Stomatal evidence for a decline in atmospheric CO_2 concentration during the Younger Dryas stadial: a comparison with Antarctic ice core records

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ABSTRACT: A recent high-resolution record of Late-glacial CO_2 change from Dome Concordia in Antarctica reveals a trend of increasing CO_2 across the Younger Dryas stadial (GS-1). These results are in good agreement with previous Antarctic ice-core records. However, they contrast markedly with a proxy CO_2 record based on the stomatal approach to CO_2 reconstruction, which records a ca. 70 ppm mean CO_2 decline at the onset of GS-1. To address these apparent discrepancies we tested the validity of the stomatal-based CO_2 reconstructions from Kråkenes by obtaining further proxy CO_2 records based on a similar approach using fossil leaves from two independent lakes in Atlantic Canada. Our Late-glacial CO_2 reconstructions reveal an abrupt ca. 77 ppm decrease in atmospheric CO_2 at the onset of the Younger Dryas stadial, which lagged climatic cooling by ca. 130 yr. Furthermore, the trends recorded in the most accurate high-resolution ice-core record of CO_2, from Dome Concordia, can be reproduced from our stomatal-based CO_2 records, when time-averaged by the mean age distribution of air contained within Dome Concordia ice (200 to 550 yr). If correct, our results indicate an abrupt drawdown of atmospheric CO_2 within two centuries at the onset of GS-1, suggesting that some re-evaluation of the behaviour of atmospheric CO_2 sinks and sources during times of rapid climatic change, such as the Late-glacial, may be required. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: atmospheric carbon dioxide concentration; stomatal density; Younger Dryas; Atlantic Canada.

Introduction

Understanding how atmospheric CO_2 concentrations fluctuated during episodes of abrupt environmental changes, particularly those characterising the Late-glacial (ca. 14 000 to 10 000 yr BP), has important implications for our ability to predict future climatic responses and potential feedback to anthropogenic increases in CO_2. A recent high-resolution ice-core record from Dome Concordia in Antarctica demonstrates that the atmospheric CO_2 concentration increased by 76 ppm in four incremental steps between the Last Glacial Maximum (ca. 18 000 yr BP) and the early Holocene (ca. 11 000 yr BP) (Monnin et al., 2001). Moreover, a trend of gradually rising CO_2 from 237 ppm to 259 ppm was recorded by these authors

across the Younger Dryas stadial (now referred to as Greenland Stadial 1 or GS-1, Björck et al., 1998), an event which marks an abrupt return to near-glacial conditions in the North Atlantic between 12 900 and 11 500 yr BP. These results are in good agreement with the general trends in atmospheric CO_2 recorded in existing Antarctic ice-cores from Byrd (Neftel et al., 1988) and Vostok (Barnola et al., 1987). However, they contrast markedly with the pattern of CO_2 change and absolute concentrations reconstructed over the same interval from changes in leaf macrofossil stomatal density (Beerling et al., 1995). This stomatal-based record from Kråkenes in Norway (Beerling et al., 1995) shows a significant and rapid decline in atmospheric CO_2 from median values of 273 to 210 ppm at the onset of GS-1. Clearly, as CO_2 records from three Antarctic ice-cores are in good agreement in terms of the general trend of CO_2 change over this important interval, further investigation of the trends derived using the stomatal approach to atmospheric CO_2 reconstructions is required.

The stomatal approach to estimating palaeo-CO_2 levels relies on a well-established inverse relationship between

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atmospheric CO_2 concentration and stomatal frequency of terrestrial leaves of many C3 species (Woodward, 1987). Stomatal frequency of some species, however, particularly grasses and C4 plants, are insensitive to changes in CO_2 concentration (Raven and Ramsden, 1989; Praet and Jiang, 1995). For those species that are CO_2 sensitive, an inverse relationship between CO_2 concentration and stomatal frequency has been demonstrated on time-scales of weeks (Woodward and Bazzaz, 1988), years (Beerling et al., 1998), centuries (Woodward, 1987; Beerling and Chaloner, 1993; Wagner et al., 1996, 1999) and millennia (McElwain et al., 1995; Rundgren and Beerling, 1999). Furthermore, a genetic basis for the response is provided by the recent identification of a CO_2 sensitive gene in Arabidopsis thaliana that is involved in the control of stomatal development from epidermal cell initials (Gray et al., 2000). The robustness of this proxy-CO_2 method is further supported by a high-resolution stomatal-based CO_2 reconstruction spanning the Holocene (Rundgren and Beerling, 1999), which demonstrates almost identical trends in atmospheric CO_2 change through the Holocene as those measured from Taylor Dome (Indermühle et al., 1999). The striking similarity in the general trends of CO_2 change spanning the Holocene reconstructed from stomatal-based and ice-core methods highlights the need to investigate further their apparent differences during the Late-glacial period.

Here we present two new Late-glacial stomatal-based CO_2 records from different lake sites in Atlantic Canada and critically compare them with existing ice-core and stomatal-based records to address these apparent discrepancies between proxy and ice-core CO_2 records. The accuracy of Late-glacial CO_2 records from Byrd in Antarctica (Neftel et al., 1988) have been questioned recently, however, owing to a large scatter of data during GS-1 (of up to 30 ppm) (see fig. 2 in Blunier et al., 1997) and in the early Holocene (of >30 ppm) (Indermühle et al., 1999; Marchal et al., 1999). Because these effects may reflect contamination within the ice, we have confined comparison of our data to CO_2 records from Dome Concordia, which are currently considered the most accurate high-resolution reconstructions available for the Late-glacial (Monnin et al., 2001). Similarly, although CO_2 reconstructions from Vostok and Taylor Dome are also considered accurate and reliable, only six measurements are available between 16 000 and 10 000 yr BP in the case of the former (Barnola et al., 1987) with no records available across GS-1 in the case of the latter (Indermühle et al., 1999).

High-resolution (ca. 20 to 37 calendar yr) proxy-CO_2 records were determined from stomatal frequencies of subfossil leaves of Dryas integrifolia, Picea mariana, P. glauca and Larix laricina obtained from sediment cores from Splan Pond and Pine Ridge Pond. The proxy-CO_2 record from Pine Ridge Pond was compared with a chironomid-derived terrestrial temperature record (Walker et al., 1992; Levesque et al., 1994) from the same core in order to investigate the phase relationship between CO_2 and temperature change on a centennial time-scale during GS-1. This was undertaken because, despite important advances in synchronisation of Arctic and Antarctica temperature records using atmospheric methane (Blunier et al., 1997; Blunier and Brook, 2001) and molecular O_2 (Sowers and Bender, 1995), the error associated with the gas age and ice age difference in Dome Concordia (±200 to ±550 yr; Monnin et al., 2001) makes it difficult to assess the phase relationships between temperature and CO_2 on centennial time-scales. This critically hampers our understanding of the cause and effect underpinning rapid climatic fluctuations.

Locality information

Pine Ridge Pond (45°34′N, 67°06′W) and Splan Pond (45°15′20″N, 67°19′50″W) are located in New Brunswick, Atlantic Canada (Fig. 1). Pine Ridge Pond is 100 m in diameter and situated within the Saint Croix Highlands at an elevation of 90 m, within the Appalachian physiographic region (Levesque et al., 1994); Splan Pond is ca. 4 ha in area, 106 m in elevation, and is located 7.2 km northwest of St Stephen, New Brunswick (Mayle and Cwynar, 1995). Both sites have AMS ^{14}C-based chronologies (Mayle et al., 1993).

Methods

Calculation of stomatal frequency

Stomatal frequency of Angiosperm leaves is conventionally expressed in terms of stomatal density (SD), the number of stomata per mm^2 leaf area, and stomatal index (SI), the proportion of stomata expressed as a percentage of epidermal cells. The arrangement of stomata into rows or bands parallel to the long axis in our three conifer species (P. mariana, P. glauca and L. laricina) prevented assessment of stomatal frequency based on area (i.e. SD and SI). Instead, stomatal frequency was calculated from the sum of the mean abaxial and adaxial number of stomata per 1 mm row length (SNL, stomatal number per length) in the case of L. laricina. In the case of P. mariana and P. glauca the abaxial surface SNL was calculated and multiplied by the mean number of stomatal rows per leaf (SDL, stomatal density per length). Stomatal frequency in D. integrifolia was measured conventionally by calculating the stomatal density on the abaxial leaf surface (see McElwain et al. (1995) and Poole and Kürschner (1999) for details on methods). Stomata were observed and counted using a Leica epifluorescence microscope (×400 magnification) within a 0.189 mm^2 graticule. The stomata of all three conifer species were counted using a Leica epifluorescence microscope (×200 magnification) along the length of a calibrated 0.8 mm scale bar.
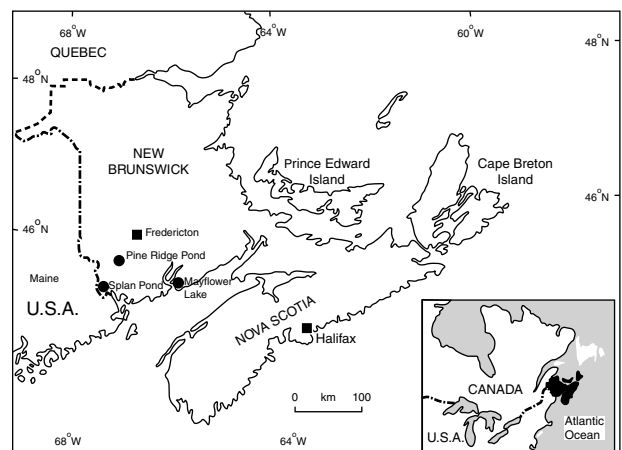

Figure 1 Site map showing the locations of Pine Ridge Pond, Splan Pond and Mayflower Lake in Atlantic Canada

Sources of historical leaf material

Leaf material was obtained from herbarium sheets from the Royal Botanical Gardens Kew of *L. laricina* (collected between 1822 and 1964), *P. mariana* (1875–1999), *P. glauca* (1851–1992) and *D. integrifolia* (1766–1988). In the case of *Picea*, both *P. mariana* and *P. glauca* were incorporated into a single training set as both species showed similar SDL response rates to CO_2. Herbarium material for all four species represented in the lake sediment cores was chosen from a wide range of localities in northern USA and Canada, within the individual species natural climatic ranges and tolerances, so as to minimise the potential effects of other biological and environmental factors on stomatal density. Herbarium leaves of cultivated trees from Scotland, North America and England were also included in the calibration data sets as no significant differences in stomatal frequencies were observed between cultivated and naturally growing trees collected in the same year and which had therefore grown in the same CO_2 concentration. Leaf material from herbarium sheets collected from high elevations was not included in the calibration data sets in order to avoid the effects of decreased CO_2 partial pressure with increasing elevation on stomatal frequency.

Calibration of historical data sets

Species- and genus-specific responses of stomatal frequency (i.e. SD, SNL and SDL) to atmospheric CO_2 concentration were determined by calibrating historical stomatal frequency data obtained from herbarium sheets from the Royal Botanical Gardens Kew against the historically recorded CO_2 concentrations at the time of growth (Neftel *et al.*, 1988; Keeling and Whorf, 2000). Mean stomatal frequency (SD, SNL and SDL) of two to ten observations per leaf was regressed against CO_2 concentration in which the leaf developed. In cases where more that one leaf was available for the same CO_2 concentration a mean stomatal frequency from three to six leaves was obtained, each with two to ten observations per leaf (i.e. *Dryas* and *Picea*). A total of 79 *D. integrifolia* leaves, 62 *Picea* and 30 *Larix* needles were utilised for the calibration data sets. Inherent variability within stomatal density caused by factors such as light intensity, temperature and water availability, although minimal compared with the effect of CO_2 concentration, are all accounted for within the 90% confidence limits of the regression equations (Fig. 2).

Calculating CO₂ concentration from stomatal frequency

Changes in atmospheric CO_2 concentration ($\hat{x}$) during GS-1 were determined from stomatal frequencies ($y' = $ SD, SDL or SNL) of subfossil leaves of *Dryas integrifolia*, *Picea mariana*, *P. glauca* and *Larix laricina* preserved in Pine Ridge Pond and Splan Pond (Tables 1 and 2). Intrinsic variation in stomatal frequency within and among leaves/needles was calculated from means of three leaves/needles per tree/shrub and five to ten areas per leaf. A total of 119 and 130 leaves were investigated from Pine Ridge Pond and Splan Pond respectively. Subfossil stomatal frequency records were calibrated against taxon-specific historic training sets (Fig. 2) using inverse regression analysis (Draper and Smith, 1981) according to $\hat{x} = \dfrac{335.4 - y'(SDL)}{0.795}$ for *Picea spp.*, $\hat{x} = $
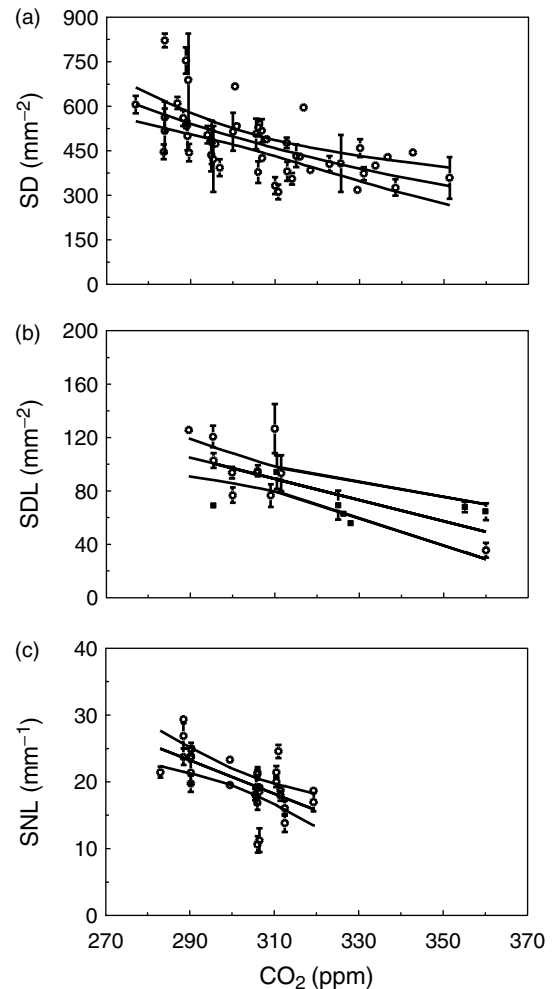

Figure 2 Regression plots of modern training sets with 90% confidence intervals between stomatal frequency and atmospheric CO_2 for (a) *Dryas integrifolia*, ($r = 0.62$, $n = 46$) (b) *Picea mariana* and *P. glauca* ($r = 0.71$, $n = 18$) and (c) *Larix laricina* ($r = 0.617$, $n = 30$). In (a) and (b) the error bars represent the standard error of a mean stomatal frequency per two to ten observations per leaf or a mean stomatal frequency (SD or SDL) from three to six leaves per CO_2 concentration, each with two to ten observations per leaf. In (c) the error bars represent the standard error of a mean stomatal frequency of two to five observations per leaf

$\dfrac{96.38 - y'(SNL)}{0.252}$ for *Larix laricina* and $\hat{x} = \sqrt{\dfrac{55990885.4}{y'(SD) + 123.9}}$ for *D. integrifolia*. Where two species occurred together at the same depth, a mean predicted CO_2 concentration was obtained. Confidence limits for the predicted CO_2 concentration were calculated by applying the following formula (adapted by P. Mitchell from Draper and Smith (1981)) for inverse confidence limits

$$\overline{x} + \left(\frac{y' - \overline{y}}{b(1-g)} \right) \pm \left(\frac{ts}{b(1-g)} \right)$$

$$\times \sqrt{\left(\frac{(1-g)}{m} + \frac{(1-g)}{n} + \frac{(y' - \overline{y})^2}{b^2 SS_x} \right)}$$

where $\overline{x}$ and $\overline{y}$ are the mean CO_2 concentration and stomatal frequency respectively of the taxon-specific historical data set (Fig. 2), y' (SD, SDL or SNL) is the subfossil stomatal frequency, t is Student's t for $(n-2)$, b is the slope, SS_x is the sum-of-squares of x, $g = \dfrac{t^2 s^2}{b^2 SS_x}$, s^2 is the residual mean square, m is the total number of leaf samples per depth and n is the

sum of observations on all leaves per depth. In the case of *D. integrifolia*, the slope (*b*) was calculated according to

$$b = \frac{1}{-\sqrt{\frac{55990885.4}{(SD + 123.9)^{\frac{1}{2}}}}}.$$

However, as no single species spans the entire Late-glacial period (Levesque *et al.*, 1994; Mayle and Cwynar, 1995), the resultant CO_2 reconstructions for both ponds (Fig. 3) are composites of several species CO_2 records; the Late-glacial interstadial (Greenland Interstadial-1, GI-1, Björck *et al.*, 1998), Younger Dryas (GS-1) and early Holocene records being predominantly derived from stomatal frequencies of *Picea*, *Dryas integrifolia* and *Larix laricina* respectively. This approach is justified by the observation that the training sets of each species respond in the same direction and by a similar rate to CO_2 concentration (Fig. 2), and where species-specific estimates overlap, both yield closely matching CO_2 values.

The phase relationship between CO_2 concentration and GS-1 temperature change was determined by comparison between maxima and minima in the Pine Ridge Pond atmospheric CO_2 curve with those from a previous independently derived chironomid (midge)-based water temperature reconstruction (Levesque *et al.*, 1994, 1997) from the same sediment core (Fig. 3).

Chronology

Pine Ridge Pond

The lower GS-1 boundary (673 cm) at Pine Ridge Pond is dated by AMS ^{14}C dating of terrestrial plant macrofossils to 10820 ± 70[14]C yr BP (Levesque *et al.*, 1994) (12 900 cal. yr BP, Stuiver *et al.*, 1998), in close agreement with the mean of 10 770 ^{14}C yr BP obtained from five nearby Late-glacial sites (Mayle *et al.*, 1993). Although four AMS dates further up the sequence are regarded as anomalously young (Levesque *et al.*, 1994), correlation with similar stratigraphical changes in chironomid-based lake surface water temperature, lithology and pollen from eight nearby AMS ^{14}C-dated Late-glacial sites, allow confident assignment of an age estimate of ca. 10 000 ^{14}C yr BP (11 400 cal. yr BP) to the upper GS-1 boundary (Levesque *et al.*, 1993, 1997; Mayle *et al.*, 1993, Mayle and Cwynar, 1995). Interpolation between upper and lower GS-1 boundary estimates reveals that 1 cm of YD sediment is equivalent to ca. 20 ^{14}C yr (37 calendar yr).

Splan Pond

Estimates of 10 930 ^{14}C yr BP and 10 120 ^{14}C yr BP were obtained for the lower and upper GS-1 boundaries respectively for Splan Pond, based on a linear regression through four GS-1 AMS ^{14}C dates (Mayle *et al.*, 1993). Interpolation between these upper and lower GS-1 boundary ages reveals that 1 cm of YD sediment is equivalent to ca. 12 ^{14}C yr (20 calendar yr). The CO_2 records from both ponds are plotted against depth, and cross-correlated by means of the onset and termination of the major climatic events (i.e. GS-1 and GI-1b), expressed by loss-on-ignition (LOI) curves (Levesque *et al.*, 1993, 1994). Onsets are defined as the depth at which the percentage LOI begins to decline, and terminations as the depth at which the percentage LOI reaches its former value. See discussion in Mayle *et al.* (1993) for justification of this approach.
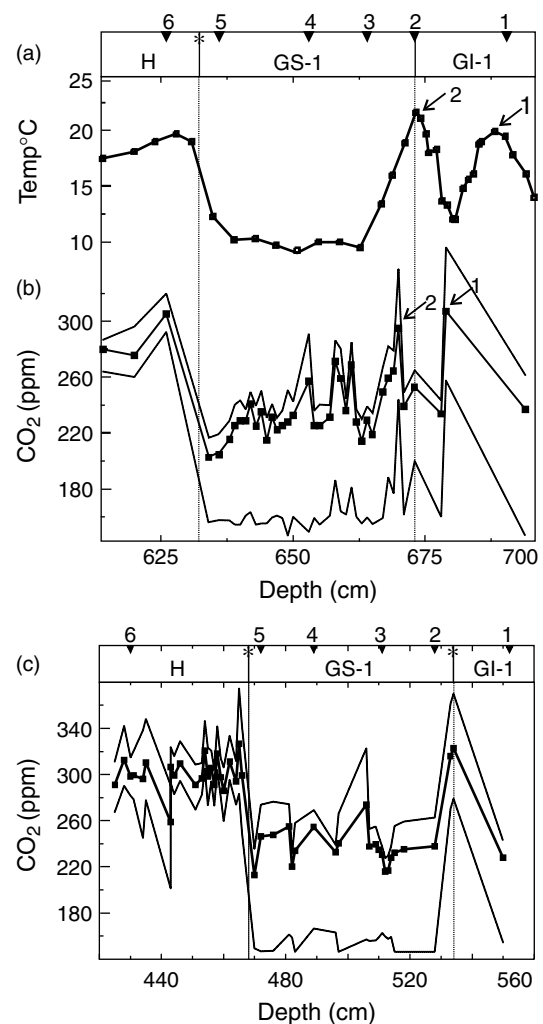

Figure 3 Atmospheric CO_2 reconstructions from fossil leaves compared with lake surface-water temperature change: (a) lake summer surface-water temperatures from Pine Ridge Pond subfossil chironomid remains (Levesque *et al.*, 1994); (b) Pine Ridge Pond CO_2; (c) Splan Pond CO_2. The envelope in (b) and (c) represents the 90% inverse confidence limits of the reconstructions calculated by inverse regression. Peaks in CO_2 were compared with peaks in temperature (indicated by numbered arrows 1 and 2) to determine their phase relationship. The onset and termination of the Younger Dryas stadial (GS-1) are defined by the decline and rise in temperature and loss-on-ignition (Levesque *et al.*, 1994; Mayle *et al.*, 1993) in Splan and Pine Ridge ponds. AMS radiocarbon dates (▲) and assigned dates (*) (see methods) are indicated by
1 = 11 490 ± 80, 2 = 10 820 ± 70, 3 = 10 160 ± 70,
4 = 10 020 ± 90, 5 = 9760 ± 70, 6 = 9500 ± 100[14]C yr in (b) and
1 = 11 640 ± 90, 2 = 10 960 ± 80, 3 = 10 970 ± 70,
4 = 10 430 ± 90, 5 = 10 090 ± 70, 6 = 8830 ± 70[14]C yr in (c).

Results

The reconstructed composite CO_2 record from Pine Ridge Pond (Fig. 3) reveals an abrupt CO_2 decline from mean Late-glacial Interstadial (GI-1a and 1c, Björck *et al.*, 1998) values of 301 ± 6 ppm to mean values for the entire GS-1 of 235 ± 2 ppm. Low CO_2 concentrations prevailed throughout GS-1 (Fig. 3) and increased abruptly at its termination to mean early Holocene values of 286 ± 16 ppm. An abrupt 77 ppm decline in atmospheric CO_2 at the onset of GS-1 is estimated to have occurred within two centuries (5 cm of sediment is equivalent to ca. 100 radiocarbon yr or 185 calendar yr) based on the age–depth relationship (Mayle *et al.*, 1993).

Table 1 Pine Ridge Pond stomatal frequency data. SD, SDL and SNL represent stomatal density, stomatal density per length and stomatal number per length respectively. See methods for calculation. m is the total number of leaf samples per depth and n is the sum of observations on all leaves per depth

Depth (cm)	Species	SD	SDL	SNL	s.e	m	n
694	*D. integrifolia*	874			39	1	3
679	*Picea sp*		89		9.4	2	7
678	*D. integrifolia*	901			38	4	7
671	*D. integrifolia*	855			50	4	12
670	*Picea sp*		100		5.8	1	7
669	*D. integrifolia*	676			30	4	14
668	*D. integrifolia*	762			65	4	14
668	*Picea sp*		120		8.5	1	5
667	*D. integrifolia*	778			30	2	8
665	*D. integrifolia*	1044			82	2	4
664	*D. integrifolia*	941			53	4	17
663	*D. integrifolia*	1097			76	2	10
662	*D. integrifolia*	954			65	5	15
661	*D. integrifolia*	652			18	4	15
660	*D. integrifolia*	882			22	5	13
659	*D. integrifolia*	711			55	2	6
658	*D. integrifolia*	637			50	5	15
657	*D. integrifolia*	924			50	5	15
655	*D. integrifolia*	978			28	2	5
654	*D. integrifolia*	978			50	4	11
653	*D. integrifolia*	722			38	1	4
650	*D. integrifolia*	911			46	3	14
649	*D. integrifolia*	955			200	1	2
648	*D. integrifolia*	978			28	4	8
647	*D. integrifolia*	1007			46	8	14
646	*D. integrifolia*	924			28	4	15
645	*D. integrifolia*	1089			53	2	4
644	*D. integrifolia*	889			89	2	2
643	*D. integrifolia*	985			79	2	6
642	*D. integrifolia*	841			48	5	13
641	*D. integrifolia*	946			29	6	17
640	*D. integrifolia*	946			95	2	7
639	*D. integrifolia*	978			101	2	4
638	*D. integrifolia*	1081			65	3	6
636	*D. integrifolia*	1232			62	4	7
634	*D. integrifolia*	1251			129	3	7
626	*L. laricina*		20		3	1	2
620	*Picea sp.*			116	5.7	1	5
614	*L. laricina*		26		0.7	2	10

Table 2 Splan Pond stomatal frequency data. SD, SDL and SNL represent stomatal density, stomatal density per length and stomatal number per length respectively. m is the total number of leaf samples per depth and n is the sum of observations on all leaves per depth

Depth (cm)	Species	SD	SDL	SNL	s.e	m	n
550	*D. integrifolia*	948			69	2	6
534	*Picea sp.*		79		1.6	1	5
533	*Picea sp.*		84		0.3	1	4
528	*D. integrifolia*	867			94	1	2
518	*D. integrifolia*	889			174	1	4
515	*D. integrifolia*	915			92	1	5
514	*D. integrifolia*	948			39	3	6
513	*D. integrifolia*	933			39	5	15
512	*D. integrifolia*	1071			44	5	19
511	*D. integrifolia*	922			48	7	24
510	*D. integrifolia*	895			42	3	7
509	*D. integrifolia*	852			37	3	6
507	*D. integrifolia*	868			56	2	10
506	*D. integrifolia*	649			32	2	9
497	*D. integrifolia*	844			n/a	1	1
496	*D. integrifolia*	926			38	7	43
489	*D. integrifolia*	743			32	3	11
483	*D. integrifolia*	898			22	1	5
482	*D. integrifolia*	1031			40	4	11
481	*D. integrifolia*	760			40	2	9
476	*D. integrifolia*	789			28	1	4
472	*D. integrifolia*	800			n/a	1	1
470	*D. integrifolia*	1111			n/a	1	1
466	*Picea sp.*		97		0.3	1	4
465	*Picea sp.*		76		0.4	1	3
464	*L. laricina*			23	1.2	4	10
462	*L. laricina*			19	0.6	4	12
460	*L. laricina*			23	4.4	1	3
459	*L. laricina*			22	1.0	5	11
458	*L. laricina*			19	1.4	3	8
457	*L. laricina*			25	2.3	4	8
456	*L. laricina*			19	1.1	6	19
455	*L. laricina*			21	1.8	2	7
454	*L. laricina*			17	1.1	4	16
453	*L. laricina*			22	1.1	6	11
451	*L. laricina*			23	1.1	2	9
446	*L. laricina*			19	1.1	3	12
444	*L. laricina*			22	1.3	3	14
443	*L. laricina*			20	1.3	5	8
443	*Picea sp.*		129		2.6	2	
435	*L. laricina*			18	2.9	1	3
434	*Picea sp.*		100		1.4	1	3
431	*L. laricina*			21	1.2	3	8
430	*L. laricina*			22	0.6	5	7
428	*L. laricina*			16	2.3	2	6
425	*L. laricina*			23	0.3	4	9

The reconstructed CO₂ record from Splan Pond mirrors that obtained from Pine Ridge Pond with mean interstadial (GI-1a) CO₂ concentrations of 319 ± 5 ppm, which decline abruptly to mean GS-1 concentrations of 234 ± 2 ppm and return to mean early Holocene values of 298 ± 2 ppm at its termination. Analysis of the phase relationship between temperature and CO₂ change at Pine Ridge Pond reveals that the onset of GS-1 cooling is marked by a 12 °C fall in summer lake surface-water temperature that appears to lead the abrupt CO₂ decline by 3.5 cm (deposited during ca. 70 ¹⁴C yr or 130 calendar yr) (Levesque *et al.*, 1994). As the stratigraphical resolution of both CO₂ and temperature records over this time interval (ca. 1 cm) is significantly higher than the observed lag and both proxy temperature and CO₂ records were derived from the same lake sediment core, we consider that this represents a robust phase relationship. Conversely, lake temperatures and CO₂ increased more or less synchronously at the termination of GS-1; however, the stratigraphical resolution of the CO₂ and temperature records over this interval is not sufficiently high at Pine Ridge Pond to establish phase relationships with the same confidence. Phase relationships could not be determined with confidence from Splan Pond because the chironomid-based temperature reconstructions and CO₂ records were derived from separate cores. Consequently, the errors associated with correlation of these separate cores (ca. 3 cm) was too large (Tables 1 and 2).

Discussion

Late-glacial CO₂ records from both Pine Ridge Pond and Splan Pond show three distinctive features that contrast markedly with Antarctic CO₂ records from Dome Concordia (Monnin *et al.*, 2001). They indicate that: (i) CO₂ concentrations were higher than typical interglacial levels (i.e. ca. 280 ppm, Petit *et al.*, 1999) by GI-1; (ii) CO₂ concentrations during GS-1 were close to typical full-glacial levels (i.e. 200 ppm, Petit *et al.*, 1999); and (iii) a rapid increase in CO₂ from near-glacial

to higher than full interglacial levels occurred during the transition from GS-1 into the early Holocene. A trend of marked reduction from high GI-1 CO_2 concentration to near-glacial CO_2 levels during GS-1 is in good general agreement with a stomatal-based record from a site in Norway (Kråkenes) (Beerling *et al.*, 1995), which shows a reduction in GI-1 CO_2 concentration from median values of 273 ppm to 210 ppm during GS-1. These records differ somewhat in the timing of CO_2 decline during the transition from GI-1 into GS-1; in Kråkenes, the CO_2 decline precedes the temperature fall into the Younger Dryas. The stomatal-based CO_2 reconstructions also differ somewhat in the general trend of CO_2 change throughout GS-1. A gradual increase in CO_2 is recorded throughout GS-1 in Kråkenes, whereas an abrupt increase in CO_2 is recorded in both Pine Ridge Pond and Splan Pond at the termination of GS-1. Some of these differences may be attributed to somewhat lower sampling resolution in the CO_2 record from Kråkenes, which has a mean sample resolution of ca. 88 calendar yr, with significant temporal gaps of >150 calendar yr. The differences in the timing of Late-glacial CO_2 change in relation to that of temperature between both stomatal-based CO_2 records highlight the need for further investigation of atmospheric CO_2 dynamics during this important interval. The importance of all three independent CO_2 records from Kråkenes (Beerling *et al.*, 1995), Pine Ridge Pond and Splan Pond is clearly that they show a consistent pattern of atmospheric CO_2 decline during the transition from GI-1 to GS-1—a feature not yet detected in ice-core records.

As the Late-glacial interstadial (GI-1a) CO_2 reconstructions for both Splan and Pine Ridge ponds were based on small leaf sample sizes ($n < 3$), further investigation was carried out on nearby AMS ^{14}C dated Mayflower Lake (Fig. 1) (Mayle and Cwynar, 1995), which contains abundant *Picea* macrofossils within GI-1a sediments. Stomatal analysis of 27 subfossil *Picea* leaves sampled over 6 cm in this lake revealed a GI-1a CO_2 concentration of 309 ± 6 ppm, confirming that GI-1a CO_2 concentrations were approximately 60 to 70 ppm higher than estimated from Antarctic ice-core reconstructions (Neftel *et al.*, 1988; Monnin *et al.*, 2001).

By the early Holocene, both ice-core measurements (Byrd, 269 ± 4 ppm (Neftel *et al.*, 1988); Taylor Dome, 268 ppm (Indermühle *et al.*, 1999); and Dome Concordia, 265 ppm (Monnin *et al.*, 2001)) and stomatal-based CO_2 reconstructions (Splan Pond, 298 ± 2 ppm; Pine Ridge Pond, 286 ± 16 ppm; Kråkenes, Norway, 275 ± 20 ppm (Beerling *et al.*, 1995); Borchert, Holland, 318 ± 26 ppm (Wagner *et al.*, 1999)) are in good agreement in terms of the direction of CO_2 change. However, CO_2 estimates based on stomatal frequency changes appear systematically higher by a minimum of ca. 10 ppm (Beerling *et al.*, 1995) to a maximum of ca. 40 ppm (Wagner *et al.*, 1999) at this time. The interhemispheric difference in atmospheric CO_2 concentration (ca. 3 ppm) is too small to account for the significant offset between ice-core and stomatal-based records, suggesting that further investigation of the potential mitigating factors in both records are required to reconcile their differences. If ice-core records of Late-glacial CO_2 change are secure, then CO_2 reconstructions from stomatal frequency data appear to consistently underestimate CO_2 levels during the stadials and overestimate them during interstadials, regardless of different reproductive biologies (i.e. in gymnosperms and angiosperms) and functional types (i.e. arctic alpine shrubs and trees). The effect could, in part, be a result of the transfer functions utilised to predict CO_2 concentrations from past changes in stomatal frequency or the mitigating effects of as yet undetermined environmental variables other than CO_2 on

stomatal frequency. The consistent discrepancies between ice-core and stomatal-based reconstructions of atmospheric CO_2 change therefore clearly merit further detailed investigation.

Both cold temperatures and/or increased water stress could potentially have reduced epidermal cell expansion in the leaves of *Dryas integrifolia* during GS-1, resulting in the observed high stomatal densities and predicted low CO_2 concentrations during this stadial (Tables 1 and 2). Calculation of stomatal indices would remove the effects of these factors on stomatal density. However, owing to the hirsute nature of *Dryas integrifolia* leaves, it was not possible to view the epidermal cell outlines unambiguously, so stomatal index could not be calculated. We note, however, that herbarium samples of *D. integrifolia* analysed for the historical training set were all derived from tundra type habitats with an arctic climate and would therefore have experienced similar temperature and water regimes during growth to those prevailing in New Brunswick during GS-1. Therefore, although reduced temperatures and precipitation may have exerted some effect on stomatal density, we believe the overriding control over the stomatal densities was CO_2 concentration during GS-1. This inference is supported by experimental observations indicating that CO_2 exerts a significantly greater effect on stomatal density and index than artificially manipulated fluctuations in temperature and humidity regimes (Beerling and Woodward, 1996; Kürschner *et al.*, 1998).

According to Tschumi and Stauffer (2000) and Indermühle *et al.* (1999), a small amount of *in situ* CO_2 production, although considerably less likely than in Greenland ice-core records (Anklin *et al.*, 1997), cannot be excluded as a possible source of error for all Antarctic ice cores or all climatic periods. Chemical impurities in ice, such as organic compounds and carbonates (indicated by Ca^{+2} levels), have been shown under certain conditions to result in *in situ* CO_2 production via oxidation and decarbonation reactions respectively (Smith *et al.*, 1997; Tschumi and Stauffer, 2000). *In situ* CO_2 depletion reactions can also occur within ice (Smith *et al.*, 1997), however, the chemistry associated with these reactions are currently not well understood. It has been suggested that the wide scatter of CO_2 estimates from closely spaced air bubbles in Byrd ice during the early Holocene (between 10 000 and 7000 yr BP) and during GS-1 may result from such *in situ* chemical reactions (Indermühle *et al*, 1999; Marchal *et al.*, 1999). No evidence for such reactions has been recorded in Dome Concordia ice (Monnin *et al.*, 2001), suggesting that chemical impurities cannot account for the discrepancies between stomatal-based and Dome Concordia ice CO_2 estimates spanning the Late-glacial.

Differences in temporal resolution may be implicated in the discrepancies between our stomatal-based CO_2 trends and Antarctic ice-core measurements. Owing to the length of time it takes for an air bubble to be enclosed within Dome Concordia ice (200 to 550 yr, Monnin *et al.*, 2001), CO_2 measurements at each interval record a time-averaged sample of 200 to 550 yr. To test for this possibility, we have time-averaged our CO_2 records from both Splan Pond and Pine Ridge Pond by 550 yr, taken as the maximum estimated pre-Holocene age distribution of air in Dome Concordia (Fig. 4). We assume a constant sedimentation rate spanning the entire interval, where 1 cm is equivalent to 20 calendar yr (Splan) and 37 calendar yr (Pine Ridge) respectively based on the age–depth relationships (Mayle *et al.*, 1993). A mean sample resolution was calculated for each locality to estimate the number of data points to be included in a running mean for each pond. In the case of Pine Ridge Pond, which possessed an average sampling resolution of 61 yr, a simple non-weighted average CO_2 concentration starting with the oldest was calculated from the next nine
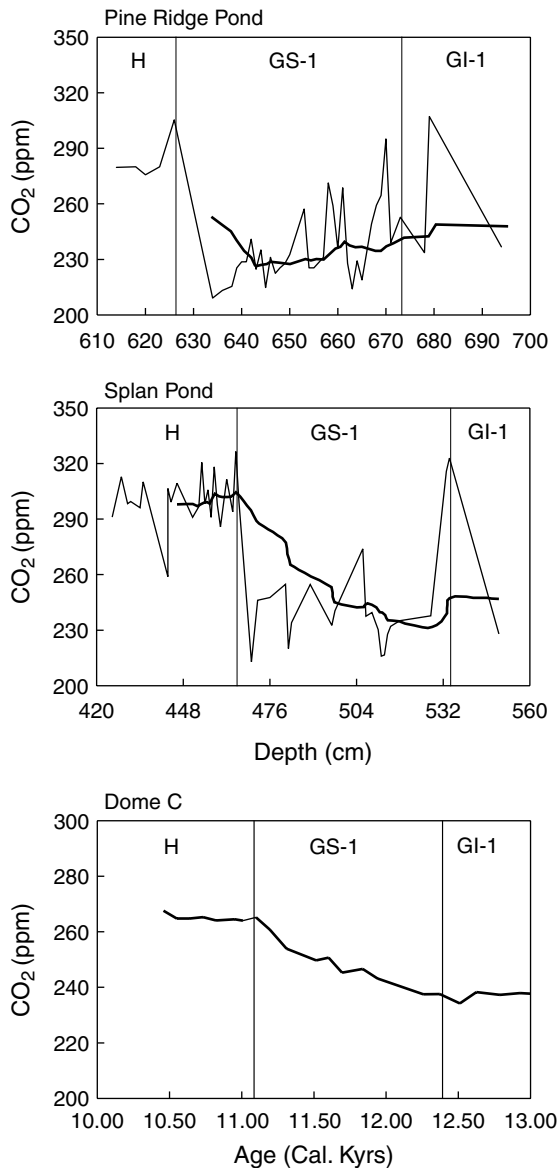
Figure 4 Comparison of time averaged (heavy black line) and non-time averaged (faint black line) CO_2 records from Pine Ridge Pond and Splan Pond with CO_2 measurements from Dome Concordia (Monnin *et al.*, 2001)

data points (= ca. 550 yr). The same exercise was repeated for Splan Pond, which possessed an average sampling resolution of 58 calendar yr. No interpolation was undertaken between individual data points. This analysis revealed a general rising trend in CO_2 concentration from ca. 245 ppm to ca. 300 ppm spanning GS-1 in Splan Pond, which mirror the trend of CO_2 increase from 230 to 275 ppm observed in Dome Concordia ice (Fig. 4).

In Pine Ridge Pond, however, although the marked CO_2 decline into GS-1 is removed by simple time averaging, the gradual trend of increase is not observed. If Dome Concordia ice is accurately recording a smoothed CO_2 signal during the Late-glacial, this suggests that the Splan Pond CO_2 record is more reliable in the latter half of GS-1 than that of Pine Ridge Pond, which shows greater amplitude fluctuations in CO_2. This gradual rising trend in CO_2 is lost in Splan Pond, however, when the time-average interval is reduced to less than 400 yr, at which point a significant reduction in atmospheric CO_2 during GS-1 re-emerges from the stomatal-based data. Consequently, based on this analysis, and our inference that a reduction in CO_2 concentration during GS-1 was the most

plausible forcing factor for increased stomatal density of *Dryas integrifolia* leaves, we suggest that the stomatal records (which effectively capture a CO_2 signal from a single growth season) may represent a higher resolution record of CO_2 change over this interval than that recorded in Dome Concordia ice. The fact that atmospheric methane measurements from ice cores in general are much less susceptible to *in situ* reactions and can be undertaken on a smaller volume of ice than required for atmospheric CO_2 measurements helps explain why the methane records from Dome Concordia are apparently less smoothed than those of atmospheric CO_2 spanning GS-1.

A reduction in atmospheric CO_2 concentration of the order of ca. 80 ppm within two centuries, as recorded here in both proxy CO_2 records, is consistent in both magnitude and rate of CO_2 decline with predictions from a three-dimensional simulation of the impact of Southern Ocean surface-nutrient depletion (owing to iron fertilisation) on atmospheric CO_2 (Sarmiento and Orr, 1991). This model predicts a 46 to 85 ppm oceanic drawdown of CO_2 within 100 yr under such a scenario. Similarly our results are consistent with the model predictions of Siegenthaler and Wenk (1984) for a rapid atmospheric CO_2 decline (within a century) in response to the effect of a reduction in thermohaline circulation (THC) on surface water chemical properties, such as that which occurred at the onset of GS-1 (Broecker *et al.*, 1985). Other simulations of the impact of a reduction in thermohaline circulation on atmospheric CO_2 predict a small net increase in CO_2 (Marchal *et al.*, 1998) qualitatively similar to that recorded in Dome Concordia spanning GS-1. It is interesting to note, however, that a sensitivity analysis of this model (Marchal *et al.*, 1998) predicts a rapid and marked (ca. 60 ppm) CO_2 decline at the onset of GS-1, similar to that identified by all three stomatal-based CO_2 reconstructions (Pine Ridge, Splan and Kråkenes), when biological cycling (determined by the rates of production versus oxidation of organic matter and the rate of production versus dissolution of $CaCO_3$) in the oceans is kept constant.

Our observation that atmospheric CO_2 decreased at the onset of GS-1, and that CO_2 change may have lagged rather than led GS-1 cooling, have important implications for our understanding of the role of atmospheric CO_2 in rapid climatic events. The temporal relationship between CO_2 and temperature demonstrated here on submillennial time-scales mirrors that observed over the longer term millennial time-scales of full-glacial–interglacial cycles (Barnola *et al.*, 1987; Petit *et al.*, 1999). The observed phase relationship in Pine Ridge suggests that CO_2 decline was not an initiator of GS-1 cooling but most probably played a significant role in amplifying initial Northern Hemisphere cooling by ca. 1 °C globally. Furthermore, owing to the apparent synchronous increase of both temperature and CO_2 at the termination of GS-1, it cannot yet be ruled out that the abrupt ca. 90 ppm CO_2 rise (Fig. 3) may have initiated early Holocene climatic warming.

Conclusions

The stomatal records from two independent localities presented here confirm the observation by Beerling *et al.* (1995) that Late-glacial plant macrofossils apparently record a decline in atmospheric CO_2 concentrations at the onset of the Younger Dryas stadial. However, owing to the differences between all three terrestrial proxy CO_2 records and those of ice cores, further investigation of plant physiological responses are required to address why stomatal based CO_2 estimates appear to

systematically overestimate interglacial (i.e. early Holocene) and interstadial (i.e. GI-1) CO_2 concentrations compared with coeval ice-core measurements. An investigation of fluctuations in macrofossil leaf stomatal frequency from the Southern Hemisphere or tropical latitudes where temperature changes were not as severe should show identical trends to those reported here (Table 1 and 2) from Atlantic Canada, if CO_2 was, as we believe, the main controlling factor throughout the Late-glacial. It is possible that a number of the short-term fluctuations recorded using the stomatal methods cannot be detected in ice cores, such as Dome Concordia, with low ice accumulation rates. According to Neftel *et al.* (1988), CO_2 fluctuation with a duration of less than twice the bubble enclosure time (equivalent to approximately 134 calendar yr in the case of Byrd ice and up to 550 calendar yr in Dome Concordia) cannot be detected in the ice or reconstructed by deconvolution. The rapid atmospheric CO_2 reduction recorded from stomatal density changes in macrofossil leaves at the onset of GS-1 (within 120 to 185 yr) may therefore have escaped detection in Antarctic-ice records. If our high-resolution records of leaf stomatal fluctuations accurately record changes in atmospheric CO_2, our results indicate that Late-glacial CO_2 change paralleled those of atmospheric CH_4 throughout the Late-glacial interval. Our findings suggest that some re-evaluation of the behaviour of atmospheric CO_2 sinks and sources during times of rapid climatic change, such as the Late-glacial, may therefore be required.

References

Anklin M, Schwander J, Stauffer B, Tschumi J, Fuchs A, Barnola JM, Raynaud D. 1997. CO_2 record between 40 and 8 kyr BP from the Greenland ice core project ice core. *Journal of Geophysical Research* **102**: 26 539–26 545.

Barnola JM, Raynaud D, Korotkevitch YS, Lorius C. 1987. Vostok ice core provides 160,000-year record of atmospheric CO_2. *Nature* **329**: 408–412.

Beerling DJ, Chaloner WG. 1993. The impact of atmospheric CO_2 and temperature change on stomatal density; observations from *Quercus robur* lammas leaves. *Annals of Botany* **71**: 431–435.

Beerling DJ, Woodward FI. 1996. Stomatal density responses to global environmental change. *Advances in Bioclimatology* **4**: 171–221.

Beerling DJ, Birks HH, Woodward FI. 1995. Rapid late-glacial atmospheric CO_2 changes reconstructed from the stomatal density record of fossil leaves. *Journal of Quaternary Science* **10**: 379–384.

Beerling DJ, McElwain JC, Osborne CP. 1998. Stomatal responses of the 'living fossil' *Ginkgo biloba* L. to changes in atmospheric CO_2 concentrations. *Journal of Experimental Botany* **49**(326): 1603–1607.

Björck S, Walker MJC, Cwynar LC, Johnsen S, Knudsen KL, Lowe JJ, Wohlfarth B, INTIMATE members. 1998. An event stratigraphy for the Last Termination in the North Atlantic region based on the Greenland ice-record: a proposal by the INTIMATE group. *Journal of Quaternary Science* **13**: 283–292.

Blunier T, Brook EJ. 2001. Timing of millennial-scale climate change in Antarctica and Greenland during the Last Glacial Period. *Science* **291**: 109–112.

Blunier T, Schwander J, Stauffer B, Stocker T, Dällenbach A, Indermühle A, Tschumi, Chappellaz J, Raynaud D, Barnola J-M. 1997. Timing of the Antarctic Cold Reversal and the atmospheric CO_2 increase with respect to the Younger Dryas event. *Geophysical Research Letters* **24**: 2683–2686.

Broecker WS, Peteet D, Rind D. 1985. Does the ocean–atmosphere system have more than one stable mode of operation? *Nature* **315**: 21–25.

Draper NR, Smith H. 1981. *Applied Regression Analysis*, 2nd edn. Wiley: New York.

Gray JE, Holroyd GH, van der Lee F, Sijmons PC, Woodward FI, Schuch W, Hetherington AM. 2000. *HIC*: a gene involved in controlling stomatal development in responses to changes in atmospheric CO_2 concentration. *Nature* **408**: 713–715.

Indermühle A, Stocker TF, Joos F, Fischer H, Smith HJ, Wahlen M, Seck B, Mastroianni D, Tschumi J, Blunier T, Meyer R, Stauffer B. 1999. Holocene carbon-cycle dynamics based on CO_2 trapped at Taylor Dome. *Nature* **398**: 121–126.

Keeling CD, Whorf TP. 2000. Atmospheric CO_2 records from sites in the SIO air sampling network. In *Trends: A Compendium of Data on Global Change*. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy: Oak Ridge, TN.

Kürschner WK, Stulen I, Wagner F, Kuiper P. 1998. Comparison of palaeobotanical observations with experimental data on the leaf anatomy of durmast oak [*Quersus petraea* (Fagaceae)] in response to environmental change. *Annals of Botany* **81**: 657–664

Levesque AJ, Mayle FE, Walker IR, Cwynar LC. 1993. A previously unrecognized late-glacial cold event in eastern North America. *Nature* **361**: 623–626.

Levesque AJ, Cwynar LC, Walker IR. 1994. A multiproxy investigation of Late-Glacial climate change and vegetation change at Pine Ridge Pond, Southwest, New Brunswick, Canada. *Quaternary Research* **42**: 316–327.

Levesque AJ, Cwynar LC, Walker IR. 1997. Exceptionally steep north–south gradients in lake temperatures during the last deglaciation. *Nature* **385**: 423–426.

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

Marchal O, Stocker TF, Joos F, Indermühle A, Blunier T, Tschumi J. 1999. Modeling the concentration of atmospheric CO_2 during the Younger Dryas climate event. *Climate Dynamics* **15**: 341–354.

Mayle FE, Cwynar LC. 1995. Impact of the Younger Dryas cooling event upon lowland vegetation of Maritime Canada. *Ecological Monographs* **65**: 129–154.

Mayle FE, Levesque AJ, Cwynar LC. 1993. Accelerator-mass-spectrometer ages for the Younger Dryas event in Atlantic Canada. *Quaternary Research* **39**: 355–360.

McElwain JC, Mitchell FJG, Jones MB. 1995. Relationship of stomatal density and index of *Salix cinerea* to atmospheric carbon dioxide concentration in the Holocene. *The Holocene* **5**(2): 216–220.

Monnin E, Indermühle A, Dällenbach A, Flückiger J, Stauffer B, Stocker TF, Raynaud D, Barnola J-M. 2001. Atmospheric CO_2 concentrations over the last glacial termination. *Science* **291**: 112–114.

Neftel A, Oeschger H, Staffelbach T, Stauffer B. 1988. CO_2 record in the Byrd ice core 50 000–5000 years BP. *Nature* **331**: 609–611.

Petit JR, Jouzel J, Raynaud D, Barkov NI, Barnola J-M, Basile I, Benders M, Chappellaz J, Davis M, Delayque G, Delmotte M, Kotlyakov VM, Legrand M, Lipenkov VY, Lorius C, Pépin L, Ritz C, Saltzman E, Stievenard M. 1999.. Climate and atmospheric history of the past 420 000 years from the Vostok ice core, Antarctica. *Nature* **399**: 429–435.

Poole I, Kürschner WM. 1999. Stomatal density and index: the practice. In *Fossil Plants and Spores: Modern Techniques*, Jones TP, Rowe NP (eds). Geological Society Publishing House: Bath; 257–260.

Praet L, Jiang XN. 1995. Effects of CO_2 enrichment, leaf position and clone on stomatal index and epidermal call density in poplar (Populus). *New Phytologist* **131**: 99–107.

Raven JA, Ramsden HJ. 1989. Similarity of stomatal index in the C4 plant *Salsola kali* L. in material collected in 1943 and in 1987: Relevance to changes in atmospheric CO$_2$ content. *Transactions of the Botanical Society Edinburgh* **45**: 223–233.

Rundgren M, Beerling D. 1999. A Holocene CO$_2$ record from the stomatal index of sub-fossil *Salix herbacea* L. leaves from northern Sweden. *The Holocene* **9**: 509–513.

Sarmiento JL, Orr JC. 1991. Three-dimensional simulations of the impact of Southern Ocean nutrient depletion on atmospheric CO$_2$ and ocean chemistry. *Limnology and Oceanography* **36**(8): 1928–1950.

Siegenthaler U, Wenk Th. 1984. Rapid atmospheric CO$_2$ variations and ocean circulation. *Nature* **308**: 624–626.

Sowers T, Bender M. 1995. Climate records covering the last deglaciation. *Science* **269**: 210–214.

Smith HJ, Wahlen M, Mastroianni D, Taylor KC. 1997. The CO$_2$ concentration of air trapped in GISP2 ice from the Last Glacial Maximum–Holocene transition. *Geophysical Research Letters* **24**: 1–4.

Stuiver M, Reimer PJ, Bard E, Beck JW, Burr GS, Hughen KA, Kromer B, McCormac G, van der Plicht J, Spurk M. 1998. INTCAL 98 radiocarbon age calibration, 24 000-0 cal. BP. *Radiocarbon* **40**: 1041–1084.

Tschumi J, Stauffer B. 2000. Reconstructing past atmospheric CO$_2$ concentrations based on ice-core analyses: open questions due to in situ production of CO$_2$ in ice. *Journal of Glaciology* **46**: 45–53.

Wagner F, Below R, Klerk PD, Dilcher D, Joosten H, Kurschner W, Visscher H. 1996. A natural experiment on plant acclimation: Lifetime stomatal frequency response of an individual tree to annual atmospheric CO$_2$ increase. *Proceedings of the National Academy of Sciences* **93**: 11 705–11 708.

Wagner F, Bohncke SJP, DeKlerk P, Dilcher DL, Kürschner WM, Visscher H. 1999. Century-scale shifts in early Holocene atmospheric CO$_2$ concentration. *Science* **284**: 1971–1973.

Walker IR, Smol JP, Engstrom DR, Birks HJB. 1992. Aquatic invertebrate, climate, scale and statistical hypothesis testing: a response to Hann, Warner and Warwick. *Canadian Journal of Fisheries and Aquatic Sciences* **49**: 1276–1280.

Woodward FI. 1987. Stomatal numbers are sensitive to increase in CO$_2$ from pre-industrial levels. *Nature* **327**: 617–618.

Woodward FI, Bazzaz FA. 1988. The response of stomatal density to CO$_2$ partial pressure. *Journal of Experimental Botany* **39**: 1771–1781.