lewis for assistance with early method development, Greg Kane for assistance with isotopic analyses, John Southon for assistance with radiocarbon dating and three anonymous referees for comments on a previous draft of this manuscript. Funding for this work was provided by NIH IRACDA Postdoctoral Fellowships awarded to J. S. M. and K. M. B., and a NSF Career Award to J. K. W.

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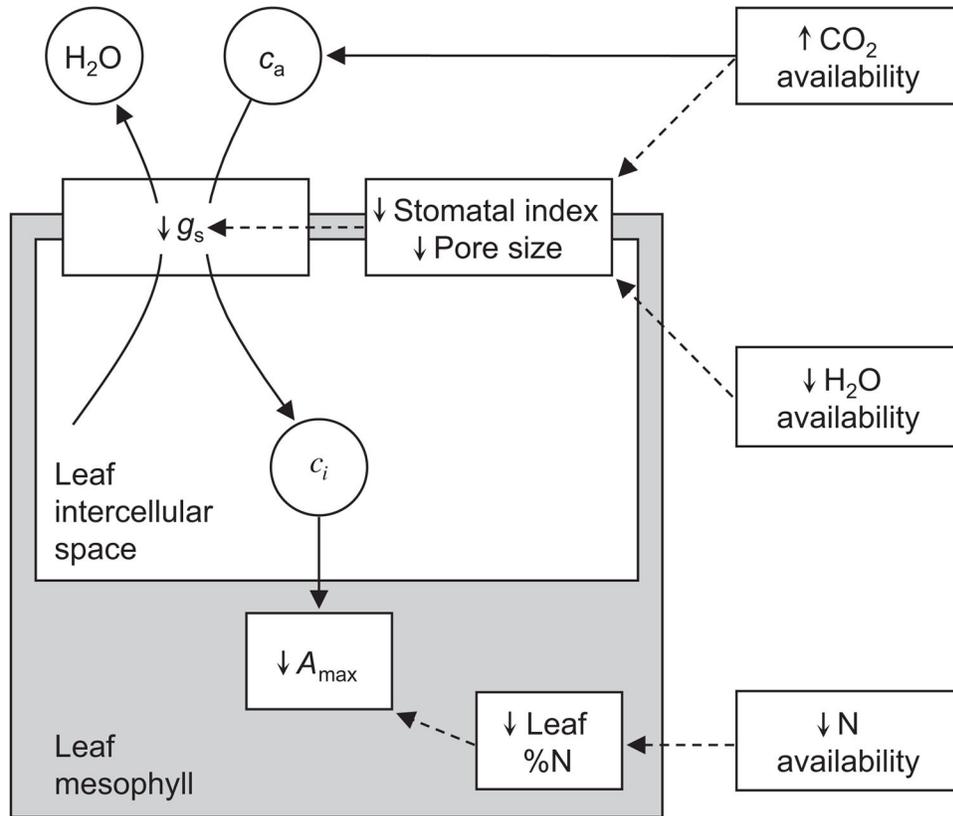
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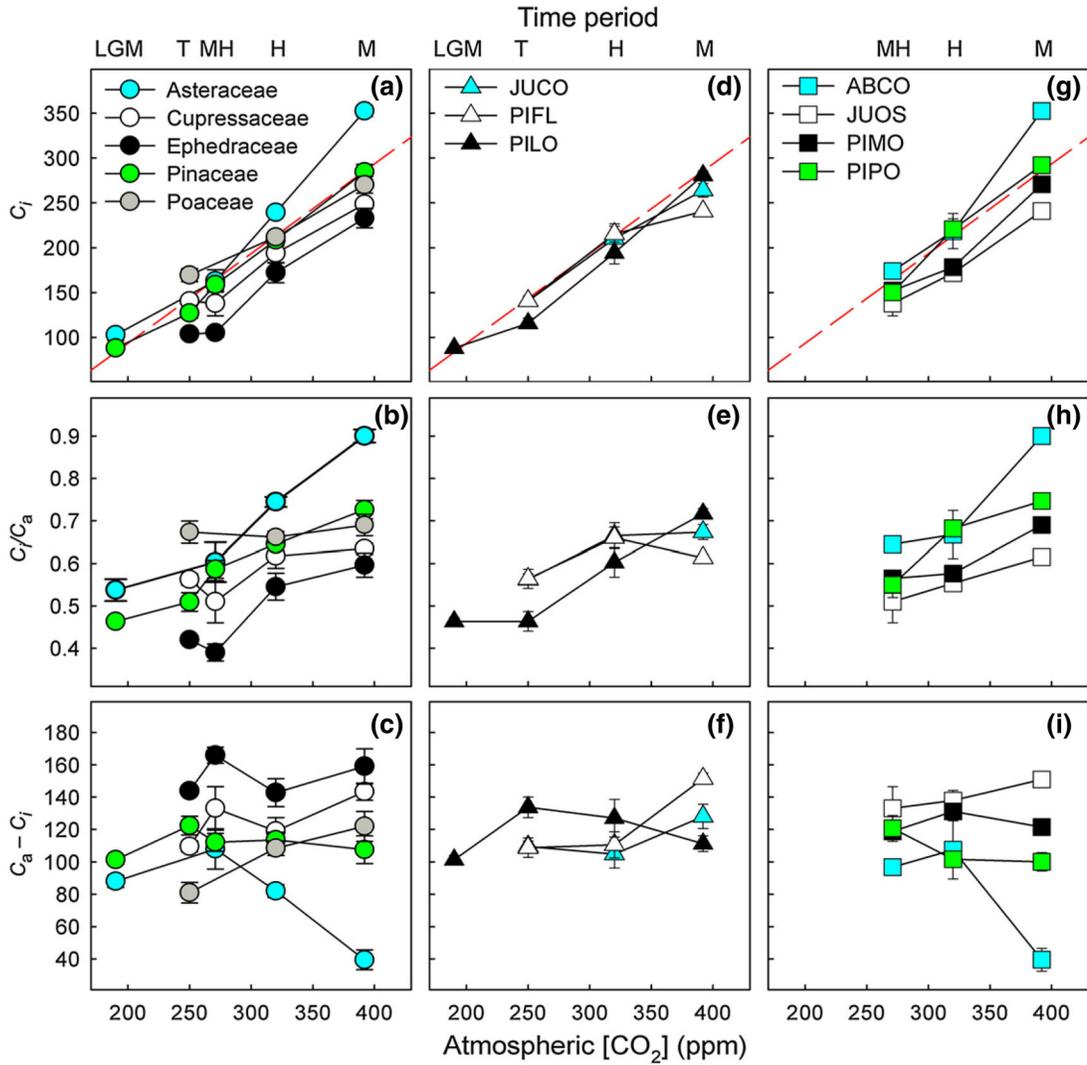


**Figure 1.** Modern (white circles) and midden (black circles) specimens were collected from the Snake Range, NV. Inset shows the location of the Snake Range within the Great Basin Desert (shaded area). Most herbaria specimens were collected from the Snake Range or other locations within the Great Basin Desert (Table S2).



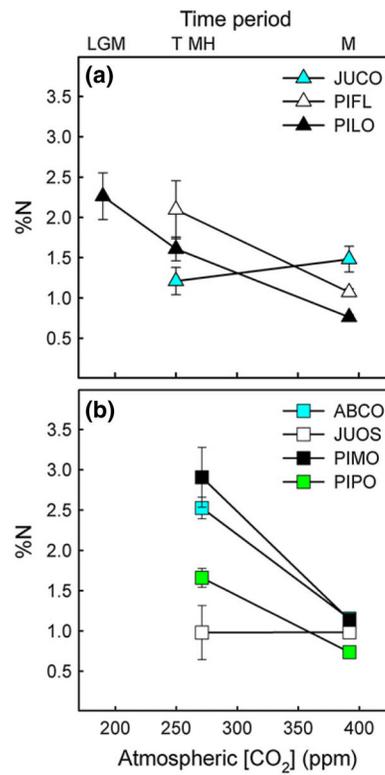
**Figure 2.**

Two components of leaf function, stomatal conductance ( $g_s$ ) and maximum photosynthetic capacity ( $A_{max}$ ), influence the concentration of CO<sub>2</sub> inside the leaf ( $c_i$ ) and plant responses to global change. First,  $g_s$  controls the influx of CO<sub>2</sub> into the leaf intercellular space, but also the efflux of H<sub>2</sub>O from the leaf mesophyll. As stomata open,  $g_s$  and  $c_i$  increase, but the water use efficiency of photosynthesis decreases for a given  $A_{max}$ . We hypothesised that plants decreased  $g_s$  in part by reducing stomatal index and/or pore size in response to rising  $c_a$  and decreasing water availability since the LGM. Second,  $A_{max}$  is directly proportional to leaf N content (DeLucia & Schlesinger 1991), and we hypothesised that decreasing N availability since the LGM reduced leaf N content, resulting in lower  $A_{max}$ . Solid lines show the flux of CO<sub>2</sub> and H<sub>2</sub>O. Dashed lines show indirect effects of global change on leaf traits.

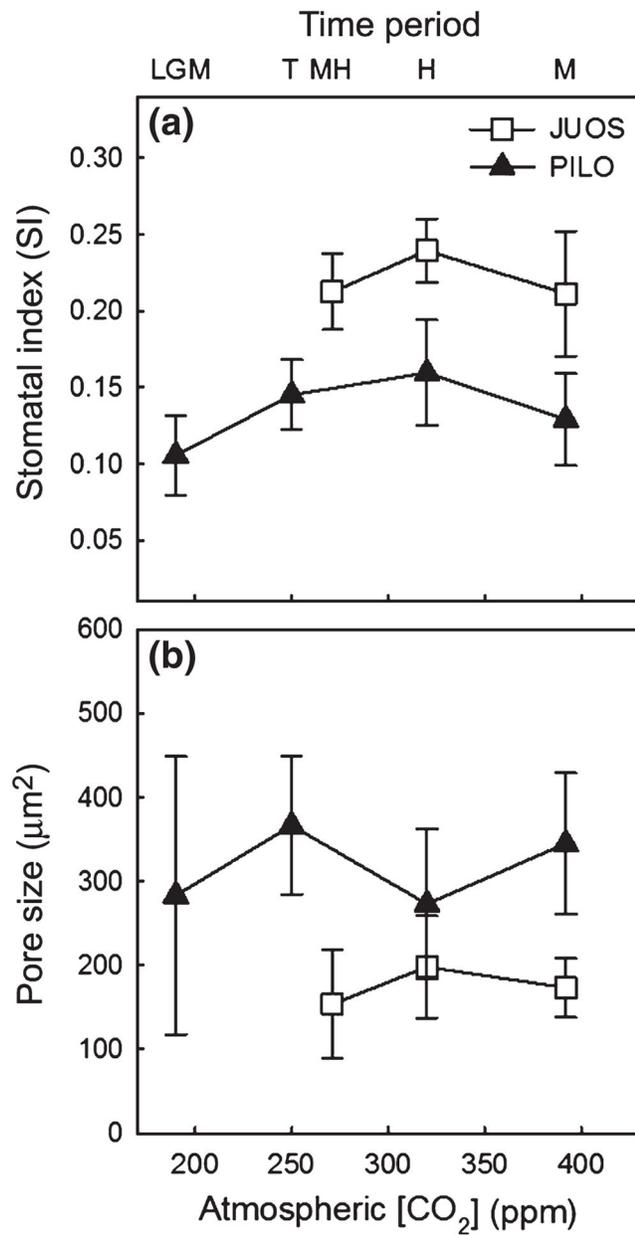


**Figure 3.**

Plant families and species differed in the slope of  $c_i$  vs.  $c_a$  as well as the magnitude and timing of changes in  $c_i/c_a$  and  $c_a - c_i$ . Panels show mean ( $\pm$  SE)  $c_i$ ,  $c_i/c_a$ , and  $c_a - c_i$  for the five plant families (a–c), dominant Transition species (d–f) and dominant Holocene species (g–i). Data are plotted against the mean  $c_a$  for each time period (LGM, last glacial maximum; T, glacial–interglacial transition; MH, mid-Holocene; H, historic and M, modern). The red dashed lines in panels a, d and g represent a slope = 1. Species abbreviations are as follows: *Juniperus communis* (JUCO), *Pinus flexilis* (PIFL), *Pinus longaeva* (PILO), *Abies concolor* (ABCO), *Juniperus osteosperma* (JUOS), *Pinus monophylla* (PIMO) and *Pinus ponderosa* (PIPO).



**Figure 4.** The dominant Transition (a) and Holocene (b) species differed in the degree to which leaf %N (mean – SE) changed over time. Data are plotted against the mean  $c_a$  for each time period. Time period and species abbreviations are as described for Figure 3.



**Figure 5.** Mean ( $\pm$  SE) stomatal index (a) and pore size (b) did not change over time for *Juniperus osteosperma* or *Pinus longaeva*. Data are plotted against the mean  $c_a$  for each time period. Time period abbreviations are as described for Figure 3.

Table 1

Hypothesised (a) and observed (b–d) responses to global change since the LGM. Observed changes in the slope of  $c_i$  vs.  $c_a$ ,  $c_i/c_a$ ,  $c_a - c_i$ , leaf %N, stomatal index and stomatal pore size are shown for each plant family (b), the dominant Transition species (c) and the dominant Holocene species (d). Results for each taxon were interpreted based on the hypothesised responses (a).

	Slope of $c_i$ vs. $c_a$		$c_i/c_a$	$c_a - c_i$	Leaf %N	Stomatal index and pore size*	Interpretation
(a) Hypothesised responses							
Hypothesis 1a	1	Increased	Constant	Constant	Constant	Constant	No change in $g_s$ or $A_{max}$
Hypothesis 1b	1	Increased	Constant	Constant	Decreased	Decreased	$g_s$ and $A_{max}$ decreased equally
Hypothesis 2	<1	Increased <sup>†</sup>	Increased	Increased	Constant	Decreased	$g_s$ decreased more than $A_{max}$
Hypothesis 3	>1	Increased	Decreased	Decreased	Decreased	Constant	$A_{max}$ decreased more than $g_s$
(b) Plant families							
Asteraceae	>1	Increased	Decreased	Decreased	–	–	$A_{max}$ decreased more than $g_s$
Poaceae	<1	Constant <sup>†</sup>	Increased	Increased	–	–	$g_s$ decreased more than $A_{max}$
Cupressaceae	<1	Increased	Increased	Increased	–	–	$g_s$ decreased more than $A_{max}$
Ephedraceae	1	Increased	Constant	Constant	–	–	No change or $g_s$ and $A_{max}$ decreased equally
Pinaceae	1	Increased	Constant	Constant	–	–	No change or $g_s$ and $A_{max}$ decreased equally
(c) Dominant transition species							
<i>Juniperus communis</i>	1	Increased	Constant	Constant	Constant	–	No change in $g_s$ or $A_{max}$
<i>Pinus flexilis</i>	<1	Increased	Increased	Increased	Decreased	–	$g_s$ decreased more than $A_{max}$
<i>Pinus longaeva</i>	1	Increased	Constant	Constant	Decreased	Constant	$g_s$ and $A_{max}$ decreased equally
(d) Dominant Holocene species							
<i>Abies concolor</i>	1	Increased	Decreased	Decreased	Decreased	–	$A_{max}$ decreased slightly more than $g_s$
<i>Juniperus osteosperma</i>	1	Increased	Constant	Constant	Constant	Constant	No change in $g_s$ or $A_{max}$
<i>Pinus monophylla</i>	1	Increased	Constant	Constant	Decreased	–	$g_s$ and $A_{max}$ decreased equally
<i>Pinus ponderosa</i>	1	Increased	Constant	Constant	Decreased	–	$g_s$ and $A_{max}$ decreased equally

\* A recent study suggests that small, dense pores might be most beneficial under low  $c_a$  (Franks & Beeling 2009). In this case, increased pore size combined with decreased SI from glacial to modern times would support the hypothesis that  $g_s$  decreased over time (Hypothesis 1b or 2).

<sup>†</sup> A very large decrease in  $g_s$  relative to  $A_{max}$  could result in constant or even decreasing  $c_i/c_a$  from glacial to modern times (see Table S1 and Fig. S1).