

Reproducibility of Holocene atmospheric CO₂ records based on stomatal frequency

Friederike Wagner*, Lenny L.R. Kouwenberg, Thomas B. van Hoof, Henk Visscher

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

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Abstract

The majority of the stomatal frequency-based estimates of CO₂ for the Holocene do not support the widely accepted concept of comparably stable CO₂ concentrations throughout the past 11,500 years. To address the critique that these stomatal frequency variations result from local environmental change or methodological insufficiencies, multiple stomatal frequency records were compared for three climatic key periods during the Holocene, namely the Preboreal oscillation, the 8.2 kyr cooling event and the Little Ice Age. The highly comparable fluctuations in the palaeo-atmospheric CO₂ records, which were obtained from different continents and plant species (deciduous angiosperms as well as conifers) using varying calibration approaches, provide strong evidence for the integrity of leaf-based CO₂ quantification.

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1. Introduction

A variety of land plants is capable of sustained adjustment of the number of leaf stomata to changing atmospheric CO₂ concentrations. Measured on fossil leaves and calibrated against modern training sets, stomatal frequency data are increasingly applied as a proxy for palaeo-atmospheric CO₂ reconstructions. The quality and quantity of fossil leaf remains preserved in lake and peat deposits of Holocene age allow the generation of stomatal frequency records from sites worldwide.

The majority of the stomatal frequency-based CO₂ estimates for the Holocene do not support the widely accepted concept of comparably stable CO₂ concentrations throughout the past 11,500 years (Indermühle et al., 1999). The available high-resolution CO₂ reconstructions based on plant fossils suggest that century-scale CO₂ fluctuations contributed to Holocene climate evolution (Rundgren and Beerling, 1999; Wagner et al., 1999a; McElwain et al., 2002; Wagner et al., 2002; Rundgren and Björck, 2003; Kouwenberg, 2004).

Within the ongoing discussion on the alternative concept of a dynamic CO₂ regime, the approach of translating observed shifts in stomatal frequencies in terms of atmospheric CO₂ changes has often been questioned. It has been suggested that these shifts are a consequence of environmental factors other than CO₂ or an artefact of improper assembling and calibration of the modern training sets (Birks et al., 1999; Indermühle et al., 1999b). These comments emphasise the necessity to examine the amplitude and duration of reconstructed CO₂ fluctuations by comparing stomatal frequency records based on taxonomically and ecologically contrasting plant species. The global nature of the CO₂ signal should be revealed by records originating from a wide geographical range.

In the present review, we discuss the reliability of stomatal frequency derived CO₂ records by comparing available data from three different time slices in the Holocene which are known to be phases of major climatic change on the Northern Hemisphere. The first in a series of century-scale Holocene climate deteriorations evident in marine, terrestrial and ice core derived climate reconstructions is the so-called Preboreal oscillation, a short-lived cool pulse recorded at ~11.2 kyr BP, soon after the end of the Younger Dryas (Björck et al., 1997, 1998). The most prominent

*Corresponding author.

E-mail address: r.wagner@bio.uu.nl (F. Wagner).

and best documented cooling in the Holocene is centred around 8.2 kyr BP (Alley et al., 1997; Barber et al., 1999), where fresh water pulses from the melt-down of the Laurentian ice-sheet are thought to have reduced the thermohaline circulation in the North Atlantic for approximately 300 years. Well known from historical records and direct instrumental measurements is the pre-industrial cooling trend of the last millennium, commonly addressed as the Little Ice Age. Interrupted by periods of relative warmth, cool pulses occurred at different times in different parts of the world. In many Northern Hemisphere regions, the Little Ice Age culminated in a series of cool pulses between the 14th and the early 19th century AD.

For all of these three events, multiple stomatal frequency records are available. By directly comparing the results from the contrasting studies, we illustrate that the harmonious variation in stomatal frequency responses cannot be a result of local environmental changes or methodological insufficiencies, but do have their origin in a common, at least hemispherically acting forcing factor, namely atmospheric CO₂ dynamics throughout the Holocene.

2. Discussion

2.1. Modern calibration data sets

The most critical issue in calculating atmospheric CO₂ concentrations from fossil stomatal frequency data is the accuracy of the modern calibration data sets. These training sets enable quantification of the response rates of individual species to atmospheric CO₂ changes and, therefore, serve as reference data for CO₂ estimates from fossil leaves. An unique opportunity to study the leaf morphological adaptation of plants to changing ambient CO₂ is provided by the well-documented continuous CO₂ increase from pre-industrial values of approximately 280 to 375 ppmv present day level. Analysis of herbarium specimens of known age allows us to tie up known historical CO₂ and corresponding stomatal frequencies. Uncertainties in the herbarium data sets due to varying sample localities can be reduced by adding data from continuous, but often less well dated, leaf sequences accumulated in e.g. young peat deposits (Wagner et al., 1996).

Fossil and herbarium leaves grown during the industrial CO₂ rise, however, do not cover CO₂ levels below 280 ppmv, which hampers the statistical modelling of the stomatal response to lower CO₂ concentrations. To obtain data from the lower CO₂ range, the plants' response to CO₂ partial pressure (in Pa) rather than CO₂ mixing ratio (in ppmv) can be utilized (Woodward and Bazzaz, 1988). Because partial pressure decreases with elevation due to the reduced air pressure,

inclusion of leaf material grown at higher altitudes allows extension of the historical training set to CO₂ levels below 28 Pa (equivalent to 280 ppmv at sea level). If the leaf material in the modern training set as well as the fossil assemblages originate from localities at the same elevation, expression of CO₂ levels in either partial pressure or mixing ratio will be of no consequence. However, if leaves from different altitudes are included in the training set or fossil stomatal frequency data, CO₂ levels must be calculated as partial pressure for stomatal frequency calibration (Fig. 1B). The estimated local barometric pressure at the site where the fossil material was derived from, can then be used to reconvert the reconstructed CO₂ partial pressure to mixing ratio, to enable quantitative comparison with other CO₂ reconstructions.

In this manner significant changes in stomatal frequency under changing atmospheric CO₂ have been demonstrated for many woody angiosperms, of which deciduous trees such as *Betula*, *Quercus* and *Ginkgo*, as well as common high latitude shrubs like *Salix* and *Dryas*, show the most pronounced responses. Conifers exhibiting a prominent decrease in stomatal frequency with increasing historical CO₂ include *Tsuga*, *Picea*, *Larix* and *Metasequoia* (see Royer et al., 2001; Kouwenberg et al., 2003). Although desirable, a general model for fossil CO₂ estimates embracing multiple genera cannot be generated, due to the highly individual responses of the various genera (Kürschner et al., 1997; Rundgren and Björck, 2003).

For broad-leaved plant species, the ratio between stomata and the total amount of epidermal cells on the leaf surface (the stomatal index) has been proven to be the most sensitive parameter to quantify their response to CO₂ changes. By applying the cell ratio rather than the pure number of stomata, undesirable effects of lateral epidermal cell expansion due to contrasting light regimes, leaf age or temporary hydrological conditions are circumvented (Poole and Kürschner, 1999). Because of the specific stomatal patterning in the narrow-leaved conifers, the number of stomata per millimetre needle length rather than the stomatal index responds to atmospheric CO₂ levels. Although this parameter is density-based, leaf age and environmental conditions do not mask the adjustment to CO₂ levels (Kouwenberg et al., 2003; Kouwenberg, 2004).

While the conifer species studied show a linear decrease in stomatal frequency under present day atmospheric CO₂, the majority of broad-leaved species currently used for CO₂ reconstructions indicate a decrease in CO₂ sensitivity at values above approximately 320 ppmv (Kürschner et al., 1997). The response patterns on species or genus level determined in the modern training sets require the application of taxon-specific statistical treatments for the individual plant categories to guarantee the best fit of the models for

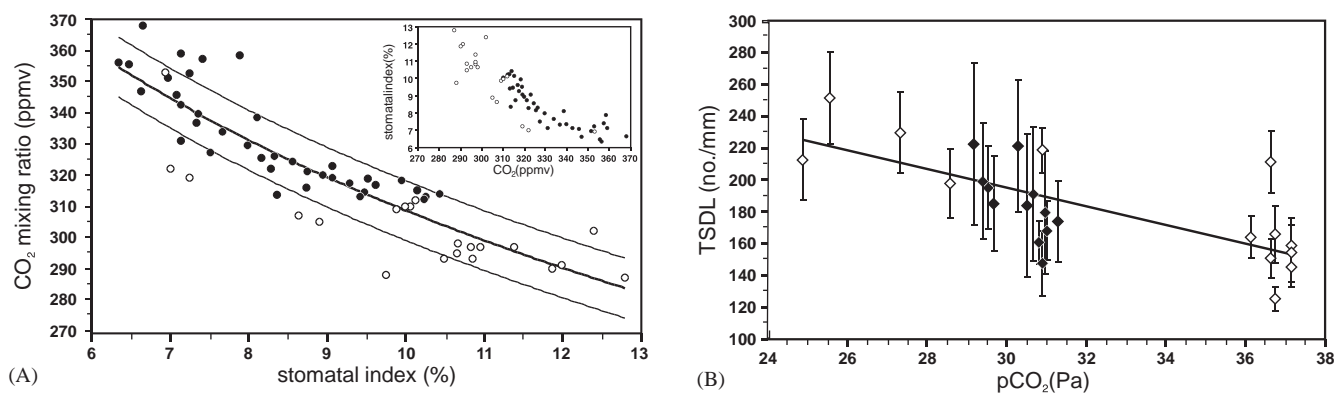


Fig. 1. Modelled relation between atmospheric CO₂ concentration and stomatal frequency in training sets consisting of leaves from herbaria and sub-fossil deposits calibrated against historical CO₂ concentrations. CO₂ mixing ratios of 290–315 ppmv were derived from shallow Antarctic ice cores (<http://www.cdiac.esd.ornl.gov/trends/co2/siple.htm>; Neftel et al., 1985), mixing ratios of 315–368 ppmv are annual means from instrumental measurements at Mauna Loa (<http://www.cdiac.esd.ornl.gov/ndps/ndp001.html>). (A) Thick black line: Model for CO₂ estimates based on linear regression of log-transformed stomatal index (SI) data for *B. pendula/pubescens* ($\text{CO}_2 = 10^{2.802 - [0.313 + \log(\text{SI}_f)]}$; $r^2 = 0.79$); thin lines indicate ± 1 RMSE (= 9.6 ppmv). Inset: historical response of SI to global atmospheric CO₂. Training set includes leaf remains from modern peats (black circles) and herbarium specimens (open circles). (B) Response of number of stomata per millimetre needle length (TSDL) of *T. heterophylla* to a pCO₂ increase from 24 to 38 Pa. CO₂ partial pressure was calculated by multiplying the CO₂ mixing ratio by local barometric pressure P_B (Pa), estimated according to Jones (1992): $P_B = 101.325/e^{[z/(29.3)/T]}$ where z is the altitude above sea level and T the air temperature in K (estimated from mean annual temperature at the closest weather station, corrected by a temperature lapse rate appropriate for the region in case of significant altitudinal difference between site and station). Black diamonds represent sub-fossil and modern needles from Jay Bath (Mount Rainier, WA), open diamonds modern and herbarium needles from other localities. Error bars indicate ± 1 SE. Solid line indicates best fit in classical regression analysis. TSDL: true stomatal density per millimetre needle length ($\text{TSDL} = -5.8581 \times \text{pCO}_2 + 371.14$; $r^2 = 0.5124$; RMSE = 42.8 ppmv).

palaeo-CO₂ estimations. Two contrasting examples of models for inferring palaeo-CO₂ based on modern training sets of broad-leaved trees and conifers, both incorporating herbarium material as well as sub-fossil leaves from peat sequences, are presented in Fig. 1.

Analysis of modern *Betula pendula* and *Betula pubescens* leaves has demonstrated a distinct reduction of the stomatal index over the post-industrial CO₂ increase from 290 to 370 ppmv, with a levelling off in the CO₂ response at values higher than 350 ppmv (Fig. 1A). The good correspondence of the stomatal index response revealed for the two closely related species allows treatment of *B. pendula* and *B. pubescens* as one single group in the model (Fig. 1A, Wagner et al., 2000). In order to accommodate the partial non-linearity of the data while maintaining the best fit, the model for CO₂ estimations from fossil material is based on a log transformation of both SI and CO₂ values in the training set (Fig. 1A).

The stomatal frequency response of *Tsuga heterophylla* in contrast is best described using a linear rather than a non-linear model. The linearity of the response over the entire CO₂ regime from 25 to 37 Pa allows the fossil CO₂ estimation by using a classical linear regression, the most conservative statistical approach (Fig. 1B).

All *Tsuga* and *Betula* data presented hereafter are calibrated according to the models above. As a consequence of the adjusted model for *Betula*, the CO₂

reconstructions based on this genus may differ slightly from initial publications (Wagner et al., 1999a, 2002).

2.2. Palaeo-atmospheric CO₂ reconstructions

Based on the well defined response rates of *Betula* and *Tsuga*, palaeo-atmospheric CO₂ records have been established for three key periods of climate change during the Holocene (Wagner et al., 1999a, 2002; Kouwenberg, 2004). Additional CO₂ records from other species are available for the Preboreal oscillation (McElwain et al., 2002; Rundgren and Björck, 2003), the 8.2 kyr cool pulse (Rundgren and Beerling, 1999) and the Little Ice Age (van Hoof, this study). The present study focuses on the comparability of independent stomatal frequency records in terms of trends in atmospheric CO₂ and the temporal synchronicity of the records. In order to emphasize the amplitude of reconstructed atmospheric CO₂ changes associated with the three Holocene cool pulses, all available records are given in normalized CO₂ concentrations (ppmv); and all ages are given in calibrated calendar years BP.

2.3. The Preboreal oscillation (Fig. 2A and D)

The Preboreal stage of the Holocene is represented in three different stomatal frequency records. *B. pubescens* and *B. pendula* leaf remains were obtained from a peat

section temporarily exposed at the Borchert archaeological excavation site in Denekamp, The Netherlands ($52^{\circ}23'N$, $7^{\circ}00'E$; 30 m a.s.l., Fig. 2A). The section

spans the period from 11,620 to 10,920 cal BP, with a high-resolution age assessment based on a wiggle matching of 18 ^{14}C datings over the 35 cm peat section

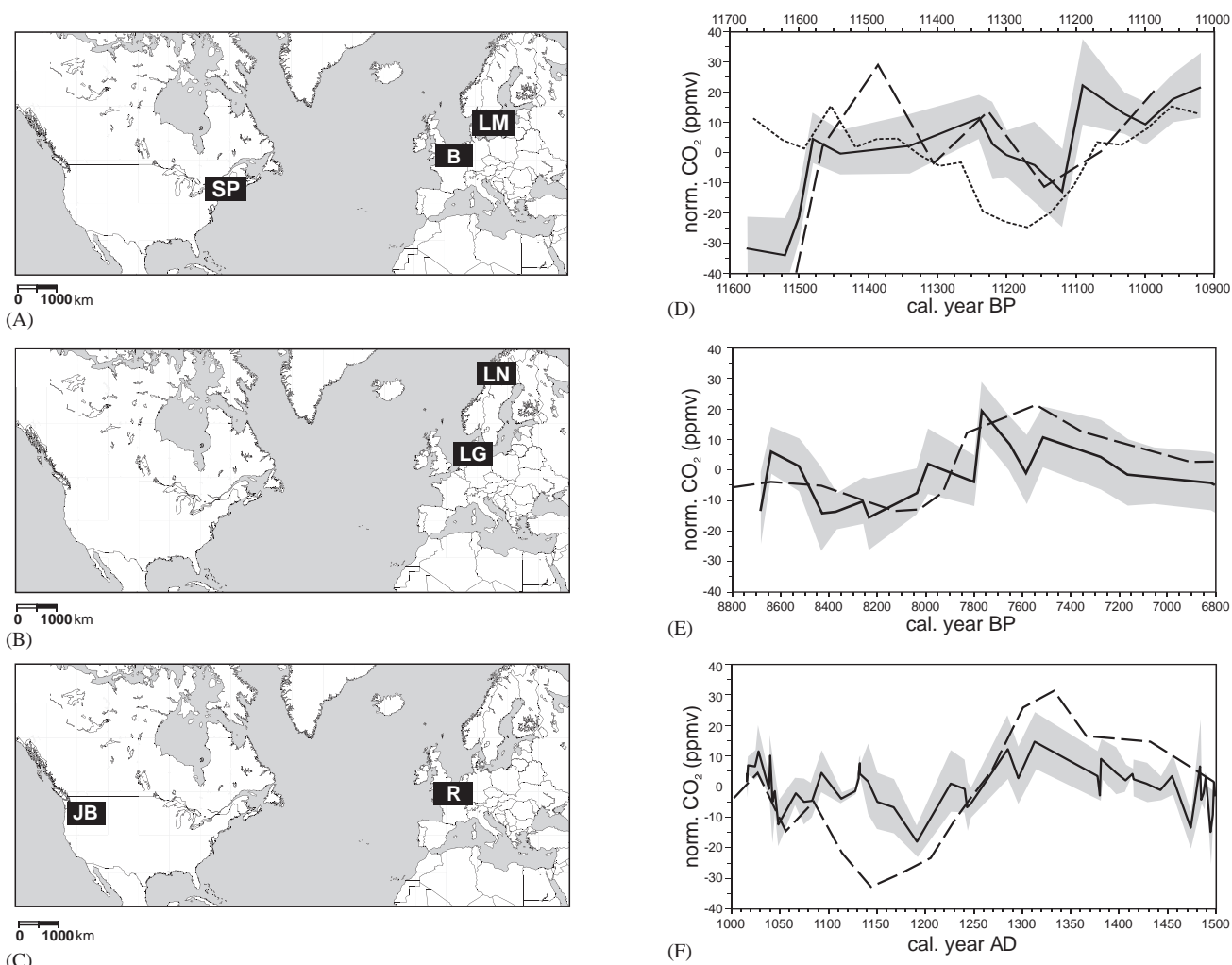


Fig. 2. Localities of the fossil assemblages (A–C) used for stomatal frequency records (D–F). (A) SP=Splan Pond (Canada), B=Borchert (Netherlands) and LM=Lake Madtjärn (Sweden). (B) LG=Lille Gribso (Denmark) and LN=Lake Njulla (Sweden). (C) JB=Jay Bath (Washington, USA) and R=Roer river (Netherlands). (D) Reconstructed normalized CO_2 mixing ratios from three stomatal records (deviations from the long-term average in each record) centred around the time of the Preboreal oscillation. The black line represents unsmoothed normalized mixing ratios based on stomatal index measurements of *B. pubescens* leaves from the Borchert section (Wagner et al., 1999a). The black dashed line represents unsmoothed normalized mixing ratios based on stomatal number per millimetre needle length of *L. laricina* from Splan Pond (McElwain et al., 2002). The dotted line represents a locally weighted average of normalized mixing ratios based on stomatal indices of *S. herbacea*, *S. polaris* and *B. nana* from Lake Madtjärn (Rundgren and Björck, 2003). The time-scale at the bottom of the figure is based on the age assessment of the Borchert and Splan Pond records. The original time-scale of the Lake Madtjärn record is shown at the top of the figure and has been shifted by 100 years (within one ^{14}C standard deviation) to facilitate comparison. The grey confidence interval represents ± 1 SE (standard error of the samples per depth) in the Borchert record. (E) Reconstructed normalized CO_2 mixing ratios based on two different stomatal frequency records (deviations from the long-term average in each record) around the 8.2 kyr cooling event. The black line represents unsmoothed normalized mixing ratios based on stomatal index of *B. pendulapubescens* from Lille Gribso (Wagner et al., 2002). The dashed line represents a five-point moving average of normalized mixing ratios based on stomatal index of *S. herbacea* from Lake Njulla (Rundgren and Beerling, 1999). The stomatal index of the training set was calibrated against CO_2 partial pressure and also expressed in Pa in the original study. Here, the reconstructed partial pressures were converted to mixing ratios using the estimated local barometric air pressure at the site (Fig. 1, Jones, 1992) to allow comparison with the other records. Both records are plotted on a common time-scale and the confidence interval represents ± 1 SE (standard error of the samples per depth) in the Lille Gribso record. (F) Reconstructed normalized mixing ratios based on two different stomatal frequency records around the onset of the Little Ice Age. The black line represents the unsmoothed normalized mixing ratios based on stomatal index of *Q. robur* leaves from the Roer river area (van Hoof et al., in prep.). The dashed line represents a three-point moving average of normalized mixing ratios based on stomatal numbers per millimetre needle length (TSDL) of *T. heterophylla* needles from Jay Bath (Kouwenberg, 2004). The TSDL of the modern training set was calibrated against CO_2 partial pressure and the reconstructed partial pressures were converted to mixing ratios using the estimated local barometric air pressure (Fig. 1, Jones, 1992). The grey confidence interval represents ± 1 SE (standard error of the samples per depth) in the Roer record.

(van der Plicht et al., 2004). The stomatal index values from this record are calibrated according to the model in Fig. 1A and provide evidence for a short-term CO₂ decrease between 11,250 and 11,080 cal BP with a CO₂ minimum at 11,120 cal BP (Fig. 2D).

This distinct minimum is supported by stomatal frequency analysis of *Larix laricina* needles preserved in a lake in New Brunswick, Canada (Fig. 2A and D, Splan Pond, 45°14'N, 67°06'W, 106 m a.s.l., age assessment based on two AMS ¹⁴C datings for this interval; McElwain et al., 2002). The timing of the CO₂ decrease documented in both records, parallels the Preboreal oscillation sensu Björck et al. (1997) which is equivalent to the GH 11.2 temperature decline documented in Greenland ice cores (INTIMATE, 1998).

Further evidence for a CO₂ decrease during this time is provided by a stomatal frequency record based on *Salix herbacea*, *Salix polaris* and *Betula nana* leaves from Lake Madtjärn in southwestern Sweden (58°35'N, 12°10'E; 135 m a.s.l.; Rundgren and Björck, 2003). The age assessment for this record is based on the age-depth model for the entire Madtjärn profile, in which the early Preboreal is covered by six AMS ¹⁴C datings. A slight temporal offset of 100 calendar years between this record and the two records above might be an artefact of the selected age-depth model for the earliest Holocene (see Rundgren and Björck, 2003 for original data).

Consistent in all records, the shifts in stomatal frequency indicate a change in the atmospheric CO₂ concentration of 20–30 ppmv associated with the Preboreal oscillation.

2.4. The 8.2 kyr BP cooling event (Fig. 2B and E)

For the time slice of the 8.2 kyr cool pulse, two stomatal frequency-based CO₂ reconstructions are available (Fig. 2E). The first record (Wagner et al., 2002) is based on the stomatal index values from European tree birch leaves derived from organic rich gyttja deposits from Lille Gribso, a small kettle hole lake North of Copenhagen, Denmark (55°58'N, 12°18'E; 45 m a.s.l., Fig. 2B). Well-preserved *B. pubescens* and *B. pendula* leaf remains occur continuously through an interval corresponding to the period between 8700 and 6800 cal BP. Chronological control is provided by a series of six AMS ¹⁴C dates measured on single birch leaves (Wagner et al., 2002).

The second record is based on *S. herbacea* leaf material from 10 horizons accumulated in a small lake close to Abisko, Northern Sweden (Lake Njulla, 68°22'N, 18°42'E; 999 m a.s.l.; Rundgren and Beerling, 1999, Fig. 2B). For the time interval of interest three ¹⁴C datings are available. The two data sets consistently reveal a century-scale interval of 30 ppmv CO₂ concentration changes with lowest CO₂ levels centred around 8.2 kyr BP.

2.5. The last millennium (Little Ice Age) (Fig. 2C and F)

For the period between AD 1000 and 1500, covering part of the Little Ice Age, two independent stomatal frequency reconstructions are shown in Fig. 2F.

T. heterophylla needle assemblages were obtained from a 91 cm sediment core drilled in Jay Bath, a shallow pond on the southern flank of Mount Rainier (Washington, USA; 46°46'N 121°46'W; 1311 m a.s.l., Fig. 2C). The age assessment for this site is established on five AMS ¹⁴C dates and one tephra layer at AD 1481 (Kouwenberg, 2004). The *T. heterophylla* stomatal frequency results in this study are calibrated according to the modern training set shown in Fig. 1B. Two CO₂ minima are evident in the record, a smaller one around AD 1050 and a very pronounced minimum centred around AD 1150 (Fig. 2F). Maximum CO₂ levels are registered during the 14th century, followed by a steady CO₂ decrease.

These initial results are confirmed in a high-resolution record of fossil *Quercus robur* leaves from an oxbow lake of the river Roer, near Sint Odiliënberg, The Netherlands (51°08'N, 6°00'E; 25 m a.s.l., Fig. 2C; van Hoof, this study). Wiggle match dating of 11 AMS ¹⁴C datings provides a precise age assessment of the 60 leaf-rich layers in this section, covering the period from AD 1000 to 1500. The CO₂ reconstruction based on *Q. robur* leaves reproduces and substantiates in detail the short-term CO₂ decrease around AD 1050. The second minimum in this high-resolution record is assigned to the late 12th to early 13th century. In good agreement with the *T. heterophylla* record, the *Q. robur* data indicate decreasing CO₂ concentrations during the late 14th and 15th century. Both records provide independent evidence for rapid CO₂ fluctuations on time-scales varying from decades to centuries. While the estimated amplitudes of 20 to 30 ppmv in the *Q. robur* record are in good agreement with the fluctuations documented for the Preboreal oscillation and the 8.2 kyr event, the maximum change up to 60 ppmv estimated in the *T. heterophylla* record exceeds the other records. The difference, however, is within or close to the standard error of the *Q. robur* record and may be caused by the comparably lower accuracy of the modern training set for *T. heterophylla* (Fig. 1B).

Temperature reconstructions for the period of the Little Ice Age based on marine and terrestrial evidence show a series of short, moderately cool pulses rather than the very pronounced single century-scale coolings of the Preboreal oscillation and the 8.2 kyr event. This pattern is also evident in the stomatal frequency-based CO₂ reconstructions.

The compilation and detailed comparison of the seven records provides an indirect but powerful assessment of the reliability of stomatal frequency analysis as a proxy

for palaeo-atmospheric CO₂ concentrations. So far, the validation of stomatal frequency as a sensitive parameter to changing CO₂ concentrations has basically been performed for individual species (see, Royer, 2001 for review). Potential influences of environmental factors other than CO₂, e.g. light, water availability and temperature, have frequently been tested in experiments under controlled growth conditions. The results obtained in a controlled and artificial environment in growth experiments, however, cannot unambiguously be transferred to responses under the natural growth conditions in the field. Field studies, on the other hand, provide insight in the intrinsic variability under natural growth conditions but observed variations are often a response to a combination of environmental parameters.

By directly comparing the generated CO₂ estimations based on independent stomatal frequency records, species-specific uncertainties may be minimized, which permits testing of the overall quality of the CO₂ reconstructions.

Taking into account the wide geographical area the investigated leaf material originates from, the difference in photoperiod over the covered latitudinal range from 45°N to 68°N could potentially affect the stomatal frequency records. Light intensity and photoperiod have been long known to strongly affect stomatal frequency (Schürmann, 1959; Kürschner et al., 1996; Poole et al., 1996; Wagner et al., 2000). While light intensity primarily regulates epidermal cell expansion and, therefore, influences stomatal densities, prolonged photoperiods lead to enhanced stomatal initiation rates quantified in the stomatal index (Schürmann, 1959; Wagner et al., 2000). The dependency on photoperiod may consequently cause erroneous data when stomatal index values from contrasting latitudes are compared to data from a restricted latitudinal range (Wagner et al., 1999b).

CO₂ estimates from high and mid-latitudes are combined in the second case study, the 8.2 kyr event, where stomatal index data of *Betula* leaves from Denmark and *S. herbacea* data from Northern Sweden are available. The calibration data for *Betula* (Fig. 1A) are based solely on leaf material from Denmark and the Netherlands, since field studies have demonstrated the high sensitivity of birch to changes in photoperiod (Wagner et al., 2000). No such dependency is known for *Salix*, and calibration data are derived from a wide latitudinal range (Rundgren and Beerling, 1999). Independent of the different approaches, the good correspondence of palaeo-CO₂ estimates from the high and mid-latitude records strongly suggests that latitudinal differences in photoperiod did not bias these data sets.

Only very sparse information is available so far on the potential influence of temperature on stomatal frequency. Controlled environment experiments with *B.*

pendula under (extremely) different growth temperatures provide evidence for a positive correlation between the stomatal index and temperature for this particular species (Wagner, 1998). No interaction between stomatal frequency and temperature was observed in experiments with cotton (Reddy et al., 1998). Comparison of spring and annual temperatures near Jay Bath with the stomatal frequency of the sub-fossil *T. heterophylla* needles during the last 90 years showed no correlation (Kouwenberg et al., 2003). Although the available data are not unequivocal, they suggest that the temperature changes are unlikely to have caused the observed changes in the individual stomatal frequency records. The three periods discussed here are generally referred to as cool pulses, but the regional temperature changes have not been uniform over the Northern Hemisphere. The good agreement of stomatal frequency records from sites located at different altitude, latitude and longitude with their individual temperature ranges minimizes the possibility of temperature changes as the responsible factor for the observed parallel changes in the record.

Effects of water availability on epidermal morphology are well known. Drought stress for instance leads to the development of distinct xeromorphic features during leaf development (Bosabalidis and Kofidis, 2002; Li and Wang, 2003). One of the most common and pronounced effect observed in growth experiments is the reduced lateral epidermal cell expansion under drought conditions (Bosabalidis and Kofidis, 2002; Li and Wang, 2003). The stomatal index, however, is not influenced by water shortage in these performed experiments. Under natural growth conditions, the comparison of actual precipitation data for the last 70 years and the stomatal frequency of *T. heterophylla* shows no correlation (Kouwenberg et al., 2003).

This observational evidence for the independence of the stomatal frequency parameters from precipitation is corroborated by the good agreement between the CO₂ records in spite of the highly regional precipitation surpluses or deficits associated with the periods of climate change studied. Again, as with temperature, the broad geographical distribution of the sites studied provides evidence for the independence of the CO₂ records from precipitation changes associated with the Preboreal oscillation, the 8.2 kyr event and the Little Ice Age.

Besides the potential impact of environmental factors other than CO₂ concentrations, genetic variations within individual plant species or hybridization of related species could be a potential cause for the changing stomatal frequency patterns. Studies on genetically controlled leaf material from *B. pendula* and *B. pubescens*, however, have shown that at least for these two species the influence of genetic difference can be neglected (Fig. 1A, Wagner et al., 2000). In cases where the species-specific stomatal frequency response

does not allow a grouping, single site CO₂ reconstructions based on multiple species can be derived by developing separate calibration data sets as demonstrated for *S. polaris* and *S. herbacea* (Rundgren and Björck, 2003). The approach of combining CO₂ estimates including a wide range of taxonomically contrasting plant types in this study a priori excludes any influence of taxonomic or genetic nature as shown by the consistency of CO₂ reconstructions derived from broad-leaved trees, herbaceous shrubs and conifers, or a combination of those.

3. Conclusions

The successful replication of stomatal frequency records in terms of timing and duration in the seven compared records provides strong evidence for the integrity of the leaf-based proxy for atmospheric CO₂ concentrations. The general coherence of the reconstructed amplitudes of atmospheric CO₂ fluctuations corroborates the assumption that a wide range of terrestrial plants shows a common response to this environmental factor independent of geographical setting, habitat conditions or taxonomy.

The agreement between stomatal frequency records from the Atlantic realm and sites located in the Pacific Northwest of the USA indicates that the observed stomatal parameter shifts are not restricted to the circum North Atlantic sector, but are at least Northern Hemispheric in nature. The demonstrated ability of stomatal frequency analysis to generate independent but highly comparable proxy records clearly meets the requirements for a palaeo-proxy in the field of global atmospheric CO₂ dynamics.

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