Evidence of changing intrinsic water use efficiency under rising atmospheric CO2 concentrations in Boreal Fennoscandia from subfossil leaves and tree ring $\delta13C$ ratios

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Journal:	*Global Change Biology*
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 $\delta^{13}C$ (*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

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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 i>P. sylvestris reaches a plateau. We suggest that northern boreal i>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.

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Running title: Palaeorecords of plants responses to rising CO_2

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Keywords: intrinsic water use efficiency, stomatal density, stomatal conductance, Fennoscandia, boreal forest, physiological forcing.

Submitted to Global Change Biology for consideration as a Primary Research Article.

7th September 2009, resubmitted February 1st 2010.

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 $\delta^{13}C$ (*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 CO_2 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 CO_2, 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

experiments at the plant level reveal the incredible speeds with which plants can alter their

stomatal numbers after exposure to raised CO_2 (Lake *et al.* 2001; Hetherington & Woodward

2003). However, physiological forcing is not well understood or constrained (National

Research Council 2003; Doutriaux-Boucher 2009). For example, warming might be offset by

increases in leaf area index due to higher temperatures and precipitation changes that may

accompany higher CO_2 levels (Betts *et al.* 2000) and there is evidence of thresholds (so-called

response limits) in plant capacity to raise water use efficiency (Kürschner *et al.* 1997).

Moreover the degree of spatial and species variability in the response of water use efficiency

and stomatal conductance is an unknown. Uncertainty in the magnitude and significance of

physiological forcing arises because most studies on the response of stomatal conductance to

rising CO_2 are extrapolated from short term, leaf 'exposure' studies (e.g. Ainsworth & Rogers

2007) which cannot take account of acclimation that may alter the stomatal response over

longer timescales (National Research Council 2003). Thus, at present, we do not know

whether measured reductions in stomatal conductance in plants exposed to raised CO_2

concentrations reflect the full range of long-term water use behaviour in forests (Leuzinger &

Körner 2007). Short term changes in stomatal opening and closure are only one component of

the system.

Palaeo-observations of changes in stomatal physiology and plant stable carbon isotope ratios

might be used to better understand the changes in water use efficiency underlying the

physiological forcing feedback. Stomatal frequency and geometry measured from subfossil

leaves and $\delta^{13}C$ ratios from tree rings represent independent records of changes in stomatal

control over water use efficiency in a palaeo-context. As such, combined analysis of these

independent parameters, within a region, might be used to investigate long-term plant

responses at differing CO_2 levels and to elucidate the physiological changes driving shifts in

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intrinsic water use efficiency (iWUE). The rate at which trees exchange CO_2 and water with

the atmosphere can be estimated from $\delta^{13}C$ as iWUE, the potential rather than actual WUE

(Ehleringer & Cerling 1995; Saurer *et al.* 2004); actual WUE being also influenced by

changes in respiratory losses over time at the whole plant level and being sensitive to vapour

pressure differences between atmosphere and leaf. Tree ring stable carbon isotope ratio

derived time series of iWUE represent the maximum potential WUE as it changes through

time. Stable carbon isotope ($\delta^{13}C$) ratios are known to provide a reliable method of

monitoring long term changes in plant physiology (Farquhar *et al.* 1982).

Whilst rising iWUE over the recent past has been widely noted in tree ring based $\delta^{13}C$ studies

(e.g. (Saurer *et al.* 2004; Waterhouse *et al.* 2004) its cause has not been attributed directly to a

specific physiological source. Nor is it clear how iWUE will change with continued rising

CO_2 in the future. We compare $\delta^{13}C$ based records of iWUE from northern Boreal *Pinus*

sylvestris and a record of maximum stomatal conductance to water vapour (g_{wmax}) derived

from subfossil *Betula nana* leaves from the same geographical area (Table 1). The ideal data

set to attribute palaeo changes in iWUE to a specific physiological cause would be measured

iWUE and stomatal density variations from the same palaeoclimate archive. Unfortunately,

for most species the stomatal geometry and density records are unknown because they simply

were not measured. With this in mind we investigate a different question – when comparing

two independent lines of proxy evidence for changes in water use efficiency, what changes do

two of the dominant Boreal species, from the same region, show?

Using subfossil leaf assemblages Kürschner *et al.* (1997) illustrated a non linear response in

stomatal frequency changes of European birch and oak species and predicted that certain

species may reach a 'response limit' and stop altering stomatal geometry and number at

between 350-400ppmv CO_2. Diverging results for different plant species point to a genus or species-specific adaptation capacity, where in certain taxa stomatal frequency response limits are already reached at present day CO_2 levels (Kürschner *et al.* 1997; Royer 2001). Waterhouse *et al.* (2004) also find that iWUE in trees across northern Europe is, in some cases, no longer rising. Clearly plant iWUE cannot rise indefinitely and stomatal conductance and frequency can never be zero. Hence future predictions of evapotranspiration flux within the Boreal forest zone might be more accurately gauged by taking account of palaeo evidence for threshold points in different species across different regions.

The C3 plants have internalised their gas exchange surfaces in order to maintain control over water budget, a necessary step as any membrane which is permeable to large CO_2 molecules is also permeable to water molecules (McCarroll & Loader 2004). The gas exchange and water balance mechanisms in plants are therefore intrinsically linked; when plants take in CO_2 through stomata they transpire water at the same time. Long term water exchange in plants can be studied via $\delta^{13}C$ ratios in annual tree ring cellulose, because $\delta^{13}C$ essentially records CO_2 gas exchange (Saurer *et al.* 2004). There is a slight offset between leaf and trunk $\delta^{13}C$ and using $\delta^{13}C$ cellulose to calculate iWUE, rather than initial photosynthate or even leaf $\delta^{13}C$, may introduce some bias into the calculated iWUE values. However, the temporal trends will remain unaffected (Waterhouse *et al.* 2004).

Increased iWUE under raised CO_2 has been reported in:*Pinus* (Runion *et al.* 1999), *Quercus* (Anderson & Tomlinson 1998; Lodge *et al.* 2001), *Fagus* (Bucher-Wallin *et al.* 2000) and *Picea* (Roberntz & Stockfors 1998) with an average gain of around 19% in northern Eurasia (Saurer et al 2004) over the industrial period, although over the wider northern hemisphere percentage changes are highly variable. Waterhouse et al (2004) report increases of between

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, CO_2 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 $\delta^{13}C$ in wood cellulose extracted from absolutely dated

annual rings of trees record variability in the internal partial pressure of CO_2 (*ci*) (Farquhar et

al. 1982) thus *ci* and iWUE were calculated from measured tree ring $\delta^{13}C$ values.

Site	Proxy	Location	Species	Source
Laanila, Finland	$\delta^{13}C$-inferred iWUE	68ºN 27ºE	*Pinus sylvestris*	Gagen *et al.* (2007) and this study.
Kiruna, Sweden Kevo, Finland	Stomata-inferred g_{wmax}	67ºN 20ºE 69°N 27°E	*Betula nana*	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 $\delta^{13}C$ in 2001,

the time series is fully described elsewhere (Gagen *et al.* 2007). Trees were sampled using a

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

Fractionation (Δ) of carbon isotopes by C3 plants is described as follows,

$$\Delta = a + (b\text{-}a) \cdot ci/ca \qquad (1)$$

where *a* (28‰) and *b* (4.4‰) are constants representing fractionation due to diffusion and carboxylation respectively and *ci* and *ca* are intercellular and atmospheric CO_2 concentrations.

Fractionation (Δ) acts upon the isotopic composition of the source gas (atmospheric CO_2) as follows:

$$\delta^{13}C \text{ plant} = \delta^{13}C \text{ atmosphere} - \Delta \qquad (2)$$

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such that post industrial time series of δ^{13}C measurements contain the industrially derived

change in atmospheric δ^{13}C which is corrected against the actual atmospheric δ^{13}C

measurements (e.g. Francey *et al*. 1999). Corrected plant δ^{13}C then reflects a pre AD 1850

δ^{13}C value of -6.4 ppmv.

Intrinsic water use efficiency is calculated as:

$$iWUE = (ca\text{-}ci) \,/\, 1.6 \tag{3}$$

where *ci* is calculated by a re-arrangement of equation 1 after applying equation 2 (for full

derivation see McCarroll *et al*. 2009).

Stable carbon isotope ratios in tree rings are commonly used as a climate proxy because *ci* is

externally forced by a control that is often climatic, whereas *ca* remains stable or its changes

can be accounted for (McCarroll *et al.* 2009). Since the fractionation constants (*a* and *b*) do

not vary in response to climate and the atmospheric concentration of CO_2 (*ca*) is known, δ^{13}C

essentially records changes in *ci* as regulated by two processes: stomatal conductance (g_s),

and photosynthetic assimilation rate (*A*). If *ca* changes through time the response of the plant

to these changes will also be recorded in tree ring δ^{13}C.

A record of maximum stomatal conductance to water vapour (g_{wmax}) from subfossil Betula

nana *leaves*

The long term g_{wmax} time series (AD 1750-2008) was obtained from measured changes in

stomatal density and stomatal geometry using standard paleoecological methods, in subfossil

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B. nana leaves sampled from a peat mire at Kiruna in northern Swedish Lapland (Table 1). In addition, *B. nana* leaves were collected from (*i*) herbarium specimens that were growing in Fennoscandia >67°N (n years=5, AD 1919, 1935, 1958, 1963, and 1965), (*ii*) from a mire at Kevo (leaves growing AD 1976-1996), and (*iii*) from modern plants growing at Kevo (69°45'N, 27°E, AD 1997-2008, 1998 missing).

Subfossil leaf fragments were picked from peat extracted from the mire, identified and separated under a binocular microscope by leaf and cuticle morphologies. For microscopic analysis, all leaf fragments were bleached in a cold 2-4% sodium hypochlorite solution, rinsed in water, and mounted on microscopic slides. Standardized, computer-aided analysis of epidermal parameters was performed on a Leica Quantimet 500C/500+ image-analysis system. The number of analysed leaf fragments in each sample varies because of different cuticle preservation in the sediments. A maximum of ten stomata-bearing alveoles/leaf fragments were analysed for epidermal cell density (*ED* [n/mm²]) and stomatal density (*SD* [n/mm²]). On each leaf fragment, twenty stomatal pores were measured for maximum pore length (*PL* [μm]) and maximum stomatal length (*SL* [μm]). Based on these measurements, maximum stomatal conductance to water vapour (g_{wmax} [mol m^{-2} s^{-1}]) was calculated using the equation proposed by Franks & Beerling (2009):

$$g_{w\max} = \frac{d}{v} \cdot SD \cdot a_{\max} \left/ \left(l + \frac{\pi}{2}\sqrt{a_{\max}/\pi} \right) \right. , \qquad (4)$$

where *d* (the diffusivity of water vapour in air [m^2 s^{-1}]), *v* (the molar volume of air [m^3 mol^{-1}]), and *l* (the depth of the pore) were taken as constants, and a_{max} is the maximal pore 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):

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

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):

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

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

Kiruna monolith from ca. 1 year (AD 2003-1955) to 3 or 5 years (between AD 1954 and 1721), and in the Kevo monolith each sample is less than 1 year's peat accumulation (AD 1976-1996).

To obtain a combined dataset, values from *B. nana* leaves growing in the same year were averaged. Subsequently, in order to extract the main trend in the g_{wmax}, g_s, S and PL records, the combined core and herbarium observations were smoothed with a robust *loess* function (locally weighted regression) (Cleveland & Devlin 1988) with a constant window width and fixed target points in time. The smoothed curve was constructed by determining fitted values at 10-year intervals with a 100-year window-width (here referred to the full span of the window). In this method the smoothing window does not change as a function of data density around the fitted values. The smoothed g_{wmax} time series is shown in Fig. 1 and the g_s, PL, S, and the unsmoothed g_{wmax} time series are shown in the Supplementary Material (Fig. S1).

Results

Changes in iWUE since the start of industrialisation to present (AD 1820 to 2002) at Laanila reveal a significant rise over time (Fig.1). There is a temporary decrease in iWUE centred on AD 1910 which is known to be climatically derived. The iWUE series is scaled from $\delta^{13}C$ values which contain the effects of climate and the period around AD 1910 is known to be a prolonged cold spell in northern Fennoscandia, detected in many tree ring proxies from the 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 $\delta^{13}C$ 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).

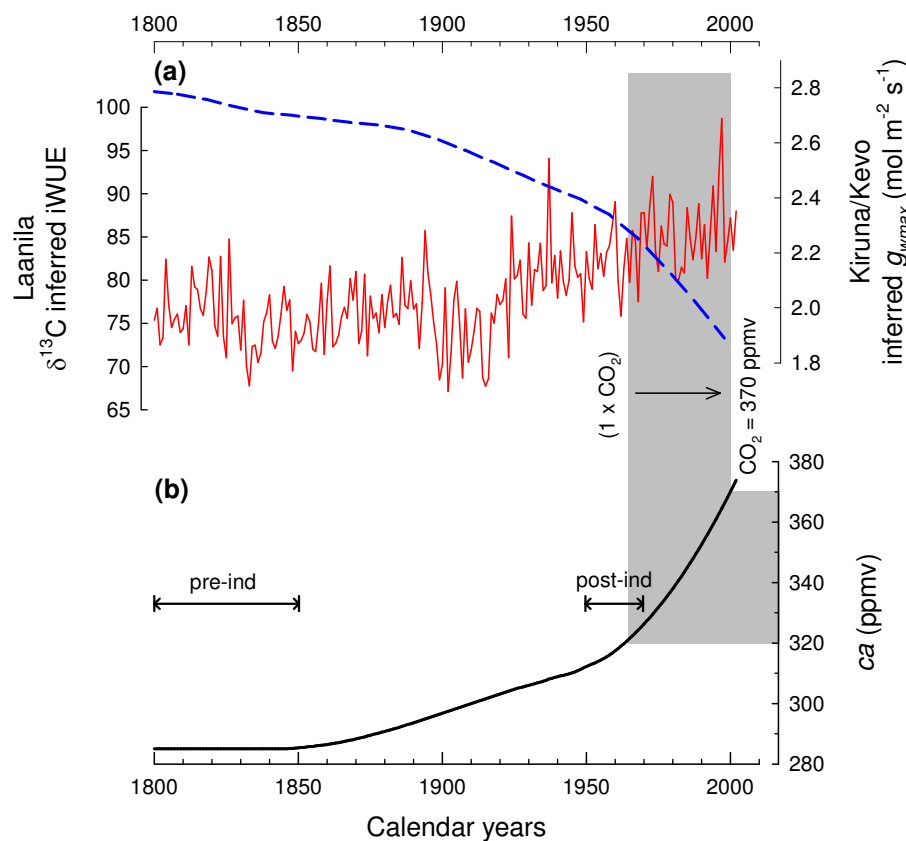**Fig 1. (a) $\delta^{13}C$ inferred intrinsic water-use efficiency (iWUE) record from *P. sylvestris* tree rings at Laanila (red line) and 10-year smoothed stomatal density and geometry inferred maximum stomatal conductance (g_{wmax}) from subfossil and modern *B. nana* leaves at Kiruna/Kevo (dashed 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 CO_2 = 370 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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g_{wmax} remained almost constant. Instead, after ~AD 1850 the steeper decline of stomatal density was not counterbalanced by an equal increase in stomatal size (see Fig. S1 and Supplementary Material). Hence, the change in g_{wmax} we observe over the study period is 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 CO_2 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 CO_2 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 CO_2. Mean global values of g_s lower by 20% with a change to doubled CO_2 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

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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 gc 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

lower than those indicated by the palaeo data. However, it should be remembered that the

Betts *et al*. (2000) data are global averages. The range of changes in iWUE displayed in the

tree ring series is considerable even within a relatively small geographical range, site

conditions clearly playing an important part in the degree to which trees are able to make

water savings. The Betts *et al*. (2000) comparison with the palaeo data reveals an interesting

divergence between the g_{wmax} and iWUE data after 1970 (Table 2). The 16% change in g_{wmax}

(AD 1967-2000) is no longer reflected in a similar magnitude change in iWUE in *P. sylvestris*

at Laanila, which has only shifted by 3% since 1970.

Discussion

A shift to static iWUE at Laanila?

Waterhouse *et al*. (2004), in a study on Finnish and British oak, beech and pine trees first

noted that, at higher levels of atmospheric CO_2 concentration, rising iWUE began to plateau

as measured in tree ring $\delta^{13}C$, much as is seen at Laanila (Fig 2). Long term trends in iWUE

as calculated from tree ring $\delta^{13}C$ essentially record the relationship between *ci* and *ca* through

time (equation 3). This relationship can be described as either passive or active depending on

whether *ci* simply tracks *ca,* or whether the tree uses strong stomatal control to limit the

change in *ci* as *ca* rises (McCarroll *et al*. 2009). If neither stomatal conductance nor

photosynthetic rate alters, then for each rise in *ca, ci* will simply drift upwards in a passive

response and iWUE will not increase. The more common response seen in trees appears to be

the active one; iWUE increasing in parallel with rising *ca* as stomatal conductance is reduced.

However, an examination of the entire Laanila iWUE time series reveals an apparent change

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 ci was calculated using equations (1) and (3)

but maintaining the average starting $\delta^{13}C$ values throughout the series length (such a curve

displays the iWUE changes that would have been seen with the maintenance of constant ci/ca

via strong active stomatal control).

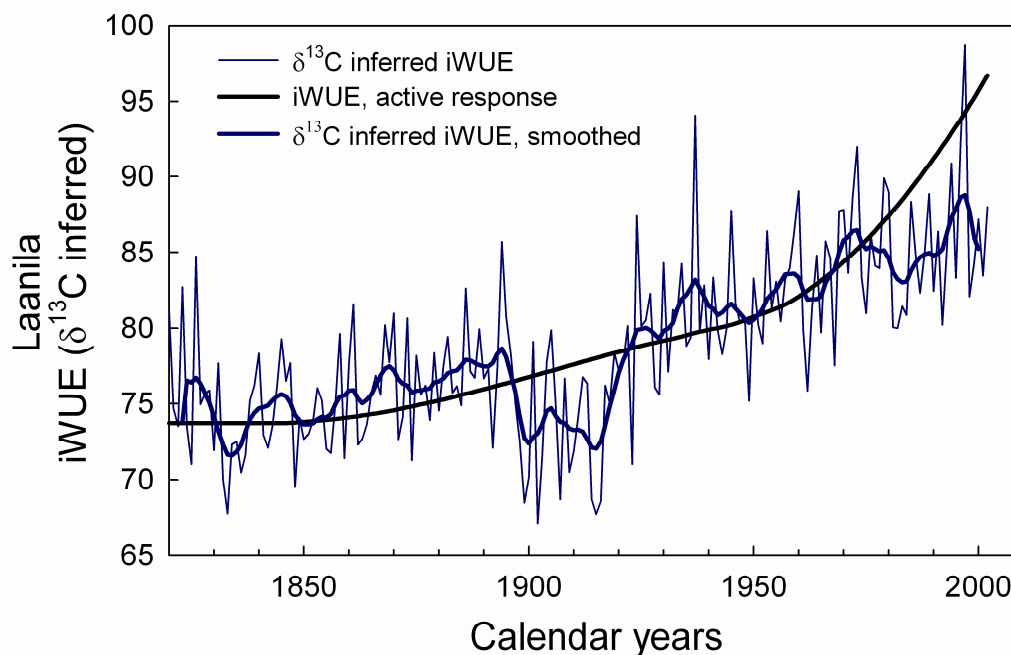**Fig. 2 Active response and measured response iWUE. Time series for actual iWUE from $\delta^{13}C$ are shown at Laanila (blue, annual values and 10-year centralised moving average) along with the trend which would have occurred had the trees maintained an active response to rising CO_2 (stable ci/ca)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

At Laanila, the measured iWUE shows a divergence from the modelled active response line

after approximately AD 1970, suggesting a change from an active to a passive response in

ci/ca. 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 $\delta^{13}C$ responds to. Simple linear correlations between Laanila $\delta^{13}C$ and

climate in fact increase in strength when the post-1970 trend in $\delta^{13}C$ is removed (see

McCarroll *et al*. 2009 and Gagen *et al*. 2007 for details). We conclude, on the basis of the divergence between the active and measured *ci/ca* response, that iWUE in *P. sylvestris* at this location is no longer rising.

The results from Laanila are unusual but not unique. Saurer *et al*. (2004) find maintenance of *ci/ca* in δ^{13}C measurements from 26 high latitude Eurasian sites between AD 1961-1990 (335 ppmv on average). However, a switch in iWUE, around AD 1970, has been seen elsewhere (e.g. Robertson 1998) whilst Marshall & Monserud (1996) also note static iWUE in various pine and fir species from sites in the western United States between ~AD 1920-1990 . A picture is emerging of a complex plastic response in long term adaptation of trees to changes in CO_2. The Laanila series supports the idea of a limit to the ability of the trees to maintain stomatal control over *ci* under higher CO_2.

For much of the 150 year record length, before the divergence at ~ AD 1970, g_{wmax} and δ^{13}C do display a ~1:1 rate change as CO_2 has risen. It is of note that these two important Boreal species record changes of similar magnitudes over the 'pre-industrial' and 'post-industrial' periods. Two independent but closely related archives, in two different species, show changes of similar magnitude over the same period in response to rising CO_2. It is notable that the measured changes in both archives in the past are also larger than those used in climate models for modelling canopy conductance changes.

In contrast to the δ^{13}C record, the g_{wmax} record from *B. nana* at Kiruna and Kevo seems to still be decreasing up to the present. The continuing decrease in stomatal conductance is observed commonly in *Betula pubescens* from various localities, suggesting that this birch species has not reached its response limit yet in this region. Kürschner *et al*. (1997) suggest a predicted

response limit of approximately 400ppmv for *B. pubescens*. The indication of a nonlinear

response, whether evident already or predicted to occur in the next decades, in the major

evergreen and deciduous species of the Boreal forest clearly does not back up the continued

use of a linear parameterisation for stomata conductance with rising CO_2 in coupled climate-

vegetation model studies.

The land surface schemes of GCMs require an accurate calculation of plant transpiration, and

the actual value of change in stomatal conductance under CO_2 doubling is a vital parameter

(Cox *et al*. 1998). At present GCM based studies of physiological forcing operate on a linear

relationship between stomatal conductance and rising CO_2 to very high *ca* levels (Sellers *et al*.

1996, Cox *et al*. 1999). The evidence presented here and elsewhere from inferred iWUE

measurements (e.g. Waterhouse *et al*. 2004) suggest that, in fact, the linear relationship

between stomatal conductance and rising CO_2 may not be valid at today's higher CO_2 levels

for all species, in all regions.

Using the linear g_s model, GCMs predict the drying effect of physiological forcing to cause an

additional 1°C warming in summer temperatures in the Boreal forest (Pollard & Thompson

1995). If this is not the case and there is a limit to the rise in iWUE derived from changes in

stomatal conductance then physiological forcing will cause a smaller additional warming than

expected. Whilst this may be good news, potentially limiting a positive feedback, it is

important to get the estimate correct.

The two species represented in this study cover a huge area of the Boreal region. In order to

account for the broad spatial variability in plants' response to changing CO_2, more records of

changing iWUE would be needed to model the canopy conductance changes through time

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over different regions. Unfortunately, for most stomatal frequency records, the geometry

changes of stomata are unknown because they simply were not measured. In order to attribute

the iWUE changes to the stomatal density and size regulation mechanism within the same

species $g_{w\max}$ records would be needed for the same species monitored for iWUE (inferred

from δ^{13}C). We note a pressing need for measurements of stomatal morphology and stomatal

frequency changes in more species, coupled to measurements of water use efficiency from the

same region, for the same species.

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