

Atmospheric CO₂ fluctuations during the last millennium reconstructed by stomatal frequency analysis of *Tsuga heterophylla* needles

Lenny Kouwenberg
Rike Wagner
Wolfram Kürschner
Henk Visscher

Palaeoecology, Laboratory of Palaeobotany and Palynology, Utrecht University, Budapestlaan 4, 3584 CD Utrecht, Netherlands

ABSTRACT

A stomatal frequency record based on buried *Tsuga heterophylla* needles reveals significant centennial-scale atmospheric CO₂ fluctuations during the last millennium. The record includes four CO₂ minima of 260–275 ppmv (ca. A.D. 860 and A.D. 1150, and less prominently, ca. A.D. 1600 and 1800). Alternating CO₂ maxima of 300–320 ppmv are present at A.D. 1000, A.D. 1300, and ca. A.D. 1700. These CO₂ fluctuations parallel global terrestrial air temperature changes, as well as oceanic surface temperature fluctuations in the North Atlantic. The results obtained in this study corroborate the notion of a continuous coupling of the preindustrial atmospheric CO₂ regime and climate.

Keywords: carbon dioxide, paleoclimate, Holocene, conifers, climate controls, paleoecology.

INTRODUCTION

Analysis of the coupling between atmospheric CO₂ levels and climate change under natural conditions in the past is crucial in the forecasting of the impact of anthropogenically produced CO₂ on the coupled climate-carbon cycle system. At present, the notion of covarying atmospheric CO₂ levels and climate is reinforced by the predictions in climate models of (1) a strong rise in global mean temperature as a result of excessive CO₂ emissions (1–7 °C; Houghton et al., 2001), and (2) significant feedback effects of these CO₂-induced climate changes on the carbon exchange between oceanic, atmospheric, and terrestrial reservoirs (Plattner et al., 2001).

However, it seems as if the role of CO₂ variability in past climate change depends on the time scale studied. The clear coupling between CO₂ and temperature on both glacial-interglacial and millennial scales detected in Antarctic ice cores (Fischer et al., 1999; EPICA Community Members, 2004) is in marked contrast with apparently stable CO₂ levels of the Holocene that reflect neither prolonged climate shifts, such as the late-Holocene gradual cooling, nor centennial-scale cooling events, such as the Preboreal Oscillation and the 8.2 ka event (Indermühle et al., 1999).

It is intriguing that only ice-core records of the last millennium support centennial-scale coupling of CO₂ and Holocene climate; CO₂ changes of 5–15 ppmv are broadly concurrent with Northern Hemisphere climate shifts (Barnola et al., 1995; Etheridge et al., 1996; Indermühle et al., 1999). Unfortunately, data from different coring localities disagree about timing and magnitude of the changes. To resolve these discrepancies and to corroborate the concept that centennial-scale CO₂ variations are involved in late Holocene climate change, stomatal frequency analysis (Woodward, 1987; Royer, 2001) of tree leaves buried in peat and lake deposits provides an alternative method for detecting and quantifying short-term CO₂ fluctuations. The inverse relationship between the number of stomata (pores on plant leaves through which gas exchange occurs) and ambient CO₂ enables the reconstruction of past CO₂ levels with a potentially annual resolution (e.g., Wagner et al., 1996). Stomatal data increasingly substantiate a much more

dynamic Holocene CO₂ evolution than suggested by ice-core data.

Coupled to centennial-scale cooling events, CO₂ changes of 20–50 ppmv occur synchronously in records from Europe and North America (Wagner et al., 2004).

So far the available stomatal and ice-core records over the last millennium have insufficient temporal resolution and chronological inaccuracies, and have not provided a picture of CO₂ dynamics that can be correlated with available high-resolution proxy temperature records (Rundgren and Beerling, 1999; Gerber et al., 2003). In this study we present a well-dated, high-resolution CO₂ record from North America, spanning the period A.D. 800–2000. In order to investigate the role of CO₂ as a forcing factor in preindustrial climate change, CO₂ data are compared with selected temperature records.

MATERIAL AND METHODS

Buried needles of the conifer *Tsuga heterophylla* were recovered from a 91 cm sediment core from Jay Bath, a shallow pond at Mount Rainier (Washington State; 46°46'N, 121°46'W; 1311 m above sea level). The core was cut into 1-cm-thick sediment samples, 3–5 identifiable needles were processed, and stomatal frequency was measured according to the quantification strategy of Kouwenberg et al. (2003).

Quantification of preindustrial CO₂ levels is based on the response rate of *Tsuga heterophylla* needles to the CO₂ increase over the last century (Fig. 1). Needles incorporated in the calibration set originate from contrasting altitudes of the Northwest Pacific region. Because the difference in air pressure with altitude affects CO₂ partial pressure, and thus the amount of CO₂ available for gas exchange, CO₂ levels are expressed as partial pressures (*p*CO₂) instead of mixing ratios. The stomatal frequency change in the calibration set is not attributable to changes in local precipitation and temperature or needle maturity (Kouwenberg et al., 2003, 2004). Reconstructed CO₂ values were calculated by using a classical linear regression, which allows a better performance at the extremes and with slight extrapolation than inverse linear regression (Osborne, 1991). To allow quantitative comparison with other CO₂ records, reconstructed CO₂ levels for the last millennium are expressed as mixing ratios (Jones, 1992; Kouwenberg, 2004).

Age vs. depth relationships for the sediment core (Fig. 2) were determined by fitting a fourth-order polynomial through a series of five accelerator mass spectrometry ¹⁴C dates and one tephra layer at 21 cm from the A.D. 1481 Mount St. Helens eruption (Mullineaux, 1996). In this way an overall averaged mean sedimentation rate of 1 cm per 27 yr is obtained for the core.

RESULTS

The reconstructed CO₂ mixing ratios at Jay Bath since A.D. 800 are presented in Figure 3. Preindustrial CO₂ values fluctuated around a long-term average of 280–290 ppmv culminating after A.D. 1850 in a sharp rise from 280 ppmv to 370 ppmv. The CO₂ record is characterized by high-frequency variability. Focusing on centennial-scale changes (the three-point moving average), the record shows prominent CO₂ minima of ~260 ppmv ca. A.D. 860 and A.D. 1150, and modest

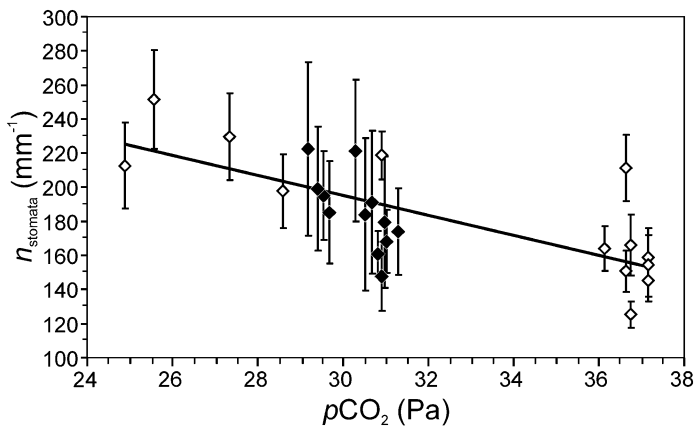


Figure 1. Response of stomatal parameters of *Tsuga heterophylla* to $p\text{CO}_2$ increase from 24 to 38 Pa. CO_2 partial pressure was calculated as CO_2 mixing ratio (in parts per million by volume) times barometric air pressure P_B (Pa). Barometric air pressure was estimated according to Jones (1992) as $P_B = 101.325/e^{(z/29.3)/T}$, where z is altitude above sea level and T air temperature in kelvin (estimated from mean annual temperature at closest weather station, corrected by temperature lapse rate in case of significant altitudinal difference between site and station). CO_2 mixing ratios of 290–315 ppmv were derived from shallow Antarctic ice cores (Neftel et al., 1985); mixing ratios of 315–368 ppmv are annual means from Mauna Loa monitoring (Keeling and Whorf, 2002). Black diamonds—needles from living trees and short peat core next to Jay Bath (Mount Rainier, Washington); open diamonds—modern and herbarium needles from other localities. Error bars indicate ± 1 standard error. Solid line indicates best fit in classical linear regression analysis (linear model showed best fit over nonlinear models); n_{stomata} is number of stomata per millimeter needle length (same as TSDL as defined in Kouwenberg et al., 2003); $n_{\text{stomata}} = -5.8581 \times p\text{CO}_2 + 371.14$; $r^2 = 0.5124$ ($P < 0.001$). This calibration data set plotted against CO_2 mixing ratios was previously published in Kouwenberg et al. (2003).

minima of 275–280 ppmv occurred ca. A.D. 1600 and A.D. 1800. In between, ca. A.D. 1000, ca. A.D. 1300, and ca. A.D. 1700, CO_2 maxima of 300 ppmv, 320 ppmv, and 300 ppmv, respectively, were estimated.

DISCUSSION

Comparison with Other CO_2 Records

Due to inherent stomatal frequency variation in tree leaves (Kürschner et al., 1997), the calculated raw proxy CO_2 data show considerable scatter. Yet the three-point average matches the industrial CO_2 rise after A.D. 1800 very well. The last six data points, which are completely independent from the calibration data set, clearly reproduce the CO_2 increase from 280 to 370 ppmv, evident from shallow Antarctic ice cores (Neftel et al., 1985) and instrumental measurements at Mauna Loa since A.D. 1958 (Keeling and Whorf, 2002). This accurate correspondence corroborates the reliability of the older reconstructed CO_2 fluctuations.

The presence of short-term CO_2 variability in the last millennium is supported by stomatal records from Europe. Despite much lower chronological accuracy, a comparable pattern of centennial-scale fluctuations was recognized in stomatal frequency data of *Salix* leaves from Sweden (Rundgren and Beerling, 1999). A very high-resolution record based on *Quercus* leaves from the Netherlands, spanning the first half of the last millennium, shows a remarkably similar decrease in CO_2 between A.D. 1000 and A.D. 1200, a sharp increase of ± 34 ppmv until A.D. 1300, and a slow decrease until A.D. 1500 (Wagner et al., 2004).

The observed CO_2 fluctuations in the Jay Bath record, up to 50 ppmv, exceed those measured in Antarctic ice cores (Fig. 3). The most prominent feature in the stomatal CO_2 reconstruction, the CO_2 mini-

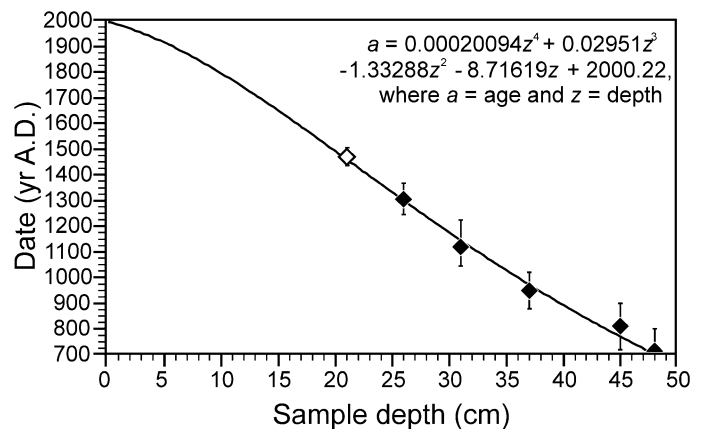


Figure 2. Age (a) vs. depth (z) diagram for Jay Bath core. White diamond—tephra layer from A.D. 1481 Mount St. Helens eruption (Mullineaux, 1996). Black diamonds— ^{14}C accelerator mass spectrometer dates converted to calendar ages by using Oxcal 3.8 (Bronk Ramsey, 1995) and INTCAL98 calibration data (Stuiver et al., 1998). Black line shows fourth-order polynomial providing best fit ($r^2 = 0.9977$). Error bars indicate 2σ (95.4%) probability ranges.

um ca. A.D. 1200 and a subsequent rise during that century, is also reflected in the South Pole and D47 ice cores, albeit with a smaller amplitude maximum of 12 ppmv (Siegenthaler et al., 1988; Barnola et al., 1995). The Law Dome ice core temporal resolution in the earlier part of the record is insufficient to have captured this short-term CO_2 event (Etheridge et al., 1996).

The discrepancies between the ice-core and stomatal reconstructions may partially be explained by varying age distributions of the air in the bubbles because of the enclosure time in the firn-ice transition zone. This effect creates a site-specific smoothing of the signal (decades for Dome Summit South [DSS], Law Dome, even more for ice cores at low accumulation sites), as well as a difference in age between the air and surrounding ice, hampering the construction of well-constrained time scales (Trudinger et al., 2003). However, the large amplitude of the CO_2 signal in the Jay Bath record, compared to the ice cores and other stomatal reconstructions, may be influenced by the relatively low amount of needles in the low- CO_2 part of the calibration data set.

Sources of Carbon

To explain substantial centennial-scale CO_2 shifts during the last millennium, a mechanism must be found to produce such rapid changes

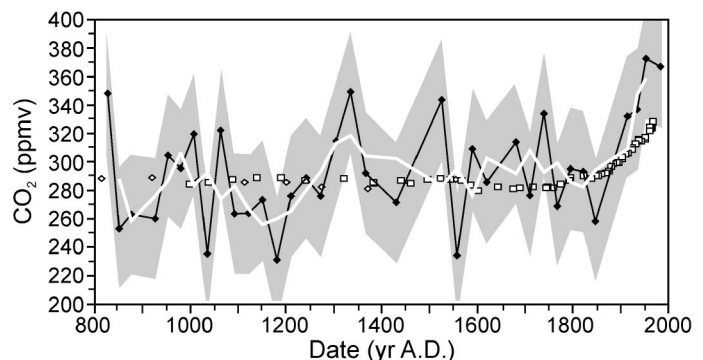


Figure 3. Reconstructed CO_2 mixing ratios based on stomatal frequency counts on *Tsuga heterophylla* needles for A.D. 800–2000. Black line connects means of 3–5 needles per sample; thick white line shows three-point moving average. Gray area indicates confidence interval of ± 1 root mean standard error. White squares— CO_2 data from Law Dome ice core (Etheridge et al., 1996); white diamonds— CO_2 data from Taylor Dome ice core (Indermühle et al., 1999).

in carbon cycle dynamics. Ruddiman (2003) argued that decadal- to centennial-scale CO₂ anomalies of 4–10 ppmv observed in ice-core records could be the result of widespread reforestation of agricultural areas in Europe and Asia, which were abandoned after outbreaks of epidemic diseases. However, although natural or anthropogenic changes in the terrestrial biosphere are likely to have played a role in short-term carbon cycle dynamics, the amount of carbon needed to cause a shift of 50 ppmv would far exceed the size of potential carbon sources and sinks in the terrestrial biosphere (Ruddiman, 2003). Moreover, the lack of high-resolution land-cover reconstructions for the last millennium does not permit a detailed comparison between centennial-scale changes in land cover and the CO₂ record.

The oceans, on the other hand, may be a much larger potential source or sink for atmospheric carbon (Raven and Falkowski, 1999). Exchange of CO₂ between atmosphere and ocean is highly dependent on physicochemical factors affecting the solubility of CO₂ in the ocean surface waters, such as alkalinity, dissolved inorganic carbon content, salinity, and temperature (Takahashi et al., 1993). **Involvement of sea-surface temperature (SST) changes in the production and depletion of atmospheric CO₂ is strongly suggested by the apparent synchronicity between the timing of CO₂ maxima and minima in the stomata-based record (within uncertainty limits), and changes in North Atlantic Ocean SST as recorded offshore the Mid-Atlantic United States** (Cronin et al., 2003) (Fig. 4B), as well as offshore West Africa (DeMenocal et al., 2000) (Fig. 4C). Provided that SST fluctuations at these sites reflect SST changes in the entire North Atlantic, temperature-driven changes in the CO₂ flux between ocean surface waters and atmosphere may be invoked as a plausible mechanism to explain at least a substantial part of the reconstructed CO₂ variations over the last millennium. For example, a modeled cooling of 2.7 °C in the Atlantic Ocean (north of lat 20°N) during the Younger Dryas produced a local *p*CO₂ decrease of 2.7 Pa (Marchal et al., 1999). Apart from SST change, climate-induced changes in salinity, carbonate chemistry, circulation, and marine biological productivity in the Atlantic or the other ocean basins, such as the Southern Ocean, may also have affected atmospheric CO₂ levels (Sarmiento and Orr, 1991; Marchal et al., 1998; Plattner et al., 2001).

CO₂ and Air Temperature

The CO₂ changes as detected in the stomatal records should be expected to significantly affect radiative forcing and thus global air temperatures. However, atmospheric CO₂ levels are influenced by temperature-induced changes in biospheric and marine feedback systems. In order to investigate the potential of rapid CO₂ changes as a forcing factor in climate over the last millennium, the CO₂ record is compared to global temperatures. Some remarkable correlations are revealed between the stomata-based CO₂ record and a multiproxy-based reconstruction of global mean temperature (Mann and Jones, 2003), particularly with respect to the timing of the warm periods and the CO₂ maxima ca. A.D. 950 and A.D. 1300 (Fig. 4D). The overall picture suggests a clear covariation between CO₂ and global temperature. Note, however, that the Southern Hemisphere contribution to the global mean temperature estimation is based on limited proxy data.

A causative link between CO₂ and terrestrial air temperature would be most prominent in Northern Hemisphere records, because models of the spatial distribution of a future temperature rise due to anthropogenic CO₂ emission indicate that the greatest warming is expected to occur at high latitudes on the Northern Hemisphere continents (Houghton et al., 2001). Similar centennial-scale fluctuations can be recognized in Northern Hemisphere air temperature records based on extratropical tree-ring records (Briffa, 2000; Esper et al., 2002) (Fig. 4E). In model sensitivity studies on the evolution of CO₂ and Northern Hemisphere temperature in response to changes in radiative forcing over the last millennium, CO₂ fluctuations of at least 20 ppmv were

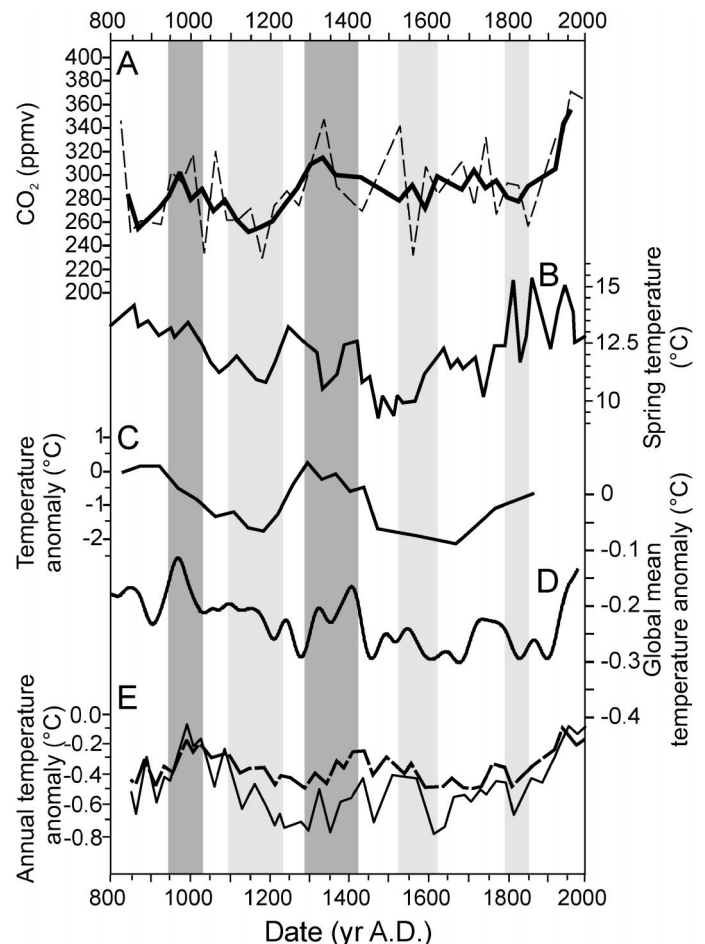


Figure 4. Comparison between reconstructed CO₂ mixing ratios and Northern Hemisphere climate records. A: CO₂ mixing ratios from stomatal counts on *Tsuga heterophylla* needles from Jay Bath. Thin dashed line connects means of 3–5 needles per sample depth, thick line three-point moving average to emphasize centennial scale trends. Dark gray bands—periods of high CO₂ mixing ratios; light gray bands—periods of low CO₂ mixing ratios (based on three-point average trends). **B:** Sea-surface temperatures of Chesapeake Bay (Mid-Atlantic United States) reconstructed from Mg/Ca ratios of ostracods (Cronin et al., 2003). **C:** Sea-surface temperature anomalies offshore West Africa as reconstructed from foraminiferal assemblages (DeMenocal et al., 2000). Shown are cold-season anomalies; warm-season anomalies have similar pattern (not depicted). **D:** Global mean temperatures (45 yr running average) from multiproxy records based on 1961–1990 reference period (Mann and Jones, 2003). **E:** Summer temperature anomalies in tree-ring records from Northern Hemisphere. Dashed line is from Briffa (2000); black line is from Esper et al. (2002).

connected with Northern Hemisphere temperature changes on the scale of the Esper tree-ring record (under very high solar forcing conditions) (Gerber et al., 2003). Such changes are larger than those observed in ice cores, but are compatible with the stomata-based record.

The CO₂ minimum at A.D. 1150 and the maximum ca. A.D. 1400 seem to correspond to terrestrial temperature changes that occurred 50–100 yr later, suggesting a forcing role for CO₂ fluctuations. However, attempts to determine leads and lags by simply comparing the CO₂ and air temperature records have some important drawbacks. The chronology of the CO₂ record contains uncertainties on the scale of several decades. Furthermore, climate changes over the last millennium are the result of the interplay between a number of forcing factors; variations in solar irradiance output and volcanic eruptions are at least as important as CO₂ in terms of influence on radiative forcing and thus on global temperature records. In order to truly assess the impact of rapid

CO₂ changes on Holocene climate, radiative forcing models must be run, including other known forcing factors as well as a more dynamic CO₂ regime for which this study provides support.

CONCLUSIONS

The centennial-scale variability in atmospheric CO₂ concentration linked to documented global and regional temperature change since A.D. 800, recognized in this study, corroborates continuous coupling of CO₂ and climate during the Holocene. For the first time, CO₂ changes inferred from stomatal frequency analysis have been related to co-eval variation in Atlantic SSTs providing evidence that **CO₂ fluctuations over the last millennium at least partly could have originated from temperature-driven changes in CO₂ flux between ocean surface waters and atmosphere.** Because the CO₂ variation also shows similarities with terrestrial air temperature trends in the extratropical Northern Hemisphere regions—which are the areas most sensitive to global warming—it may be hypothesized that throughout the last millennium, CO₂ could have served as a forcing factor for terrestrial air temperature.

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REFERENCES CITED

Barnola, J.M., Anklin, M., Porcheron, J., Raynaud, D., Schwander, J., and Stauffer, B., 1995, CO₂ evolution during the last millennium as recorded by Antarctic and Greenland ice: *Tellus*, v. 47B, p. 264–272.

Briffa, K.R., 2000, Annual climate variability in the Holocene: Interpreting the message of ancient trees: *Quaternary Science Reviews*, v. 19, p. 87–105.

Bronk Ramsey, C., 1995, Radiocarbon calibration and analysis of stratigraphy: The OxCal program: *Radiocarbon*, v. 37, p. 425–430.

Cronin, T.M., Dwyer, G.S., Kamiya, T., Schwede, S., and Willard, D.A., 2003, Medieval Warm Period, Little Ice Age and 20th century temperature variability from Chesapeake Bay: *Global and Planetary Change*, v. 36, p. 17–29.

DeMenocal, P., Ortiz, J., Guilderson, T., and Sarnthein, M., 2000, Coherent high- and low-latitude climate variability during the Holocene Warm Period: *Science*, v. 288, p. 2198–2202.

EPICA (European Project for Ice Coring in Antarctica) Community Members, 2004, Eight glacial cycles from an Antarctic ice core: *Nature*, v. 429, p. 623–628.

Esper, J., Cook, E.R., and Schweingruber, F.H., 2002, Low-frequency signals in long tree-ring chronologies for reconstructing past temperature variability: *Science*, v. 295, p. 2250–2253.

Etheridge, D.M., Steele, L.P., Langenfelds, R.L., Francey, R.J., Barnola, J.M., and Morgan, V.I., 1996, Natural and anthropogenic changes in atmospheric CO₂ over the last 1000 years from air in Antarctic ice and firn: *Journal of Geophysical Research*, v. 101, no. D2, p. 4115–4128.

Fischer, H., Wahlen, M., Smith, J., Mastroianni, D., and Deck, B., 1999, Ice core records of atmospheric CO₂ around the last three glacial terminations: *Science*, v. 283, p. 1712–1714.

Gerber, S., Joos, F., Brügger, P., Stocker, T.F., Mann, M.E., Sitch, S., and Scholze, M., 2003, Constraining temperature variations over the last millennium by comparing simulated and observed atmospheric CO₂: *Climate Dynamics*, v. 20, p. 281–299.

Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., and Xiaosu, D., 2001, *Climate Change 2001: The scientific basis contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change (IPCC)*: Cambridge, UK, Cambridge University Press, 944 p.

Indermühle, A., Stocker, T.F., Joos, F., Fischer, H., Smith, H.J., Wahlen, M., Deck, B., Mastroianni, D., Tschumi, J., Blunier, T., Meyer, R., and Stauffer, B., 1999, Holocene carbon-cycle dynamics based on CO₂ trapped in ice at Taylor Dome, Antarctica: *Nature*, v. 398, p. 121–126.

Jones, H.G., 1992, *Plants and microclimate*: Cambridge, UK, Cambridge University Press, 428 p.

Keeling, C.D., and Whorf, T.P., 2002, Atmospheric CO₂ concentrations—Mauna Loa Observatory, Hawaii, 1958–2002: <http://cdiac.esd.ornl.gov/ndps/ndp001.html> (September 2004).

Kouwenberg, L.L.R., 2004, Application of conifer needles in the reconstruction of Holocene CO₂ levels [Ph.D. thesis]: Utrecht, Laboratory of Paleobotany and Palynology (LPP) Foundation, LPP Contributions series 16, 130 p.

Kouwenberg, L.L.R., McElwain, J.C., Kürschner, W.M., Wagner, F., Beerling, D.J., Mayle, F.E., and Visscher, H., 2003, Stomatal frequency adjustment of four conifer species to historical changes in atmospheric CO₂: *American Journal of Botany*, v. 90, p. 610–619.

Kouwenberg, L.L.R., Kürschner, W.M., and Visscher, H., 2004, Changes in stomatal frequency and size during elongation of *Tsuga heterophylla* needles: *Annals of Botany*, v. 94, p. 1–9.

Kürschner, W.M., Wagner, F., Visscher, E.H., and Visscher, H., 1997, Predicting the response of leaf stomatal frequency to a future CO₂ enriched atmosphere: Constraints from historical observations: *Geologische Rundschau*, v. 86, p. 512–517.

Mann, M.E., and Jones, P.D., 2003, Global surface temperatures over the past two millennia: *Geophysical Research Letters*, v. 30, no. 15, 1820, doi: 10.1029/2003GL017814.

Marchal, O., Stocker, T.F., and Joos, F., 1998, Impact of oceanic reorganizations on the ocean carbon cycle and atmospheric carbon dioxide content: *Paleoceanography*, v. 13, p. 225–244.

Marchal, O., Stocker, T.F., Joos, F., Indermühle, A., Blunier, T., and Tschumi, J., 1999, Modelling the concentration of atmospheric CO₂ during the Younger Dryas climate event: *Climate Dynamics*, v. 15, p. 341–354.

Mullineaux, D.M., 1996, Pre-1980 tephra-fall deposits erupted from Mount St. Helens, Washington: U.S. Geological Survey Professional Paper, v. 1563, p. 1–99.

Neftel, A., Moor, E., Oeschger, H., and Stauffer, B., 1985, Evidence from polar ice cores for the increase in atmospheric CO₂ in the past two centuries: *Nature*, v. 315, p. 45–47.

Osborne, C., 1991, Statistical calibration: A review: *International Statistical Review*, v. 59, p. 309–336.

Plattner, G.K., Joos, F., Stocker, T.F., and Marchal, O., 2001, Feedback mechanisms and sensitivities of ocean carbon uptake under global warming: *Tellus*, v. 53B, p. 564–592.

Raven, J.A., and Falkowski, P.G., 1999, Oceanic sinks for atmospheric CO₂: *Plant, Cell and Environment*, v. 22, p. 741–755.

Royer, D.L., 2001, Stomatal density and stomatal index as indicators of paleoatmospheric CO₂ concentration: Review of Palaeobotany and Palynology, v. 114, p. 1–28.

Ruddiman, W., 2003, The anthropogenic greenhouse era began thousands of years ago: *Climatic Change*, v. 61, p. 261–293.

Rundgren, M., and Beerling, D.J., 1999, A Holocene CO₂ record from the stomatal index of subfossil *Salix herbacea* L. leaves from northern Sweden: *The Holocene*, v. 9, p. 509–513.

Sarmiento, J.L., and Orr, J.C., 1991, Three-dimensional simulations of the impact of Southern Ocean nutrient depletion on atmospheric CO₂ and ocean chemistry: *Limnology and Oceanography*, v. 36, p. 1928–1950.

Siegenthaler, U., Friedli, H., Loetscher, H., Moor, E., Neftel, A., Oeschger, H., and Stauffer, B., 1988, Stable-isotope ratios and concentration of CO₂ in air from polar ice cores: *Annals of Glaciology*, v. 10, p. 151–156.

Stuiver, M., Reimer, P.J., Bard, E., Beck, J.W., Burr, G.S., Hughen, K.A., Kromer, B., McCormac, G., van der Plicht, J., and Spurk, M., 1998, INTCAL98 radiocarbon age calibration, 24,000–0 cal. BP: *Radiocarbon*, v. 40, p. 1041–1084.

Takahashi, T., Olafsson, J., Goddard, J.G., Chipman, D.W., and Sutherland, S.C., 1993, Seasonal variation of CO₂ and nutrients in the high-latitude surface ocean: A comparative study: *Global Biogeochemical Cycles*, v. 7, p. 843–878.

Trudinger, C.M., Rayner, P.J., Enting, I.G., Heimann, M., and Scholze, M., 2003, Implications of ice core smoothing for inferring CO₂ flux variability: *Journal of Geophysical Research*, v. 108, no. D16, 4492, doi: 10.1029/2003JD003562.

Wagner, F., Below, R., De Klerk, P., Dilcher, D.L., Joosten, H.J.H., Kürschner, W.M., and Visscher, H., 1996, A natural experiment on plant acclimation: Lifetime stomatal frequency response of an individual tree to annual atmospheric CO₂ increase: *National Academy of Sciences Proceedings*, v. 93, p. 11,705–11,708.

Wagner, F., Kouwenberg, L.L.R., van Hoof, T.B., and Visscher, H., 2004, Reproducibility of Holocene atmospheric CO₂ records based on stomatal frequency: *Quaternary Science Reviews*, v. 23, p. 1947–1954.

Woodward, F.I., 1987, Stomatal numbers are sensitive to increases in CO₂ concentration from the pre-industrial levels: *Nature*, v. 327, p. 617–618.

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