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► To cite this version:

Mary Gagen, Walter Finsinger, Rike Wagner, Danny Mccarrol, Neil Loader, et al.. Evidence of changing intrinsic water use efficiency under rising atmospheric CO₂ concentrations in Boreal Fennoscandia from subfossil leaves and tree ring $\delta^{13}\text{C}$ ratios. *Global Change Biology*, 2010, 17 (2), pp.1064. 10.1111/j.1365-2486.2010.02273.x . hal-00599517

HAL Id: hal-00599517

<https://hal.science/hal-00599517>

Submitted on 10 Jun 2011

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Evidence of changing intrinsic water use efficiency under rising atmospheric CO₂ concentrations in Boreal Fennoscandia from subfossil leaves and tree ring $\delta^{13}\text{C}$ ratios

Journal:	<i>Global Change Biology</i>
Manuscript ID:	GCB-10-0103
Wiley - Manuscript type:	Primary Research Articles
Date Submitted by the Author:	31-Jan-2010
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Keywords:	intrinsic water use efficiency, physiological forcing, boreal forest, stomatal conductance, stable isotope dendroclimatology, stomatal density
Abstract:	Investigating the many internal feedbacks within the climate system is a vital component of the effort to quantify the full effects of future anthropogenic climate change. The stomatal apertures of plants tend to close and decrease in number under elevated CO ₂ concentrations, increasing water use efficiency and reducing canopy evapotranspiration. Experimental and modelling studies reveal huge variations in these changes such that the warming associated with reduced evapotranspiration (known as physiological forcing) is neither well understood or constrained. Palaeo-observations of changes in stomatal response and plant water use efficiency under rising CO ₂ might be used to better understand the processes underlying the physiological forcing feedback and to link measured changes in plant water use efficiency to a specific physiological change in stomata. Here we use time series of tree ring $\delta^{13}\text{C}$ (<i>Pinus sylvestris</i>) and subfossil leaf (<i>Betula nana</i>) measurements of stomatal density and geometry to derive records of changes in intrinsic water use efficiency and maximum stomatal conductance

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	<p>in the Boreal zone of northern Finland and Sweden. We investigate the rate of change in both proxies, over the recent past. The two independent lines of evidence from these two different Boreal species indicate increased intrinsic water use efficiency and reduced maximum stomatal conductance of similar magnitude from pre-industrial times (ca. AD 1850) to around AD 1970. After this maximum stomatal conductance continues to decrease to AD 2000 in <i>B. nana</i> but intrinsic water use efficiency in <i>i>P. sylvestris</i> reaches a plateau. We suggest that northern boreal <i>i>P. sylvestris</i> might have reached a threshold in its ability to increase water use efficiency and we discuss the implication for modelling stomatal conductance in the land surface schemes of General Circulation Models.</p>
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For Review Only

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6 **Evidence of changing intrinsic water use efficiency under rising**
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8 **atmospheric CO₂ concentrations in Boreal Fennoscandia from subfossil**
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10 **leaves and tree ring $\delta^{13}\text{C}$ ratios.**
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17 Running title: Palaeorecords of plants responses to rising CO₂
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52 **Keywords:** intrinsic water use efficiency, stomatal density, stomatal conductance,
53 Fennoscandia, boreal forest, physiological forcing.
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57 *Submitted to Global Change Biology for consideration as a Primary Research Article.*
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59 *7th September 2009, resubmitted February 1st 2010.*
60

Abstract:

Investigating the many internal feedbacks within the climate system is a vital component of the effort to quantify the full effects of future anthropogenic climate change. The stomatal apertures of plants tend to close and decrease in number under elevated CO₂ concentrations, increasing water use efficiency and reducing canopy evapotranspiration. Experimental and modelling studies reveal huge variations in these changes such that the warming associated with reduced evapotranspiration (known as physiological forcing) is neither well understood or constrained. Palaeo-observations of changes in stomatal response and plant water use efficiency under rising CO₂ might be used to better understand the processes underlying the physiological forcing feedback and to link measured changes in plant water use efficiency to a specific physiological change in stomata. Here we use time series of tree ring $\delta^{13}\text{C}$ (*Pinus sylvestris*) and subfossil leaf (*Betula nana*) measurements of stomatal density and geometry to derive records of changes in intrinsic water use efficiency and maximum stomatal conductance in the Boreal zone of northern Finland and Sweden. We investigate the rate of change in both proxies, over the recent past. The two independent lines of evidence from these two different Boreal species indicate increased intrinsic water use efficiency and reduced maximum stomatal conductance of similar magnitude from pre-industrial times (ca. AD 1850) to around AD 1970. After this maximum stomatal conductance continues to decrease to AD 2000 in *B. nana* but intrinsic water use efficiency in *P. sylvestris* reaches a plateau. We suggest that northern boreal *P. sylvestris* might have reached a threshold in its ability to increase water use efficiency and we discuss the implication for modelling stomatal conductance in the land surface schemes of General Circulation Models.

Introduction

Plant gas exchange plays an important role in the positive climate feedback known as physiological forcing, because models indicate that the atmosphere dries out as evapotranspiration reduces under raised atmospheric CO₂ concentrations (Cox *et al.* 1999). Evapotranspiration reduces because the stomatal apertures of plants tend to close, decrease in number and alter their geometry (Woodward 1987; Woodward & Kelly 1995; Kürschner *et al.* 1997; Gray *et al.* 2000; Lake *et al.* 2001) when exposed to elevated CO₂, increasing water use efficiency (WUE unit carbon gain for each unit of water lost), and ultimately reducing evapotranspiration (Cox *et al.* 1999; Bunce 2004; Young *et al.* 2006). The climatic impact of the stomatally derived reduction in evapotranspiration is described by the feedback term physiological forcing. Climate model-based studies have shown that reductions in plant stomatal conductance can lead to enhanced regional and global warming via physiological forcing (Sellers *et al.* 1996; Sellers *et al.* 1997; Cox *et al.* 1999; Betts *et al.* 2000) whilst

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3 experiments at the plant level reveal the incredible speeds with which plants can alter their
4 stomatal numbers after exposure to raised CO₂ (Lake *et al.* 2001; Hetherington & Woodward
5 2003). However, physiological forcing is not well understood or constrained (National
6 Research Council 2003; Doutriaux-Boucher 2009). For example, warming might be offset by
7 increases in leaf area index due to higher temperatures and precipitation changes that may
8 accompany higher CO₂ levels (Betts *et al.* 2000) and there is evidence of thresholds (so-called
9 response limits) in plant capacity to raise water use efficiency (Kürschner *et al.* 1997).
10 Moreover the degree of spatial and species variability in the response of water use efficiency
11 and stomatal conductance is an unknown. Uncertainty in the magnitude and significance of
12 physiological forcing arises because most studies on the response of stomatal conductance to
13 rising CO₂ are extrapolated from short term, leaf 'exposure' studies (e.g. Ainsworth & Rogers
14 2007) which cannot take account of acclimation that may alter the stomatal response over
15 longer timescales (National Research Council 2003). Thus, at present, we do not know
16 whether measured reductions in stomatal conductance in plants exposed to raised CO₂
17 concentrations reflect the full range of long-term water use behaviour in forests (Leuzinger &
18 Körner 2007). Short term changes in stomatal opening and closure are only one component of
19 the system.

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46 Palaeo-observations of changes in stomatal physiology and plant stable carbon isotope ratios
47 might be used to better understand the changes in water use efficiency underlying the
48 physiological forcing feedback. Stomatal frequency and geometry measured from subfossil
49 leaves and $\delta^{13}\text{C}$ ratios from tree rings represent independent records of changes in stomatal
50 control over water use efficiency in a palaeo-context. As such, combined analysis of these
51 independent parameters, within a region, might be used to investigate long-term plant
52 responses at differing CO₂ levels and to elucidate the physiological changes driving shifts in
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3 intrinsic water use efficiency (iWUE). The rate at which trees exchange CO₂ and water with
4 the atmosphere can be estimated from δ¹³C as iWUE, the potential rather than actual WUE
5 (Ehleringer & Cerling 1995; Saurer *et al.* 2004); actual WUE being also influenced by
6 changes in respiratory losses over time at the whole plant level and being sensitive to vapour
7 pressure differences between atmosphere and leaf. Tree ring stable carbon isotope ratio
8 derived time series of iWUE represent the maximum potential WUE as it changes through
9 time. Stable carbon isotope (δ¹³C) ratios are known to provide a reliable method of
10 monitoring long term changes in plant physiology (Farquhar *et al.* 1982).
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25 Whilst rising iWUE over the recent past has been widely noted in tree ring based δ¹³C studies
26 (e.g. (Saurer *et al.* 2004; Waterhouse *et al.* 2004) its cause has not been attributed directly to a
27 specific physiological source. Nor is it clear how iWUE will change with continued rising
28 CO₂ in the future. We compare δ¹³C based records of iWUE from northern Boreal *Pinus*
29 *sylvestris* and a record of maximum stomatal conductance to water vapour (g_{wmax}) derived
30 from subfossil *Betula nana* leaves from the same geographical area (Table 1). The ideal data
31 set to attribute palaeo changes in iWUE to a specific physiological cause would be measured
32 iWUE and stomatal density variations from the same palaeoclimate archive. Unfortunately,
33 for most species the stomatal geometry and density records are unknown because they simply
34 were not measured. With this in mind we investigate a different question – when comparing
35 two independent lines of proxy evidence for changes in water use efficiency, what changes do
36 two of the dominant Boreal species, from the same region, show?
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55 Using subfossil leaf assemblages Kürschner *et al.* (1997) illustrated a non linear response in
56 stomatal frequency changes of European birch and oak species and predicted that certain
57 species may reach a ‘response limit’ and stop altering stomatal geometry and number at
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3 between 350-400ppmv CO₂. Diverging results for different plant species point to a genus or
4 species-specific adaptation capacity, where in certain taxa stomatal frequency response limits
5 are already reached at present day CO₂ levels (Kürschner *et al.* 1997; Royer 2001).
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10 Waterhouse *et al.* (2004) also find that iWUE in trees across northern Europe is, in some
11 cases, no longer rising. Clearly plant iWUE cannot rise indefinitely and stomatal conductance
12 and frequency can never be zero. Hence future predictions of evapotranspiration flux within
13 the Boreal forest zone might be more accurately gauged by taking account of palaeo evidence
14 for threshold points in different species across different regions.
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24 The C3 plants have internalised their gas exchange surfaces in order to maintain control over
25 water budget, a necessary step as any membrane which is permeable to large CO₂ molecules is
26 also permeable to water molecules (McCarroll & Loader 2004). The gas exchange and water
27 balance mechanisms in plants are therefore intrinsically linked; when plants take in CO₂
28 through stomata they transpire water at the same time. Long term water exchange in plants
29 can be studied via $\delta^{13}\text{C}$ ratios in annual tree ring cellulose, because $\delta^{13}\text{C}$ essentially records
30 CO₂ gas exchange (Saurer *et al.* 2004). There is a slight offset between leaf and trunk $\delta^{13}\text{C}$
31 and using $\delta^{13}\text{C}$ cellulose to calculate iWUE, rather than initial photosynthate or even leaf
32 $\delta^{13}\text{C}$, may introduce some bias into the calculated iWUE values. However, the temporal
33 trends will remain unaffected (Waterhouse *et al.* 2004).
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51 Increased iWUE under raised CO₂ has been reported in: *Pinus* (Runion *et al.* 1999), *Quercus*
52 (Anderson & Tomlinson 1998; Lodge *et al.* 2001), *Fagus* (Bucher-Wallin *et al.* 2000) and
53 *Picea* (Roberntz & Stockfors 1998) with an average gain of around 19% in northern Eurasia
54 (Saurer *et al.* 2004) over the industrial period, although over the wider northern hemisphere
55 percentage changes are highly variable. Waterhouse *et al.* (2004) report increases of between
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24% to 48% in a variety of species, between ~AD 1930 and 1980. However, at some sites iWUE has remained static (Marshall & Monserud 1996) or has actually shown decreases (Saurer *et al.* 2004). The rates of change, even where iWUE is rising, also seem to alter over time (Waterhouse *et al.* 2004). It is not surprising that changes in iWUE are spatially variable because water usage is strongly dependant upon site conditions; the level of moisture stress experienced at a site, local pollution effects or other stress factors and the species sensitivity to moisture variability. With this in mind, CO₂ response limits may well need to be investigated on regional and species basis.

Materials and Methods

Stable carbon isotope measurements from annual tree rings as an estimate of intrinsic water use efficiency.

Stable carbon isotope dendroclimatology was used to produce a time series of iWUE averaged from eight individually measured *Pinus sylvestris* trees at a site (Laanila) in northern Finnish Lapland (Table 1). Measurements of $\delta^{13}\text{C}$ in wood cellulose extracted from absolutely dated annual rings of trees record variability in the internal partial pressure of CO₂ (*ci*) (Farquhar *et al.* 1982) thus *ci* and iWUE were calculated from measured tree ring $\delta^{13}\text{C}$ values.

Site	Proxy	Location	Species	Source
Laanila, Finland	$\delta^{13}\text{C}$ -inferred iWUE	68°N 27°E	<i>Pinus sylvestris</i>	Gagen <i>et al.</i> (2007) and this study.
Kiruna, Sweden Kevo, Finland	Stomata-inferred $g_{w\max}$	67°N 20°E 69°N 27°E	<i>Betula nana</i>	Finsinger and Wagner-Cremer (2009) and this study

Table 1 – Site information.

At Laanila, mature *P. sylvestris* trees of over 200 years in age were sampled for $\delta^{13}\text{C}$ in 2001, the time series is fully described elsewhere (Gagen *et al.* 2007). Trees were sampled using a

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3 12 mm increment borer. Following cross dating, individual annual latewood bands were cut
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5 from the cores and extracted to α -cellulose using a modified batch processing method (Loader
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7 *et al.* 1997, Rinne *et al.* 2005). The first 50 years of material from each tree was removed
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9 prior to analysis to avoid including 'juvenile effects' in $\delta^{13}\text{C}$ (McCarroll & Loader 2004).
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11 $\delta^{13}\text{C}$ time series were then corrected for changes in atmospheric $\delta^{13}\text{C}$ of CO_2 (Francey *et al.*
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13 1999, McCarroll & Loader 2004). Cellulose $\delta^{13}\text{C}$ ratios were measured either offline by
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15 sealed-tube combustion followed by cryogenic distillation and analysis on a VG Micromass
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17 602C dual inlet stable isotope ratio mass spectrometer (SIRMS) or online via an ANCA
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19 Elemental Analyser coupled to a PDZ Europa 20/20 SIRMS. Sample precision was 0.1‰ as
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21 measured via in-house and IAEA cellulose standards and reference gas.
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29 Fractionation (Δ) of carbon isotopes by C3 plants is described as follows,
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$$\Delta = a + (b-a) \cdot ci/ca \quad (1)$$

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38 where a (28‰) and b (4.4‰) are constants representing fractionation due to diffusion and
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40 carboxylation respectively and ci and ca are intercellular and atmospheric CO_2
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42 concentrations.
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48 Fractionation (Δ) acts upon the isotopic composition of the source gas (atmospheric CO_2) as
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50 follows:
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$$\delta^{13}\text{C plant} = \delta^{13}\text{C atmosphere} - \Delta \quad (2)$$

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3 such that post industrial time series of $\delta^{13}\text{C}$ measurements contain the industrially derived
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5 change in atmospheric $\delta^{13}\text{C}$ which is corrected against the actual atmospheric $\delta^{13}\text{C}$
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8 measurements (e.g. Francey *et al.* 1999). Corrected plant $\delta^{13}\text{C}$ then reflects a pre AD 1850
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10 $\delta^{13}\text{C}$ value of -6.4 ppmv.
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15 Intrinsic water use efficiency is calculated as:
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$$18 \quad i\text{WUE} = (ca-ci) / 1.6 \quad (3)$$

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24 where ci is calculated by a re-arrangement of equation 1 after applying equation 2 (for full
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26 derivation see McCarroll *et al.* 2009).
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32 Stable carbon isotope ratios in tree rings are commonly used as a climate proxy because ci is
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34 externally forced by a control that is often climatic, whereas ca remains stable or its changes
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36 can be accounted for (McCarroll *et al.* 2009). Since the fractionation constants (a and b) do
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38 not vary in response to climate and the atmospheric concentration of CO_2 (ca) is known, $\delta^{13}\text{C}$
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40 essentially records changes in ci as regulated by two processes: stomatal conductance (g_s),
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42 and photosynthetic assimilation rate (A). If ca changes through time the response of the plant
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44 to these changes will also be recorded in tree ring $\delta^{13}\text{C}$.
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51 *A record of maximum stomatal conductance to water vapour (g_{wmax}) from subfossil Betula*
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53 *nana leaves*
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58 The long term g_{wmax} time series (AD 1750-2008) was obtained from measured changes in
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60 stomatal density and stomatal geometry using standard paleoecological methods, in subfossil

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3 *B. nana* leaves sampled from a peat mire at Kiruna in northern Swedish Lapland (Table 1). In
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5 addition, *B. nana* leaves were collected from (i) herbarium specimens that were growing in
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7 Fennoscandia >67°N (n years=5, AD 1919, 1935, 1958, 1963, and 1965), (ii) from a mire at
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9 Kevo (leaves growing AD 1976-1996), and (iii) from modern plants growing at Kevo
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11 (69°45'N, 27°E, AD 1997-2008, 1998 missing).
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17 Subfossil leaf fragments were picked from peat extracted from the mire, identified and
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19 separated under a binocular microscope by leaf and cuticle morphologies. For microscopic
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21 analysis, all leaf fragments were bleached in a cold 2-4% sodium hypochlorite solution, rinsed
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23 in water, and mounted on microscopic slides. Standardized, computer-aided analysis of
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25 epidermal parameters was performed on a Leica Quantimet 500C/500+ image-analysis
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27 system. The number of analysed leaf fragments in each sample varies because of different
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29 cuticle preservation in the sediments. A maximum of ten stomata-bearing alveoles/leaf
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31 fragments were analysed for epidermal cell density (*ED* [n/mm²]) and stomatal density (*SD*
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33 [n/mm²]). On each leaf fragment, twenty stomatal pores were measured for maximum pore
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35 length (*PL* [μm]) and maximum stomatal length (*SL* [μm]). Based on these measurements,
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37 maximum stomatal conductance to water vapour (*g_{wmax}* [mol m⁻² s⁻¹]) was calculated using the
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39 equation proposed by Franks & Beerling (2009):
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$$g_{wmax} = \frac{d}{v} \cdot SD \cdot a_{max} / \left(l + \frac{\pi}{2} \sqrt{a_{max} / \pi} \right), \quad (4)$$

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49 where *d* (the diffusivity of water vapour in air [m² s⁻¹]), *v* (the molar volume of air [m³ mol⁻¹]),
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51 and *l* (the depth of the pore) were taken as constants, and *a_{max}* is the maximal pore
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53 aperture that was approximated as $\pi(PL/2)^2$. Values for standard gas constants *d* and *v* were
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those for 25°C (Franks and Farquhar, 2001). Stomatal size (S [μm^2]) was inferred based on the SL measurements with the equation following Franks & Beerling (2009):

$$S = SL \cdot (SL/2), \quad (5).$$

In addition, stomatal conductance g_s was calculated from the reciprocal of maximum stomatal resistance (g_s [m s^{-1}] = $1/R_s$) following Parlange & Waggoner (1970):

$$R_s = \left(\frac{l}{\pi(PL \cdot PW)} + \frac{\ln(4 \cdot PL/PW)}{\pi \cdot PL} \right) / d \cdot SD, \quad (6)$$

where the half pore width (PW) was calculated as being equal to half PL . The latter equation differs from equation (4) because it does not include an “end-correction term” ($\pi/2\sqrt{(a_{max}/\pi)}$) accounting for diffusion shells at the outside end of stomatal pores (Franks & Farquhar 2001). The significance of the differences between equations (4) and (6) is explored in the Supplementary Material.

Determination of the age of sediment samples from the Kevo and Kiruna monoliths is based on radiocarbon-dated samples consisting of individual *B. nana* leaves, *Sphagnum* leaves, stems and branches (Hicks 2004; Goslar *et al.* 2005; Barnekow *et al.* 2007). In both monoliths, several samples analysed were deposited immediately prior to, or following, the peak in atmospheric ^{14}C values at ~ AD 1965 derived from the testing of nuclear weapons. Therefore, it was possible to assign calendar ages to the radiocarbon dates with high levels of precision. Control points were subsequently used to construct age-depth chronologies following Goslar *et al.* (2005). The resulting age range of samples analysed varies in the

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3 Kiruna monolith from ca. 1 year (AD 2003-1955) to 3 or 5 years (between AD 1954 and
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6 1721), and in the Kevo monolith each sample is less than 1 year's peat accumulation (AD
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8 1976-1996).
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12 To obtain a combined dataset, values from *B. nana* leaves growing in the same year were
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14 averaged. Subsequently, in order to extract the main trend in the g_{wmax} , g_s , S and PL records,
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16 the combined core and herbarium observations were smoothed with a robust *loess* function
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18 (locally weighted regression) (Cleveland & Devlin 1988) with a constant window width and
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20 fixed target points in time. The smoothed curve was constructed by determining fitted values
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22 at 10-year intervals with a 100-year window-width (here referred to the full span of the
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24 window). In this method the smoothing window does not change as a function of data density
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26 around the fitted values. The smoothed g_{wmax} time series is shown in Fig. 1 and the g_s , PL, S,
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28 and the unsmoothed g_{wmax} time series are shown in the Supplementary Material (Fig. S1).
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41 Results

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46 Changes in iWUE since the start of industrialisation to present (AD 1820 to 2002) at Laanila
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48 reveal a significant rise over time (Fig.1). There is a temporary decrease in iWUE centred on
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50 AD 1910 which is known to be climatically derived. The iWUE series is scaled from $\delta^{13}C$
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52 values which contain the effects of climate and the period around AD 1910 is known to be a
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54 prolonged cold spell in northern Fennoscandia, detected in many tree ring proxies from the
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56 region (see Gagen *et al.* 2007 for details). However, there is no long term change in the
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climatic variables which control tree ring $\delta^{13}\text{C}$ at this site, of the order of magnitude capable of explaining an increase in iWUE of 12%, seen between AD 1850 and AD 2002 (Fig. 1).

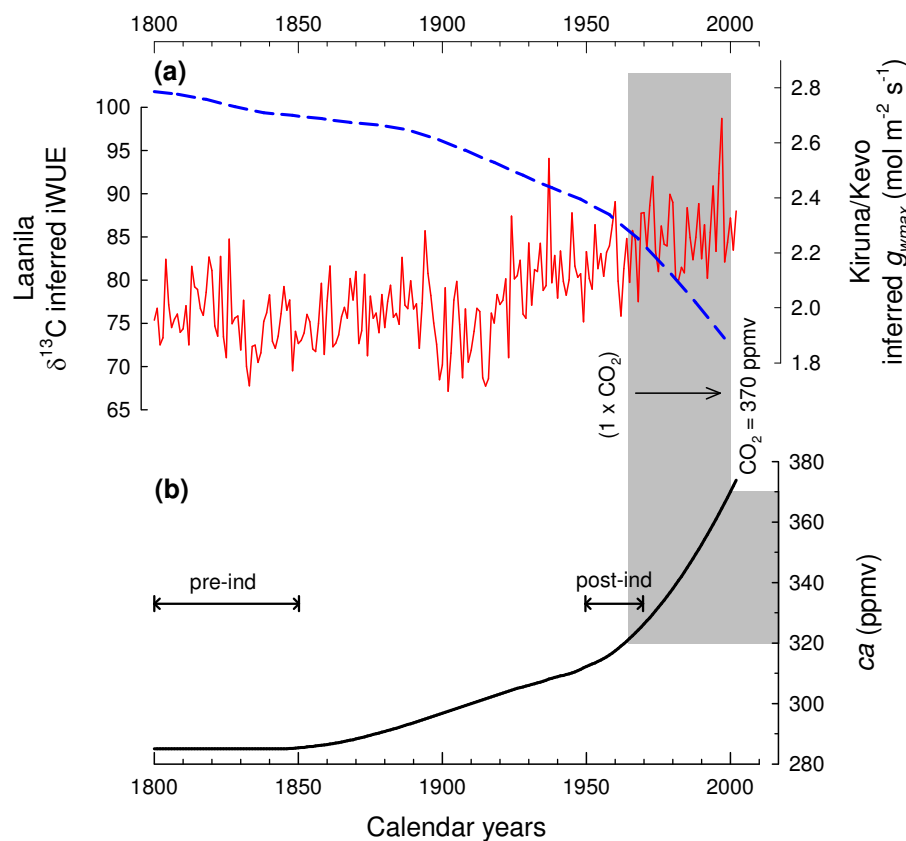


Fig 1. (a) $\delta^{13}\text{C}$ inferred intrinsic water-use efficiency (iWUE) record from *P. sylvestris* tree rings at Laanila (red line) and 10-year smoothed stomatal density and geometry inferred maximum stomatal conductance (g_{wmax}) from subfossil and modern *B. nana* leaves at Kiruna/Kevo (dashed blue line). (b) Atmospheric CO_2 concentration (ca) (black). The two reference periods for comparing pre and post-industrial changes (pre-ind and post-ind, respectively) in iWUE and g_{wmax} are indicated. The change from pre- to post-ind are 12% for iWUE and 15% for g_{wmax} . Grey band indicates the reference period between 1 x CO_2 (AD 1967) and $\text{CO}_2 = 370 \text{ ppmv}$ (AD 2000) (see Table 2).

Maximum stomatal conductance to water vapour (g_{wmax}), as inferred from the *B. nana* leaf measurements of stomatal density and geometry, declines from AD 1800 (Fig. 1) with a slight increase in the rate of decline from AD 1850, when ca begins to rise more steeply. Between AD 1850 and AD 2000 a 15% decrease of g_{wmax} is observed. It is notable that prior to ca AD 1850, changes in stomatal density were accompanied by changes in stomatal size such that

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3 g_{wmax} remained almost constant. Instead, after ~AD 1850 the steeper decline of stomatal
4 density was not counterbalanced by an equal increase in stomatal size (see Fig. S1 and
5 Supplementary Material). Hence, the change in g_{wmax} we observe over the study period is
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mainly related to a change in stomatal density.

The pre- to post-industrial percentage changes in tree ring iWUE and leaf stomatal conductance were calculated using the AD 1800-1850 (pre-ind) and AD 1950-1970 (post-ind) averages. Over this time period the stomata density and geometry-inferred g_{wmax} record shows a 15% decrease and the $\delta^{13}C$ inferred iWUE record from Laanila shows a 12% increase, very similar magnitudes of change in two different archives. It is reasonable to assume that, given the close locations of Kiruna and Laanila the *B. nana* and *P. sylvestris* sampled for the two records have experienced similar changes in climate as CO₂ has risen such that changes due to drying/wetting are controlled for. Similar magnitude shifts in g_{wmax} and iWUE are thus of note as they suggest that changes in assimilation rate have not been experienced and g_{wmax} and iWUE have changed in a ~1:1 relationship over the pre-ind and post-ind periods. However, there is a clear change in rate between the iWUE and g_{wmax} records after ~ AD 1970 (Fig. 1)

Coupled biosphere-atmosphere models have been used to study the physiological forcing feedback. Leaf-scale models of stomatal conductance (g_s) (Collatz *et al.* 1991) describe a parameterization which results in a linear decrease in g_s with rising CO₂ concentrations (e.g. Sellers *et al.* 1996; Cox *et al.* 1999; Betts *et al.* 2000). Betts *et al.* (2000) used coupled models of global vegetation and climate to examine the response of g_s to doubled CO₂. Mean global values of g_s lower by 20% with a change to doubled CO₂ if only stomatal changes are considered with no associated change in leaf area index, but lower by only 12% when the stomatal response and the response in vegetation are included. We compared the % changes in

our $iWUE$ and g_{wmax} records to the modelled g_c (g_c = canopy conductance; daytime mean canopy conductance is the net transpirational conductance of all stomata integrated over the canopy depth) changes from Betts *et al.* (2000) over the same time period (Table 2). The percentage changes were calculated between $1xCO_2$ (323ppmv ~AD 1967) and the year AD 2000 (~370 ppmv). For the Betts *et al.* (2000) data, percentage change AD 1967-2000 was obtained by calculating the linear trend for modelled g_c at $1 x CO_2$ and at $2 x CO_2$ for their ‘RP’ (no associated change in vegetation and leaf area index kept at $1 x CO_2$) and ‘RPS’ (stomatal response and response in vegetation combined) experiments (see Table 1 in Betts *et al.* 2000 and Table 2 below). Although the absolute values of inferred g_{wmax} and g_s are different, the % changes of those values over the period AD 1967 to 2000 are virtually the same (Table S1 and Fig. S2 in Supplementary Material).

Model inferred g_c changes	2 x CO_2 ‘RP’	2 x CO_2 ‘RPS’
g_c at $1x CO_2$ (323 ppmv; AD 1967)	6.09	6.09
g_c at $2x CO_2$ (646 ppmv)	4.89	5.35
% change g_c from $1x$ to $2x CO_2$	-19.70%	-12.15%
% change g_c from $1x CO_2$ to 370 ppmv (AD 2000)	-2.9%	-1.8%
Proxy inferred $iWUE$ and g_{wmax} changes		
Site	Kiruna/Kevo	Laanila
% change from $1x CO_2$ to 370 ppmv (AD 2000)	-16%	-3%

Table 2. Comparison between conductance changes used by Betts *et al.* (2000) and proxy-inferred $iWUE$ and g_{wmax} changes. Canopy conductance (g_c [$mm s^{-1}$]) and % change in g_c were calculated via a linear trend for modelled g_c at $1 x CO_2$ and at $2 x CO_2$ for ‘RP’ (no associated change in vegetation and leaf area index kept at $1 x CO_2$) and ‘RPS’ (stomatal response and response in vegetation combined) modelling experiments (from Table 1 – Betts *et al.* 2000). These changes are compared to g_{wmax} and $iWUE$ changes (lower panel). The percentage changes of g_{wmax} and $iWUE$ were calculated between $1xCO_2$ (323ppmv ~AD 1967) and the year ~AD 2000 (~370 ppmv).

Modelled changes in stomatal conductance based on changes in stomatal apertures only (Betts *et al.* 2000) could clearly not account for the larger changes in $iWUE$ or g_{wmax} seen in the palaeo record (Table 2 and Fig. 1). Were the Betts *et al.* (2000) data used to project the linear change in g_c back to the pre-ind period (AD 1800-1850) the modelled changes would be much

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3 lower than those indicated by the palaeo data. However, it should be remembered that the
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5 Betts *et al.* (2000) data are global averages. The range of changes in iWUE displayed in the
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7 tree ring series is considerable even within a relatively small geographical range, site
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9 conditions clearly playing an important part in the degree to which trees are able to make
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11 water savings. The Betts *et al.* (2000) comparison with the palaeo data reveals an interesting
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13 divergence between the g_{wmax} and iWUE data after 1970 (Table 2). The 16% change in g_{wmax}
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15 (AD 1967-2000) is no longer reflected in a similar magnitude change in iWUE in *P. sylvestris*
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17 at Laanila, which has only shifted by 3% since 1970.
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25 Discussion

26 27 28 29 *A shift to static iWUE at Laanila?*

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34 Waterhouse *et al.* (2004), in a study on Finnish and British oak, beech and pine trees first
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36 noted that, at higher levels of atmospheric CO₂ concentration, rising iWUE began to plateau
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38 as measured in tree ring $\delta^{13}C$, much as is seen at Laanila (Fig 2). Long term trends in iWUE
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40 as calculated from tree ring $\delta^{13}C$ essentially record the relationship between ci and ca through
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42 time (equation 3). This relationship can be described as either passive or active depending on
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44 whether ci simply tracks ca , or whether the tree uses strong stomatal control to limit the
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46 change in ci as ca rises (McCarroll *et al.* 2009). If neither stomatal conductance nor
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48 photosynthetic rate alters, then for each rise in ca , ci will simply drift upwards in a passive
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50 response and iWUE will not increase. The more common response seen in trees appears to be
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52 the active one; iWUE increasing in parallel with rising ca as stomatal conductance is reduced.
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54 However, an examination of the entire Laanila iWUE time series reveals an apparent change
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56 in the response of the trees over the last few decades. Fig. 2 shows the measured iWUE time
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series for Laanila and also the trend that would have occurred had the trees maintained an active response to rising CO_2 throughout. Active c_i was calculated using equations (1) and (3) but maintaining the average starting $\delta^{13}\text{C}$ values throughout the series length (such a curve displays the $i\text{WUE}$ changes that would have been seen with the maintenance of constant c_i/c_a via strong active stomatal control).

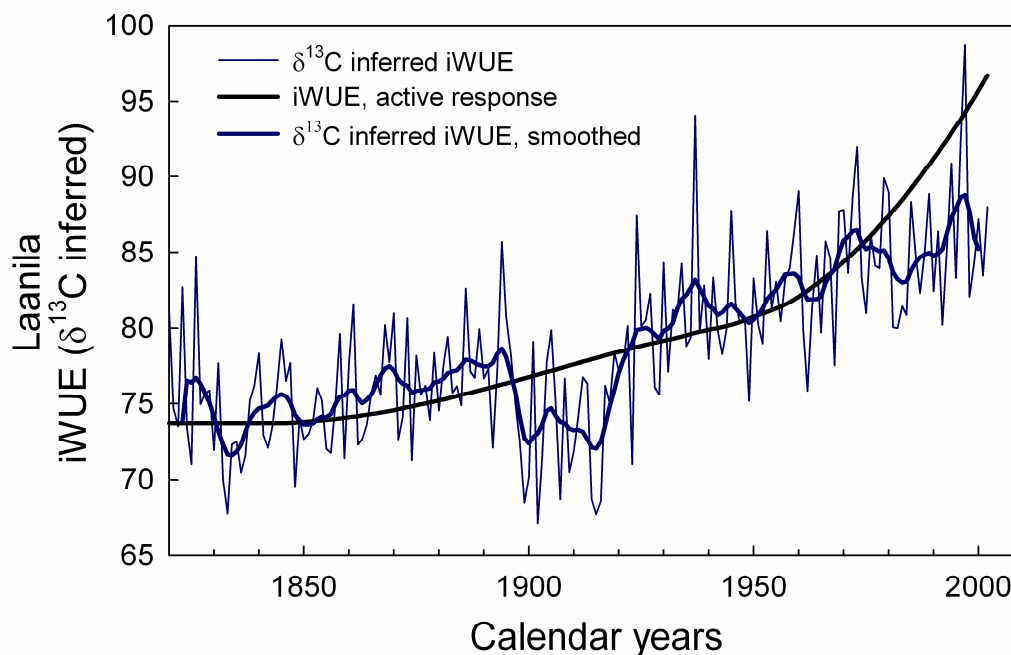


Fig. 2 Active response and measured response $i\text{WUE}$. Time series for actual $i\text{WUE}$ from $\delta^{13}\text{C}$ are shown at Laanila (blue, annual values and 10-year centralised moving average) along with the trend which would have occurred had the trees maintained an active response to rising CO_2 (stable c_i/c_a) (black).

At Laanila, the measured $i\text{WUE}$ shows a divergence from the modelled active response line after approximately AD 1970, suggesting a change from an active to a passive response in c_i/c_a . This divergence could be provoked by a change in climate, as occurred in the AD 1900s at Laanila. However, climate data are available for the last few decades from very close to Laanila and the modern divergence can be seen to be unrelated to a change in any of the climatic variables that $\delta^{13}\text{C}$ responds to. Simple linear correlations between Laanila $\delta^{13}\text{C}$ and climate in fact increase in strength when the post-1970 trend in $\delta^{13}\text{C}$ is removed (see

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3 McCarroll *et al.* 2009 and Gagen *et al.* 2007 for details). We conclude, on the basis of the
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5 divergence between the active and measured *ci/ca* response, that iWUE in *P. sylvestris* at this
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7 location is no longer rising.
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12 The results from Laanila are unusual but not unique. Saurer *et al.* (2004) find maintenance of
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14 *ci/ca* in $\delta^{13}\text{C}$ measurements from 26 high latitude Eurasian sites between AD 1961-1990 (335
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16 ppmv on average). However, a switch in iWUE, around AD 1970, has been seen elsewhere
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18 (e.g. Robertson 1998) whilst Marshall & Monserud (1996) also note static iWUE in various
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20 pine and fir species from sites in the western United States between ~AD 1920-1990 . A
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22 picture is emerging of a complex plastic response in long term adaptation of trees to changes
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24 in CO_2 . The Laanila series supports the idea of a limit to the ability of the trees to maintain
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26 stomatal control over *ci* under higher CO_2 .
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34 For much of the 150 year record length, before the divergence at ~ AD 1970, $g_{w\max}$ and $\delta^{13}\text{C}$
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36 do display a ~1:1 rate change as CO_2 has risen. It is of note that these two important Boreal
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38 species record changes of similar magnitudes over the 'pre-industrial' and 'post-industrial'
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40 periods. Two independent but closely related archives, in two different species, show changes
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42 of similar magnitude over the same period in response to rising CO_2 . It is notable that the
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44 measured changes in both archives in the past are also larger than those used in climate
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46 models for modelling canopy conductance changes.
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53 In contrast to the $\delta^{13}\text{C}$ record, the $g_{w\max}$ record from *B. nana* at Kiruna and Kevo seems to still
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55 be decreasing up to the present. The continuing decrease in stomatal conductance is observed
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57 commonly in *Betula pubescens* from various localities, suggesting that this birch species has
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59 not reached its response limit yet in this region. Kürschner *et al.* (1997) suggest a predicted
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3 response limit of approximately 400ppmv for *B. pubescens*. The indication of a nonlinear
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5 response, whether evident already or predicted to occur in the next decades, in the major
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7 evergreen and deciduous species of the Boreal forest clearly does not back up the continued
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9 use of a linear parameterisation for stomata conductance with rising CO₂ in coupled climate-
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11 vegetation model studies.
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17 The land surface schemes of GCMs require an accurate calculation of plant transpiration, and
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19 the actual value of change in stomatal conductance under CO₂ doubling is a vital parameter
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21 (Cox *et al.* 1998). At present GCM based studies of physiological forcing operate on a linear
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23 relationship between stomatal conductance and rising CO₂ to very high *ca* levels (Sellers *et al.*
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25 1996, Cox *et al.* 1999). The evidence presented here and elsewhere from inferred iWUE
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27 measurements (e.g. Waterhouse *et al.* 2004) suggest that, in fact, the linear relationship
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29 between stomatal conductance and rising CO₂ may not be valid at today's higher CO₂ levels
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31 for all species, in all regions.
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39 Using the linear g_s model, GCMs predict the drying effect of physiological forcing to cause an
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41 additional 1°C warming in summer temperatures in the Boreal forest (Pollard & Thompson
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43 1995). If this is not the case and there is a limit to the rise in iWUE derived from changes in
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45 stomatal conductance then physiological forcing will cause a smaller additional warming than
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47 expected. Whilst this may be good news, potentially limiting a positive feedback, it is
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49 important to get the estimate correct.
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55 The two species represented in this study cover a huge area of the Boreal region. In order to
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57 account for the broad spatial variability in plants' response to changing CO₂, more records of
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59 changing iWUE would be needed to model the canopy conductance changes through time
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3 over different regions. Unfortunately, for most stomatal frequency records, the geometry
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5 changes of stomata are unknown because they simply were not measured. In order to attribute
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7 the iWUE changes to the stomatal density and size regulation mechanism within the same
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9 species g_{wmax} records would be needed for the same species monitored for iWUE (inferred
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11 from $\delta^{13}C$). We note a pressing need for measurements of stomatal morphology and stomatal
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13 frequency changes in more species, coupled to measurements of water use efficiency from the
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15 same region, for the same species.
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22 **Acknowledgments:**

23
24 This research was funded by the European Union (Millennium 017008), and the British
25
26 Natural Environment Research Council (NJL NE/B501504 GHFY). MHG is supported by an
27
28 RCUK Fellowship. WF is thankful to the AXA Research Fund for financial support. This
29
30 work was developed following the Millennium Project's Northern Group Workshop held at
31
32 the Royal Swedish Academy of Sciences' Abisko Scientific Research Station. Ongoing
33
34 thanks to all our friends in the Millennium consortium.
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