

# Variation in leaf physiology of *Salix arctica* within and across ecosystems in the High Arctic: test of a dual isotope ( $\Delta^{13}\text{C}$ and $\Delta^{18}\text{O}$ ) conceptual model

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Received: 27 December 2005 / Accepted: 23 October 2006 / Published online: 15 November 2006  
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**Abstract** Leaf carbon isotope discrimination ( $\Delta^{13}\text{C}$ ) varies with the balance between net photosynthesis ( $A$ ) and stomatal conductance ( $g_s$ ). Inferences that can be made with  $\Delta^{13}\text{C}$  are limited, as changes could reflect variation in  $A$  and/or  $g_s$ . Investigators have suggested that leaf  $\delta^{18}\text{O}$  enrichment above source water ( $\Delta^{18}\text{O}$ ) may enable differentiation between sources of variation in  $\Delta^{13}\text{C}$ , as leaf  $\Delta^{18}\text{O}$  varies with transpiration rate ( $E$ ), which is closely correlated with  $g_s$  when leaves experience similar leaf to air vapor pressure differences. We examined leaf gas exchange of *Salix arctica* at eight sites with similar air temperatures and relative humidities but divergent soil temperatures and soil water contents near Pituffik, Greenland (76°N, 38°W). We found negative correlations at the site level between  $g_s$  and  $\Delta^{18}\text{O}$  in bulk leaf tissue ( $r^2 = 0.62$ , slope =  $-17.9\text{‰}/\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ,  $P = 0.02$ ) and leaf  $\alpha$ -cellulose ( $r^2 = 0.83$ , slope =  $-11.5\text{‰ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ,  $P < 0.01$ ), consistent with the notion that leaf water enrichment declines with increasing  $E$ . We also found negative correlations at the site-level between intrinsic water-use efficiency (iWUE) and  $\Delta^{13}\text{C}$  in bulk leaf tissue ( $r^2 = 0.65$ , slope =  $-0.08\text{‰}/\mu\text{mol CO}_2/\text{mol H}_2\text{O}$ ,  $P = 0.02$ ) and leaf  $\alpha$ -cellulose ( $r^2 = 0.50$ , slope =  $-0.05\text{‰}/[\mu\text{mol CO}_2/\text{mol H}_2\text{O}]$ ,  $P = 0.05$ ). When increasing  $\Delta^{13}\text{C}$  was driven by increasing  $g_s$  alone, we found negative slopes between  $\Delta^{13}\text{C}$  and  $\Delta^{18}\text{O}$  for bulk leaf tissue ( $-0.664$ ) and

leaf  $\alpha$ -cellulose ( $-1.135$ ). When both  $g_s$  and  $A_{\text{max}}$  increased, we found steeper negative slopes between  $\Delta^{13}\text{C}$  and  $\Delta^{18}\text{O}$  for bulk leaf tissue ( $-2.307$ ) and leaf  $\alpha$ -cellulose ( $-1.296$ ). Our results suggest that the dual isotope approach is capable of revealing the qualitative contributions of  $g_s$  and  $A_{\text{max}}$  to  $\Delta^{13}\text{C}$  at the site level. In our study, bulk leaf tissue was a better medium than leaf  $\alpha$ -cellulose for application of the dual isotope approach.

**Keywords** Greenland · Photosynthesis · Stomatal conductance

## Introduction

Ecologists have long held an interest in factors governing the balance between photosynthetic  $\text{CO}_2$  assimilation and  $\text{H}_2\text{O}$  loss in terrestrial plants (Schimper 1898; Warming 1909). In recent years, leaf carbon isotope discrimination ( $\Delta^{13}\text{C}$ ) has emerged as powerful tool for examining the balance between net photosynthesis ( $A$ ) and stomatal conductance to water vapor ( $g_s$ ) (Farquhar et al. 1982, 1989a, 1989b).  $\Delta^{13}\text{C}$  is of limited use in studies that require information about individual gas exchange variables, as changes in  $\Delta^{13}\text{C}$  could reflect variation in  $A$  and/or  $g_s$ . To address this limitation, investigators have suggested incorporating analyses of leaf  $\delta^{18}\text{O}$  enrichment above source water ( $\Delta^{18}\text{O}$ ) to differentiate between changes in  $\Delta^{13}\text{C}$  driven by changes in  $A$  versus  $g_s$  (Farquhar et al. 1989b; Farquhar and Lloyd 1993). This suggestion builds upon the recognition that  $\Delta^{18}\text{O}$  varies with transpiration rate ( $E$ ), which is closely correlated with  $g_s$  when leaves experience similar leaf to air vapor pressure differences.

Communicated by Dan Yakir.

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### Carbon isotope theory

Plant material  $\delta^{13}\text{C}$  is depleted relative to that of atmospheric  $\text{CO}_2$  as a result of two fractionation steps during  $\text{CO}_2$  assimilation. The first fractionation step occurs during  $\text{CO}_2$  diffusion into the leaf, as  $^{13}\text{CO}_2$  diffuses more slowly than  $^{12}\text{CO}_2$ . The second fractionation step occurs during carboxylation, as ribulose 1,5-bisphosphate (RuBP) carboxylase/oxygenase (RuBisCo) reacts more readily with  $^{12}\text{CO}_2$ . Carbon isotope discrimination during photosynthesis is, therefore, expressed as follows (Farquhar et al. 1982, 1989a):

$$\delta^{13}\text{C}_p = \delta^{13}\text{C}_a - a - (b - a)(c_i/c_a), \quad (1)$$

where  $\delta^{13}\text{C}_p$  and  $\delta^{13}\text{C}_a$  are the carbon isotope ratios (expressed relative to the Pee Dee Belemnite standard) of leaf tissue and atmospheric  $\text{CO}_2$ , respectively;  $a$  is the fractionation during diffusion (4.4‰);  $b$  is the fractionation during carboxylation (27‰); and  $c_i$  and  $c_a$  are  $\text{CO}_2$  concentrations in leaf intercellular spaces and in the atmosphere, respectively. Equation 1 indicates that, when  $\delta^{13}\text{C}_a$  is known,  $c_i/c_a$  drives variation in  $\delta^{13}\text{C}_p$ . The carbon isotope composition of plant material may also be expressed as discrimination relative to  $\delta^{13}\text{C}$  of atmospheric  $\text{CO}_2$  as follows (O’Leary 1993):

$$\Delta^{13}\text{C} = \frac{\delta^{13}\text{C}_a - \delta^{13}\text{C}_p}{1 + \delta^{13}\text{C}_p/1000}. \quad (2)$$

Intercellular  $\text{CO}_2$  concentrations may vary with  $A$  or  $g_s$ , while atmospheric  $\text{CO}_2$  concentrations are relatively stable under well-mixed conditions on timescales from days to weeks. The balance between  $A$  and  $g_s$  is often expressed as a ratio and referred to as the intrinsic water-use efficiency (iWUE) (Farquhar et al. 1982).

### Oxygen isotope theory

The  $\delta^{18}\text{O}$  value of soil water reflects a weighted average of  $\delta^{18}\text{O}$  in precipitation and near-surface evaporative enrichment. Oxygen isotopes in soil water are not fractionated during uptake by plant roots (Allison et al. 1984). Thus, xylem water  $\delta^{18}\text{O}$  is equivalent to source water  $\delta^{18}\text{O}$  ( $\delta^{18}\text{O}_s$ ) (Ehleringer and Dawson 1992). Leaf water is enriched above xylem water during transpiration because  $\text{H}_2^{18}\text{O}$  diffuses at a slower rate and has a lower vapor pressure than  $\text{H}_2^{16}\text{O}$ . The isotopic composition of leaf water at the sites of evaporation ( $\delta^{18}\text{O}_e$ ) is expressed as follows (Craig and Gordon 1965; Dongman et al. 1974; Farquhar and Lloyd 1993):

$$\delta^{18}\text{O}_e = \delta^{18}\text{O}_s + \varepsilon^* + \varepsilon_k + (\delta^{18}\text{O}_v - \delta^{18}\text{O}_s - \varepsilon_k) \frac{e_a}{e_i}, \quad (3)$$

where  $\varepsilon^*$  is the temperature-dependent fractionation associated with vapor pressure depression by  $\text{H}_2^{18}\text{O}$  (Majoube 1971),  $\varepsilon_k$  is the kinetic fractionation as water diffuses through the stomata and boundary layer,  $\delta^{18}\text{O}_v$  is the isotopic composition of atmospheric water vapor, and  $e_a$  and  $e_i$  are atmospheric and leaf intercellular vapor pressures, respectively. The isotopic composition of leaf water at the sites of evaporation can also be described as the enrichment above source water ( $\Delta^{18}\text{O}_e$ ), approximated by:

$$\Delta^{18}\text{O}_e = \varepsilon^* + \varepsilon_k + (\Delta^{18}\text{O}_v - \varepsilon_k) \frac{e_a}{e_i}, \quad (4)$$

where  $\Delta^{18}\text{O}_v$  is the isotopic composition of atmospheric water vapor relative to  $\delta^{18}\text{O}_s$  (Farquhar and Lloyd 1993). In natural systems,  $\Delta^{18}\text{O}_v$  is generally close to  $-\varepsilon^*$  and, therefore, proportional to  $1 - e_a/e_i$  (Barbour et al. 2000). Holding  $e_a$  constant, Eq. 4 predicts that reductions in  $e_i$  will lead to corresponding reductions in  $\Delta^{18}\text{O}_e$ . Increasing rates of transpiration ( $E$ ) cool the leaf, reduce  $e_i$  and should, therefore, reduce  $\Delta^{18}\text{O}_e$ .

Investigators have consistently found that bulk leaf water is less enriched than water at the sites of evaporation (e.g., Allison et al. 1985; Yakir et al. 1990; Flanagan et al. 1991), with the magnitude of this discrepancy increasing with  $E$  (Walker et al. 1989; Flanagan et al. 1994). Farquhar and Lloyd (1993) suggested that this discrepancy reflects changes in the mixing of water within the leaf and described a Péclet effect ( $\wp$ ) as the ratio of the convective flux of unenriched source water to the sites of evaporation, opposed by the back-diffusion of enriched water into the leaf as follows:

$$\wp = \frac{EL}{CD}, \quad (5)$$

where  $L$  is a scaled distance (m) over which the effect occurs, accounting for tortuosity,  $C$  is the molar density of water ( $55 \times 10^3 \text{ mol m}^{-3}$ ) and  $D$  is the diffusivity of  $\text{H}_2^{18}\text{O}$  in water ( $2.66 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ ). The enrichment of leaf water, relative to source water, over the effective length ( $\Delta^{18}\text{O}_L$ ) is then described as (Farquhar and Lloyd 1993):

$$\Delta^{18}\text{O}_L = \frac{\Delta^{18}\text{O}_e(1 - e^{-\wp})}{\wp}. \quad (6)$$

The Péclet effect, therefore, reinforces the effect of  $E$  on the enrichment of leaf water. With increasing  $E$ , mixing of water within the leaf becomes increasingly

dominated by the convective flux to the sites of evaporation, thereby reducing the enrichment of bulk leaf water.

When leaf water reaches the chloroplasts, carbonic anhydrase (CA) catalyzes the near-complete exchange of  $^{18}\text{O}/^{16}\text{O}$  between  $\text{H}_2\text{O}$  and  $\text{CO}_2$ . Further enrichment occurs during biosynthesis. Cellulose, for instance, is approximately 27‰ enriched relative to water at the site of synthesis, as a result of exchange between carbonyl-oxygen and water (Sternberg and DeNiro 1983; Sternberg 1989; Yakir and DeNiro 1990). Leaf cellulose and bulk leaf tissue, therefore, carry a  $\delta^{18}\text{O}$  value that reflects both source water  $\delta^{18}\text{O}$  and leaf water enrichment, which is expected to vary with  $E$ . Much like carbon isotope discrimination, the oxygen isotope composition of leaf cellulose and bulk leaf tissue may be expressed as enrichment above source water (Barbour et al. 2000):

$$\Delta^{18}\text{O}_p = \frac{\delta^{18}\text{O}_p - \delta^{18}\text{O}_s}{1 + \delta^{18}\text{O}_s/1000}, \quad (7)$$

where  $\delta^{18}\text{O}_p$  is that of bulk leaf tissue or leaf  $\alpha$ -cellulose and  $\delta^{18}\text{O}_s$  is that of the source (xylem) water.

Combining carbon and oxygen isotope ratios in a dual isotope conceptual model

Leaf water enrichment varies with  $E$ , which is closely correlated with  $g_s$  when leaves experience similar leaf to atmosphere vapor pressure differences. Two processes limit leaf water enrichment when rates of  $E$  and  $g_s$  are relatively high. Transpiration reduces leaf temperature and the water vapor pressure of leaf intercellular spaces, thereby increasing  $e_a/e_i$ . Transpiration also affects mixing of water within the leaf. When rates of  $E$  are relatively high, mixing is dominated by the convective flux of unfractionated water to the sites of evaporation, which tends to reduce leaf water enrichment. Building upon the recognition that leaf water enrichment varies with  $E$ , several authors have suggested that changes in the magnitude of leaf water enrichment may enable differentiation between changes in  $c_i/c_a$  ( $\Delta^{13}\text{C}$ ) that are driven by changes in  $g_s$  and those that are driven by changes in  $A$  (Farquhar et al. 1989b; Farquhar and Lloyd 1993; Yakir and Israeli 1995; Saurer et al. 1997; Farquhar et al. 1998; Barbour and Farquhar 2000; Scheidegger et al. 2000; Barbour et al. 2005). The dual isotope model is characterized by three simple predictions:

- (1) When variation in  $\Delta^{13}\text{C}$  is the consequence of changes in  $g_s$ , a negative slope between  $\Delta^{18}\text{O}$  and  $\Delta^{13}\text{C}$  is predicted because, for instance, increasing

$g_s$  is expected to increase  $c_i/c_a$  ( $\Delta^{13}\text{C}$ ) and simultaneously reduce leaf water enrichment ( $\Delta^{18}\text{O}$ );

- (2) When variation in  $\Delta^{13}\text{C}$  is the consequence of changes in  $A$ , a slope not significantly different to zero is predicted, because increasing  $A$  will reduce  $c_i/c_a$  ( $\Delta^{13}\text{C}$ ), but it should have no effect on leaf water enrichment ( $\Delta^{18}\text{O}$ );
- (3) When variation in  $\Delta^{13}\text{C}$  is the consequence of changes in both  $A$  and  $g_s$ , a steeper negative slope is predicted because, for instance, increasing  $g_s$  is expected to increase  $c_i/c_a$  ( $\Delta^{13}\text{C}$ ) and simultaneously reduce leaf water enrichment ( $\Delta^{18}\text{O}$ ), while increasing  $A$  is expected to counteract some of the effect of  $g_s$  on  $c_i/c_a$  ( $\Delta^{13}\text{C}$ ), producing a greater change in  $\Delta^{18}\text{O}$  per unit change in  $\Delta^{13}\text{C}$  (Barbour et al. 2005).

In this study, we tested the predictions of the dual isotope conceptual model in *Salix arctica* across four ecosystems of the same type and along two hill slopes, where soil temperature and soil water content vary considerably across sites. We compared isotope-based inferences with direct measurements of leaf gas exchange. The High Arctic growing season is limited to less than 90 days and *S. arctica* maintains fully expanded leaves for as few as 40 days. Therefore, adequate temporal coverage was obtained with relatively few measurements of leaf-level gas exchange and stem water  $\delta^{18}\text{O}$ .

## Materials and methods

### Study species

*Salix arctica* is a widespread, woody, long-lived Arctic-alpine dwarf willow. It is deciduous, dioecious and occurs in a wide range of habitats throughout much of the circumpolar Arctic. *Salix arctica* exhibits an unusual pattern of shoot development, where ramets develop as solely vegetative or reproductive, producing catkins as well as leaves. Leaves are produced as a single cohort that expands within two weeks of snowmelt. *Salix arctica* has been the subject of detailed physiological investigations (Dawson and Bliss 1989a, 1989b, 1993) and climate change experiments (Jones et al. 1999) in the Canadian High Arctic.

### Site and treatment descriptions

Measurements and collections were made at eight sites near Pituffik (Thule), Greenland: two hill slopes, each

comprised of three sites, and the control plots associated with two multifactor climate manipulations (Fig. 1). Data from the Thule air base (USAF) in Pituffik show a mean annual air temperature of  $-11.4^{\circ}\text{C}$  and mean annual precipitation of 12.3 cm between 1978 and 2004. In 2004, June and July air temperatures were slightly cooler than average, while August air temperatures were slightly warmer than the long-term mean. Precipitation during June, July and August of 2004 was nearly double the long-term mean.

Control plots of the multifactor climate manipulations and the upper zones of both hill slopes are prostrate dwarf-shrub, herb tundra (CAVM 2003) (Table 1). Vascular plant cover at both sites is approximately 50% and the soils are Typic Haploturbels (Soil Survey staff 1998). Areas without plant cover are characterized by frost boils, which typically grade from bare soil near the active center to 100% cryptogamic cover at the periphery. These sites will be referred to as W-Xeric and F-Xeric throughout the text, reflecting their association with a warming and watering experiment ( $76^{\circ}33'\text{N}$ ,  $68^{\circ}34'\text{W}$ ) and a fertilization experiment ( $76^{\circ}29'\text{N}$ ,  $68^{\circ}26'\text{W}$ ), respectively. Effects of the climate manipulations on various aspects of ecosystem structure and function will be discussed in future papers.

Sites were established on a north- ( $76^{\circ}31'\text{N}$ ,  $68^{\circ}26'\text{W}$ ) and a south-facing slope ( $76^{\circ}34'\text{N}$ ,  $68^{\circ}39'\text{W}$ ) of two separate basaltic outcrops. Three distinct ecosystems are present on both hill slopes. The xeric upper zones of the hill slopes are prostrate dwarf-shrub, herb tundra. Vascular plant cover ranges from approximately 25% at the north-facing site to 50% at the south-facing hill slope. The mesic middle zones are

hemiprostrate dwarf-shrub tundra (CAVM 2003), dominated by *Cassiope tetragona*, with vascular plant-cover that approaches 100%. The hydric lower zones are sedge/grass moss wetlands (CAVM 2003), where the soil surface is a mosaic of hollows, which have shallow standing water throughout the growing season, and raised hummocks. *Salix arctica* is generally confined to the tops and sides of the hummocks. Sites along the north- and south-facing hill slopes are referred to as Xeric, Mesic or Hydric, depending upon their slope position, and preceded by their aspect throughout the text (e.g., S-Hydric refers to the sedge/grass moss wetland at the base of the south-facing hill slope). The hill slopes were included in the study to maximize observed differences in leaf physiology. They were not intended to describe differences in leaf physiology on north- versus south-facing slopes in general.

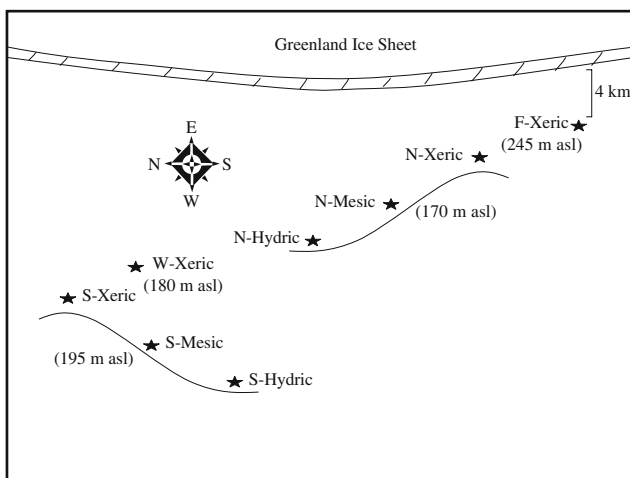
#### Microclimate monitoring

Hourly air temperatures and relative humidity at 20 cm were monitored at W-Xeric, F-Xeric and in the Mesic sites on the hill slopes with HOBO pro series loggers housed in radiation shields (Onset Computer Corp., Bourne, MA, USA). Hourly soil temperatures at 5 and 10 cm were monitored in two replicates at each of the eight sites with Hobo outdoor four-channel external temperature loggers (Onset Computer Corp.). Measurements began on 13 June, immediately following snowmelt, and ceased on 20 August, before snow pack development. The hydric sites have a deeper winter snow pack and a shorter growing season. Therefore, soil temperature measurements began later at these sites: on 26 June at S-Hydric and on 30 June at N-Hydric.

Snow depth measurements were made at each site in the first week of April 2004. Soil water content was measured in the upper 12 cm ( $n = 4$ , 3 subsamples/replicate) during gas exchange measurements at each site with a hand-held HydroSense TDR probe (Campbell Scientific, Logan, UT, USA).

#### Precipitation measurement and sampling

Snow cores (3.8 cm diameter) were collected from each site in early April 2004. Snow samples were sealed in Ziploc bags, melted in the laboratory, transferred to Nalgene bottles and frozen until analysis. Precipitation during the 2004 growing season was measured using plastic rain gauges installed at ground level. Gauges were read following cessation of an individual event. Nalgene bottles (125 ml) fitted with nylon filters and



**Fig. 1** Map depicting generalized locations of the eight study sites near Pituffik (Thule), Greenland (not to scale)

**Table 1** Microclimate characteristics at each of the eight sites between 13 June and 20 August 2004 at the study sites

Site	Air temperature (°C)	RH (%)	Soil temperature (5 cm, °C)	Soil temperature (10 cm, °C)	VWC (%)	Snow depth (cm)
W-Xeric	4.51	84.2	5.52 (0.22)	5.18 (0.33)	19.6 (4.5)	6.4 (3.4)
F-Xeric	4.45	–	5.68 (0.40)	5.33 (0.37)	17.4 (3.2)	–
N-Hydric	4.75	86.2	5.06 (0.01)	4.45 (0.01)	45.8 (5.8)	55.6 (4.5)
N-Mesic	4.39	85.5	4.21 (0.32)	3.70 (0.01)	19.3 (1.1)	15.6 (4.9)
N-Xeric	4.39	85.5	6.16 (0.07)	5.32 (0.20)	14.0 (1.2)	9.8 (5.4)
S-Hydric	4.57	84.8	5.85 (0.27)	5.09 (0.22)	31.8 (6.3)	29.9 (5.1)
S-Mesic	4.18	84.1	4.12 (0.15)	3.76 (0.06)	47.7 (12.0)	19.3 (5.1)
S-Xeric	4.18	84.1	5.47 (0.68)	4.86 (0.92)	25.3 (3.5)	7.9 (2.5)

Snow depth measurements were made on one date in early April 2004. Soil volumetric water contents (0–12 cm) were averaged over four replicates at each site, or in each zone, on three dates during the 2004 growing season ( $n = 12$ ). Hydric sites were released from snow cover later than the other sites. S-Hydric temperature and RH data are for 26 June–20 August, while those for N-Hydric are for 30 June–20 August. Standard deviations appear within parentheses

10 cm diameter funnels were used to collect samples from each event. Fog samples were collected using a slightly concave, 3 m<sup>2</sup> sheet-metal collector, mounted on a rebar tripod, such that fog condensed on the near-vertical sheet metal face and ran to a Nalgene collection vessel like that described above. Rain and fog samples were collected within 12 h following cessation of the event and frozen until analysis.

#### Gas exchange measurements

Leaf-level gas exchange measurements were made using a LI-6400 open-flow portable photosynthesis system equipped with a CO<sub>2</sub> mixer (LI-COR Biosciences, Lincoln, NE, USA). Sex (Dawson and Bliss 1989b; Jones et al. 1999) and reproductive investment (Dawson and Bliss 1993) are sources of variation in *S. arctica* leaf physiology. To reduce nonclimatic variation, measurements were taken only on fully expanded near-apical leaves of female vegetative ramets.

On each sampling date, a 15 m tape was placed through the center of each zone, perpendicular to the hill slope, and leaves were randomly selected using the aforementioned criteria. Measurements of midday leaf-level gas exchange ( $n = 4$ ) were taken at each of the eight sites in early, mid- and late July 2004. Measurements were taken with the irradiance level set at 2,000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , a constant flow rate of 400  $\mu\text{mol/s}$ , a leaf temperature of 15 °C and a reference CO<sub>2</sub> concentration of 400  $\mu\text{mol mol}^{-1}$ .

Leaves were harvested, dried for 24 h at 60 °C, scanned individually at 300 dpi and analyzed using unsupervised classifications in Erdas Imagine to determine projected leaf areas (Leica Geosystems, Atlanta, GA, USA). On one occasion, ten leaves were harvested from each zone of the south-facing hill slope, scanned, dried and scanned again to develop a cor-

rection for reductions in leaf area with drying. This exercise revealed a strong correlation between wet and dry leaf areas ( $r^2 = 0.99$ ,  $P < 0.01$ ,  $n = 30$ ).

#### Stem water potentials

Immediately following gas exchange measurements, midday water potentials were measured ( $n = 4$ ) on an unbranched cut stem of an adjacent ramet with a Scholander-type portable pressure chamber (Soilmoisture Equipment Corp. Santa Barbara, CA, USA). Care was taken to limit variation in stem diameters and leaf areas.

#### Stable isotope and leaf nutrient analyses

Following gas exchange and water potential measurements, leaf and non-green stem material were harvested from a third adjacent vegetative female ramet for isotope and nutrient analyses. Stem samples were immediately placed in glass dram vials fitted with Teflon cap liners. Parafilm was wrapped around the cap/vial junctions. Samples were placed on ice in a portable field cooler and frozen upon return to the laboratory. Leaf samples were dried for 24 h at 60 °C then ground to a fine powder.

Stem water was extracted by cryogenic vacuum distillation (Ehleringer and Osmond 1989; Ehleringer et al. 2001). Frozen stem samples were diced to increase surface area, weighed and submersed in liquid nitrogen (–196 °C) during evacuation of the vacuum line. Following evacuation, individual samples were isolated and incrementally heated to vaporize the stem water. Water vapor was trapped in a tube cooled by liquid nitrogen. Following extraction, samples were weighed, dried for 48 h at 60 °C and weighed again to determine the percent of stem water extracted during cryogenic vacuum distillation. Samples were elimi-

nated from subsequent analyses if <98% of stem water was extracted or if there was evidence of a vacuum leak during extraction.

To measure stable oxygen isotope ratios in stem waters, snow and summer precipitation, 0.2 ml of each sample was transferred to a 1.0 ml glass vial, aspirated with 10% CO<sub>2</sub> and 90% He in a glove bag, and equilibrated for 10 h at 30°C. The isotopic composition of CO<sub>2</sub> in the headspace was measured using a multi-prep sampler interfaced with a dual-inlet VG-Optima stable isotope ratio mass spectrometer (Micromass UK Ltd., Manchester, UK) (Epstein and Mayeda 1953). Deionized water (DI), cooked water (CW) and water from Cameron Pass, Colorado (CP), of known isotopic composition, were included every five samples. The standard deviations of these embedded standards were as follows: DI = 0.16‰, CW = 0.25‰ and CP = 0.21‰. Oxygen isotope ratios are reported relative to Vienna standard mean ocean water (VSMOW) as follows:

$$\delta = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1,000, \quad (8)$$

where  $\delta$  is reported in per mille (‰) and  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the isotope ratios of the sample and standard, respectively.

Stable carbon and oxygen isotope ratios were analyzed in bulk leaf tissue and leaf  $\alpha$ -cellulose. Leaf holocellulose was isolated using the method described by Leavitt and Danzer (1993), where toluene and ethanol are used to remove lipids in a Soxhlet apparatus, boiling water is used to remove soluble sugars, and sodium chlorite and glacial acetic acid are used to remove lignin and proteins. Holocellulose was subsequently reduced to  $\alpha$ -cellulose by washing the samples in 17% (w/v) NaOH (Roden and Ehleringer 1999).

Carbon and nitrogen concentrations in bulk leaf material and carbon isotope ratios in leaf  $\alpha$ -cellulose were analyzed using a Carlo Erba elemental analyzer (Thermo Electron Corporation, Milan, Italy) interfaced with an Isochrom stable isotope ratio mass spectrometer (Micromass UK Ltd.). Oxygen isotope ratios in bulk leaf tissue and leaf  $\alpha$ -cellulose were measured using a EuroVector Pyrolysis unit (EuroVector, Milan, Italy) interfaced with a VG-Optima stable isotope ratio mass spectrometer (Micromass UK Ltd.) operated in continuous flow mode. One vacuum oil working standard was included every ten samples and two cellulose working standards were included every six samples in the carbon and oxygen analyses, respectively. The standard deviation of the embedded vacuum oil standards was 0.16‰, whilst that of the cellulose standards was 0.48‰. Carbon and oxygen

isotope ratios are reported in  $\delta$  notation relative to the Pee Dee Belemnite (PDB) and VSMOW standards, respectively, and in  $\Delta$  notation relative to  $\delta^{13}\text{C}_a$  and stem water  $\delta^{18}\text{O}$ , respectively.  $\delta^{13}\text{C}_a$  was not measured, but held at  $-8.0\text{‰}$  in all of our calculations (approximated using data in Francey et al. 1999).

#### Modeling variation in leaf $\alpha$ -cellulose $\Delta^{18}\text{O}$

Barbour et al. (2004) presented a model of leaf  $\alpha$ -cellulose  $\Delta^{18}\text{O}$  that accounts for variation in source water  $\delta^{18}\text{O}$ , atmospheric water vapor  $\delta^{18}\text{O}$ , atmospheric vapor pressure, transpiration and exchange of oxygen atoms between leaf water, sucrose and cellulose in the developing cell. Sites in the present study were selected to limit variation in atmospheric vapor pressure, such that variation in leaf physiology would drive variation in leaf  $\alpha$ -cellulose  $\Delta^{18}\text{O}$ . There were, however, subtle differences in relative humidity and air temperature across the sites, which should give rise to differences in leaf  $\alpha$ -cellulose  $\Delta^{18}\text{O}$  in the absence of variation in leaf physiology. Differences in leaf  $\alpha$ -cellulose  $\Delta^{18}\text{O}$  that could be attributed to differences in air temperature and relative humidity were assessed by holding leaf physiology constant and varying atmospheric vapor pressures in the Barbour et al. (2004) model. Several variables, specific to individual plant species, are required to accurately model leaf  $\alpha$ -cellulose  $\Delta^{18}\text{O}$ : the effective length ( $L$ ) of the Péclet effect, the proportion of exchangeable oxygen in cellulose ( $p_{\text{ex}}$ ), the proportion of unenriched xylem water present in the cell during cellulose synthesis ( $p_x$ ), and the effect of changes in  $E$  on leaf temperature. These values have not been determined for *S. arctica*. Consequently, unknown variables were held at values intermediate among published values for other plant species ( $L = 0.02$  m,  $p_{\text{ex}} = 0.56$ ,  $p_x = 0.45$ ). Leaf temperature was varied by 1 °C, while holding all other variables constant, to provide a sense of the importance of changes in  $E$  and consequent changes in leaf temperature for leaf  $\alpha$ -cellulose.

#### Statistical analyses

Correlations between stem water  $\delta^{18}\text{O}$ , leaf cellulose  $\delta^{18}\text{O}$  and bulk leaf tissue  $\delta^{18}\text{O}$ ,  $\Delta^{18}\text{O}$  and  $g_s$  and  $\Delta^{13}\text{C}$  and iWUE were examined using the regression (REG) procedure in SAS 9.1 (SAS Institute, Cary, NC, USA). Variation in  $A_{\text{max}}$ ,  $g_s$ ,  $E$ ,  $c_i/c_a$ , iWUE, stem water potentials and leaf nitrogen content across the sites was examined using analysis of variance (ANOVA) in the general linear model (GLM) procedure of SAS 9.1. Comparisons of interest were made using Tukey's honest significant difference (HSD).

## Results

### Microclimate

There were subtle variations in air temperature (0.33 °C) and relative humidity (2.1%) across the sites between 13 June and 20 August 2004 (Table 1). Soil temperature varied by 2.04 °C at 5 cm and 1.57 °C at 10 cm across the sites. At both hill slope sites, the coldest soils were observed in the Mesic zones. At the north-facing hill slope, volumetric soil water content was lowest in the Xeric, intermediate in the Mesic and highest in the Hydric zone. This pattern roughly mirrored variations in winter snow depth. The south-facing hill slope deviated from this pattern in the sense that the highest soil water contents were found in the Mesic zone, where winter snow depths were intermediate between the Xeric and Hydric zones.

### Variation in leaf physiology within and across ecosystems

There was no evidence of a significant difference in any of the physiological variables examined across the four Xeric sites (Table 2). At the north-facing hill slope, there was a trend toward increasing  $g_s$ , increasing  $E$ , increasing  $c_i/c_a$ , declining iWUE and increasing leaf N moving down slope from Xeric to Mesic to Hydric. There was, however, no evidence of a corresponding increase in  $A_{\max}$ . At the south-facing hill slope, there were few significant differences across sites.

Comparison of the Mesic and Hydric sites across hill slopes revealed the greatest differences in leaf physiology. In the Mesic sites,  $A_{\max}$ ,  $g_s$ ,  $E$ ,  $c_i/c_a$ , stem  $\psi$  and leaf N increased, while iWUE declined from the south- to the north-facing hill slope. In the Hydric sites,  $g_s$ ,  $E$ ,  $c_i/c_a$ , stem  $\psi$  and leaf N increased, while iWUE declined from the south- to the north-facing hill slope. In

contrast with the Mesic sites, there was no evidence of a change in  $A_{\max}$  from S-Hydric to N-Hydric.

### $\delta^{18}\text{O}$ of precipitation and stem waters

Snow collected in early April 2004 had an average  $\delta^{18}\text{O}$  value of  $-28.1\text{‰}$ . Precipitation during the 2004 growing season was concentrated in July and August, with few measurable events during June (Fig. 2). The amount-weighted  $\delta^{18}\text{O}$  value of 2004 summer rain was  $-19.3\text{‰}$ . Source water was enriched relative to both winter snow and summer rain, with an average  $\delta^{18}\text{O}$  value of  $-15.1\text{‰}$  and a range of  $1.9\text{‰}$  across sites (Fig. 3). There was no evidence of a trend in source water  $\delta^{18}\text{O}$  at the north-facing transect, but there was trend of source water enrichment along the south facing transect, where source water in S-Xeric was depleted relative to source water in S-Hydric.

### Correlations among leaf $\alpha$ -cellulose $\delta^{18}\text{O}$ , bulk leaf tissue $\delta^{18}\text{O}$ and stem water $\delta^{18}\text{O}$

Simple linear regression of leaf  $\alpha$ -cellulose  $\delta^{18}\text{O}$  on bulk leaf tissue  $\delta^{18}\text{O}$  revealed a positive correlation ( $r^2 = 0.16$ ,  $P < 0.01$ ,  $n = 42$ ), with an estimated slope of 0.45. Simple linear regression of site-level leaf  $\alpha$ -cellulose  $\delta^{18}\text{O}$  on stem water  $\delta^{18}\text{O}$  revealed a positive correlation ( $r^2 = 0.49$ ,  $P = 0.05$ ,  $n = 8$ ), with a slope estimate of 1.04 (Fig. 4). The corresponding regression of bulk leaf tissue  $\delta^{18}\text{O}$  on stem water  $\delta^{18}\text{O}$  revealed no evidence of a correlation ( $r^2 = 0.02$ ,  $P = 0.71$ ,  $n = 8$ ).

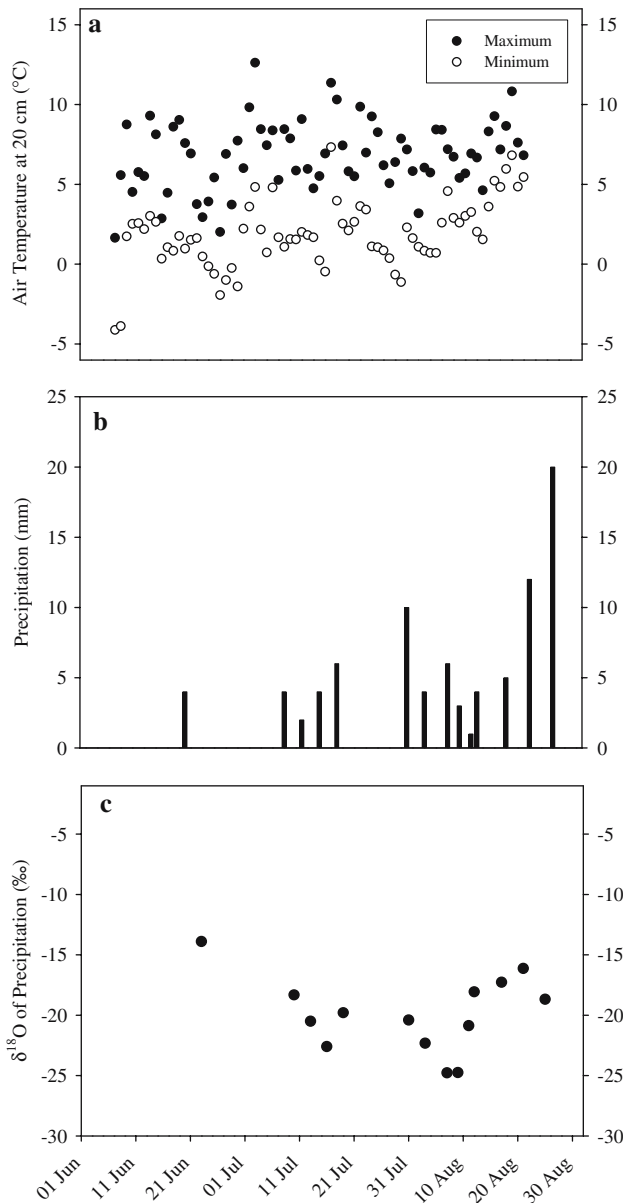
### $\Delta^{18}\text{O}$ versus $g_s$

Simple linear regression of site-level bulk leaf tissue  $\Delta^{18}\text{O}$  on  $g_s$  revealed a significant negative correlation ( $r^2 = 0.62$ ,  $P = 0.02$ ,  $n = 8$ ), with an estimated slope of

**Table 2** Variation in gas exchange physiology and water relations of *Salix arctica* at each site, across three sampling dates during July 2004

Site	$A_{\max}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$g_s$ (mol H <sub>2</sub> O $\text{m}^{-2} \text{s}^{-1}$ )	$E$ (mmol $\text{m}^{-2} \text{s}^{-1}$ )	$c_i/c_a$	iWUE ( $\mu\text{mol CO}_2 /$ mol H <sub>2</sub> O)	Stem $\psi$ (MPa)	Leaf N (mg/g)
W-Xeric	19.3 (3.5) <sup>ab</sup>	0.333 (0.100) <sup>ab</sup>	3.20 (0.86) <sup>abc</sup>	0.719 (0.068) <sup>a</sup>	62.0 (17.1) <sup>a</sup>	-0.50 (0.10) <sup>b</sup>	24.8 (2.6) <sup>bcd</sup>
F-Xeric	20.2 (4.7) <sup>ab</sup>	0.338 (0.106) <sup>ab</sup>	3.54 (0.99) <sup>abc</sup>	0.711 (0.078) <sup>a</sup>	63.6 (19.2) <sup>a</sup>	-0.59 (0.16) <sup>ab</sup>	25.0 (3.8) <sup>cd</sup>
N-Hydric	18.5 (1.9) <sup>ab</sup>	0.408 (0.092) <sup>a</sup>	4.06 (0.80) <sup>cd</sup>	0.781 (0.039) <sup>a</sup>	47.1 (9.5) <sup>a</sup>	-0.58 (0.17) <sup>b</sup>	37.4 (7.2) <sup>a</sup>
N-Mesic	21.4 (4.3) <sup>a</sup>	0.367 (0.066) <sup>ab</sup>	3.74 (0.69) <sup>bcd</sup>	0.726 (0.060) <sup>a</sup>	59.9 (14.5) <sup>a</sup>	-0.52 (0.10) <sup>b</sup>	30.4 (4.9) <sup>bc</sup>
N-Xeric	19.3 (2.6) <sup>ab</sup>	0.306 (0.047) <sup>ab</sup>	3.15 (0.60) <sup>abc</sup>	0.712 (0.027) <sup>a</sup>	63.3 (6.5) <sup>a</sup>	-0.50 (0.07) <sup>b</sup>	22.8 (4.7) <sup>d</sup>
S-Hydric	20.0 (3.4) <sup>ab</sup>	0.327 (0.099) <sup>ab</sup>	3.23 (0.94) <sup>abc</sup>	0.702 (0.089) <sup>a</sup>	66.3 (22.2) <sup>a</sup>	-0.62 (0.12) <sup>ab</sup>	31.2 (4.6) <sup>ab</sup>
S-Mesic	16.9 (3.1) <sup>b</sup>	0.265 (0.062) <sup>b</sup>	2.72 (0.61) <sup>a</sup>	0.707 (0.056) <sup>a</sup>	65.7 (13.9) <sup>a</sup>	-0.66 (0.11) <sup>ab</sup>	25.6 (3.1) <sup>bcd</sup>
S-Xeric	16.9 (3.3) <sup>b</sup>	0.276 (0.063) <sup>b</sup>	2.81 (0.52) <sup>ab</sup>	0.717 (0.058) <sup>a</sup>	63.0 (14.2) <sup>a</sup>	-0.75 (0.16) <sup>a</sup>	22.1 (2.8) <sup>d</sup>

Standard deviations appear within parentheses. Entries marked with a different superscript are significantly different at  $\alpha = 0.05$

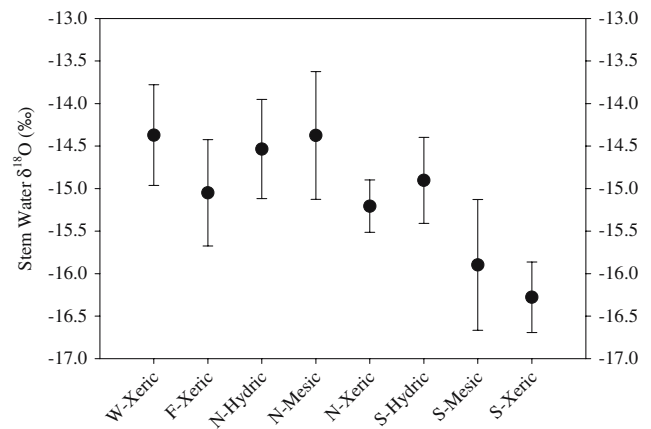


**Fig. 2a–c** Daily maximum and minimum air temperature at 20 cm (°C) (a), precipitation (mm, date is that of collection following cessation) (b) and δ<sup>18</sup>O of precipitation (‰) during the 2004 growing season at W-Xeric (c)

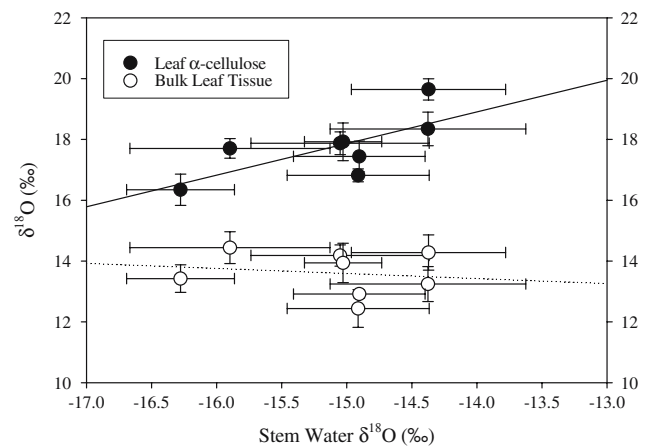
–17.9 (Fig. 5). Simple linear regression of site-level leaf α-cellulose Δ<sup>18</sup>O on *g<sub>s</sub>* also revealed a significant negative correlation ( $r^2 = 0.83$ ,  $P < 0.01$ ,  $n = 8$ ), with an estimated slope of –11.5.

Δ<sup>13</sup>C versus iWUE

Simple linear regression of site-level bulk leaf tissue Δ<sup>13</sup>C on iWUE revealed a significant negative correlation ( $r^2 = 0.65$ ,  $P = 0.02$ ,  $n = 8$ ), with an estimated slope of –0.08 (Fig. 6). Simple linear regression of site-



**Fig. 3** Stem water δ<sup>18</sup>O (‰) at each site over three sampling dates in July 2004. Bars are 1.0 SE

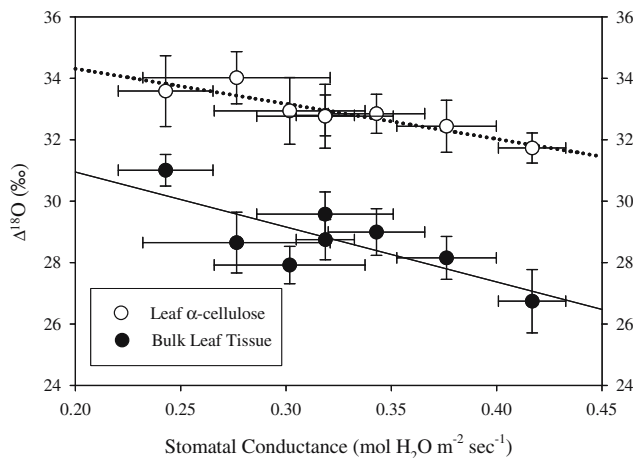


**Fig. 4** Simple linear regressions of leaf α-cellulose δ<sup>18</sup>O (‰) and bulk leaf tissue δ<sup>18</sup>O (‰) on stem water δ<sup>18</sup>O (‰). Data were averaged across three sampling dates during July 2004. Bars are 1.0 SE

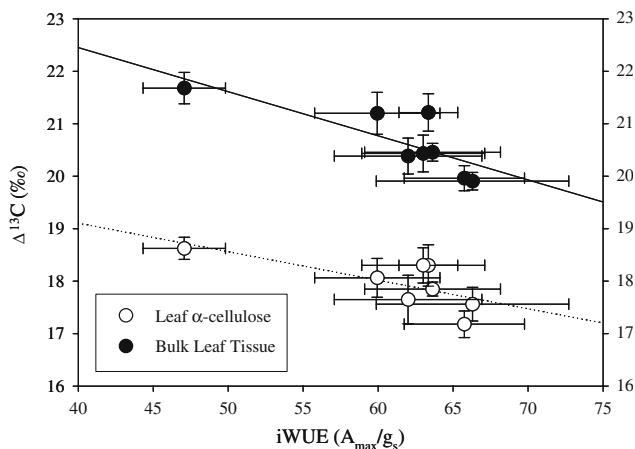
level leaf α-cellulose Δ<sup>13</sup>C on iWUE also revealed a significant negative correlation ( $r^2 = 0.50$ ,  $P = 0.05$ ,  $n = 8$ ), with an estimated slope of –0.05. Correlations between Δ<sup>13</sup>C and  $c_i/c_a$  were qualitatively similar, though slightly weaker.

Variability in  $A_{max}/g_s$  and corresponding variability in Δ<sup>18</sup>O/Δ<sup>13</sup>C

Comparison of S-Hydric with N-Hydric revealed an increase in *g<sub>s</sub>*, coincident with a nonsignificant reduction in  $A_{max}$ , giving rise to a slope estimate of –19.07 μmol CO<sub>2</sub>/mol H<sub>2</sub>O (Fig. 7a). Increasing *g<sub>s</sub>* manifested itself as an increase in  $c_i/c_a$ , with a slope of 1.02 (Fig. 7c). Corresponding isotope measurements in leaf α-cellulose revealed an increase in Δ<sup>13</sup>C and a slight



**Fig. 5** Simple linear regressions of  $\Delta^{18}\text{O}$  (‰) on stomatal conductance ( $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ ) for bulk leaf tissue and leaf  $\alpha$ -cellulose. Data were averaged across three sampling dates during July 2004. Bars are 1.0 SE



**Fig. 6** Simple linear regressions of  $\Delta^{13}\text{C}$  (‰) on intrinsic water-use efficiency ( $\text{iWUE}$ ,  $\mu\text{mol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$ ) for bulk leaf tissue and leaf  $\alpha$ -cellulose. Data were averaged across three sampling dates during July 2004. Bars are 1.0 SE

reduction in  $\Delta^{18}\text{O}$ , with a slope of  $-1.135$  (Fig. 7e). Isotope measurements made on bulk leaf tissue revealed an increase in  $\Delta^{13}\text{C}$  and a slight reduction in  $\Delta^{18}\text{O}$ , with a slope of  $-0.664$  (Fig. 7g).

Comparison of S-Mesic and N-Mesic revealed an increase in  $g_s$ , coincident with an increase in  $A_{\text{max}}$ , yielding a slope estimate of  $44.86 \mu\text{mol CO}_2 / \text{mol H}_2\text{O}$  (Fig. 7b). Increasing  $g_s$  manifested itself as an increase in  $c_i/c_a$ , with a slope of  $5.32$  (Fig. 7d). Corresponding isotope measurements in leaf  $\alpha$ -cellulose revealed an increase in  $\Delta^{13}\text{C}$  and a reduction in  $\Delta^{18}\text{O}$ , with a slope of  $-1.296$  (Fig. 7f). Isotope measurements made on bulk leaf tissue revealed an increase in  $\Delta^{13}\text{C}$  and a reduction in  $\Delta^{18}\text{O}$ , with a slope of  $-2.307$  (Fig. 7h).

## Modeling variation in leaf $\alpha$ -cellulose $\Delta^{18}\text{O}$

Leaf  $\alpha$ -cellulose  $\Delta^{18}\text{O}$  declined by  $0.4\text{‰}$  from S-Hydric to N-Hydric when  $E$  and  $g_s$  were held constant, but the atmospheric vapor pressure was allowed to vary as observed during the 2004 growing season. Leaf  $\alpha$ -cellulose  $\Delta^{18}\text{O}$  declined by a further  $0.4\text{‰}$  from S-Hydric to N-Hydric when  $E$  and  $g_s$  were allowed to vary as observed, without incorporating the unknown effect of increasing  $E$  on leaf temperature. Leaf  $\alpha$ -cellulose  $\Delta^{18}\text{O}$  declined by  $0.3\text{‰}$  from S-Mesic to N-Mesic when  $E$  and  $g_s$  were held constant, but the atmospheric vapor pressure was allowed to vary as observed during the 2004 growing season. Leaf  $\alpha$ -cellulose  $\Delta^{18}\text{O}$  declined by a further  $0.5\text{‰}$  from S-Hydric to N-Hydric when  $E$  and  $g_s$  were allowed to vary as observed, without incorporating the unknown effect of increasing  $E$  on leaf temperature. The cooling effect of increasing  $E$  on leaf  $\alpha$ -cellulose  $\Delta^{18}\text{O}$  is probably large. Cooling leaf temperatures by  $1^\circ\text{C}$  further reduced leaf  $\alpha$ -cellulose  $\Delta^{18}\text{O}$  by  $1.1\text{--}1.2\text{‰}$ .

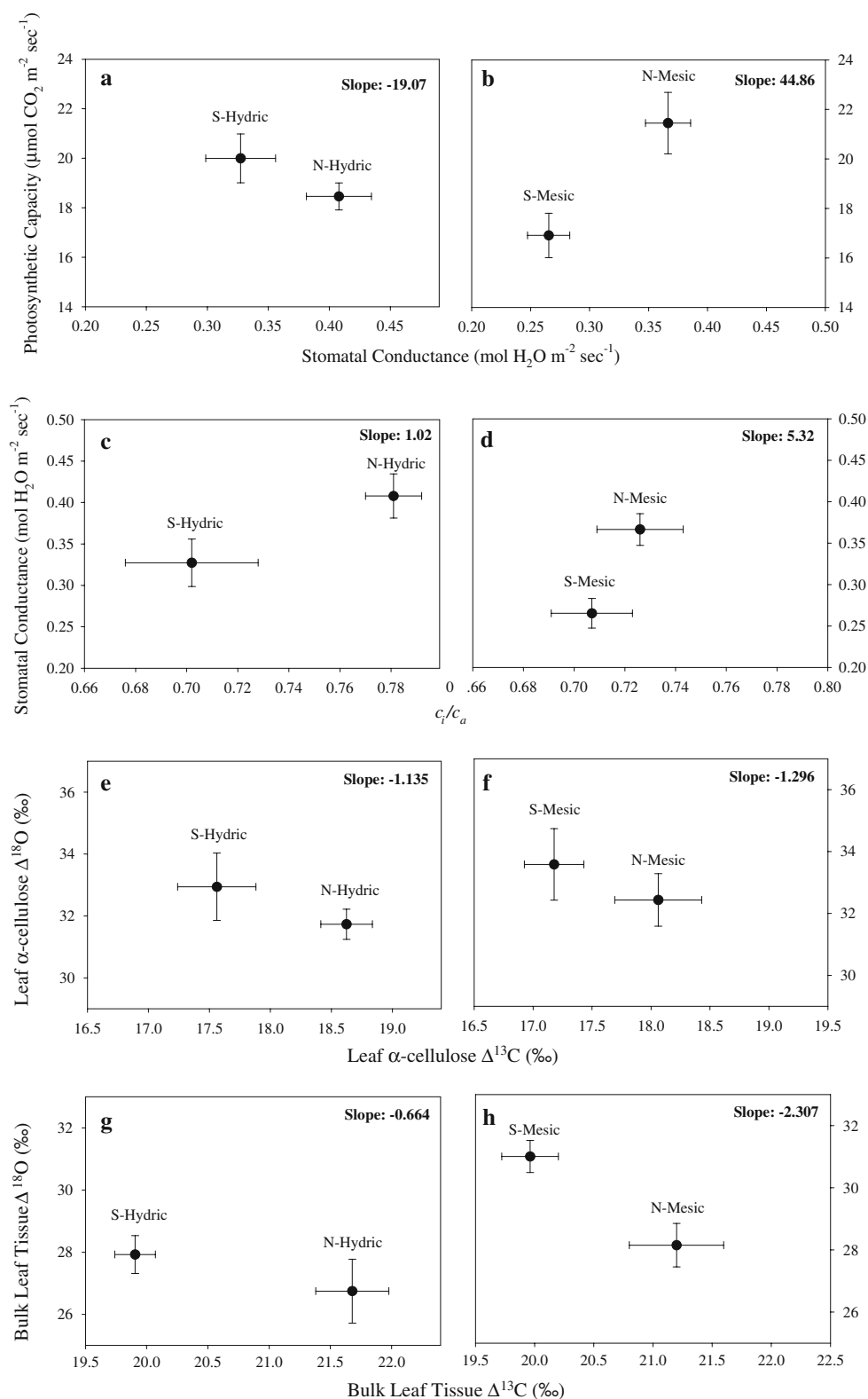
## Discussion

### Variation in leaf physiology within and across ecosystems

Early studies of plant physiology in dry ecosystems of the High Arctic suggested that plants may experience and tolerate extremely low soil water potentials (Teeri 1973). We found limited evidence of water stress at our sites during July of 2004, generally consistent with more contemporary studies of plant water relations in the High Arctic (e.g., Gold and Bliss 1995). Precipitation during the 2004 growing season was, however, nearly double the long-term mean for Pituffik.

There was no evidence of a significant difference in any of the physiological variables examined across the four Xeric sites. At the north-facing hill slope, there was a trend toward increasing  $g_s$ , increasing  $E$ , increasing  $c_i/c_a$ , declining  $\text{iWUE}$  and increasing leaf N from Xeric to Mesic to Hydric. There was, however, no evidence of a corresponding increase in  $A_{\text{max}}$ . Increases in  $g_s$  and  $E$  probably reflect a progressive increase in the availability of water from Xeric to Mesic to Hydric, in contrast with earlier observations. Dawson and Bliss (1989a) found lower rates of  $g_s$  overall and a decline in  $g_s$  from Xeric to Hydric sites, where they concluded that low soil temperatures were restricting  $g_s$ . Soil temperatures at our sites were generally similar to those of Dawson and Bliss (1989a), although differences between Xeric and Hydric sites

**Fig. 7a–g** Comparison of leaf gas exchange characteristics and the isotopic composition of bulk leaf tissue and leaf  $\alpha$ -cellulose between S-Hydric and N-Hydric and between S-Mesic and N-Mesic. **a** and **b** depict variation in photosynthetic capacity ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and stomatal conductance ( $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ). **c**, **d** display corresponding changes in the relationships between stomatal conductance and the ratio of intercellular to atmospheric  $\text{CO}_2$  concentrations ( $c_i/c_a$ ). Panels **e** and **f** show how divergent patterns of variation in leaf gas exchange physiology manifest in plots of  $\Delta^{18}\text{O}$  (‰) on  $\Delta^{13}\text{C}$  (‰) for leaf  $\alpha$ -cellulose, while **g**, **h** show how the same changes in leaf gas exchange physiology manifest in plots of  $\Delta^{18}\text{O}$  on  $\Delta^{13}\text{C}$  for bulk leaf tissue. Data were averaged across three sampling dates during July 2004. Bars are 1.0 SE



were more limited in our study. The coldest soil temperatures in our study were found in the Mesic sites, where soil water contents are moderate-to-high, microtopography is limited and soil bulk density is much

higher than in the Hydric sites. The lowest rates of  $g_s$  ( $0.265 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) in our study were observed at S-Mesic, consistent with the notion that cold soil temperatures may restrict  $g_s$ , despite moderate-to-high soil

water availability and low atmospheric demand. Similar soil temperatures at N-Mesic were, however, coincident with high rates of  $g_s$  ( $0.367 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ). Further study across a wider range of soil temperatures is required to resolve the role of soil temperature as a control on  $g_s$  across the Pituffik landscape.

The observed increase in leaf N from Xeric to Mesic to Hydric may be explained by declines in nitrogen productivity (sensu Ingestad 1979), as a result of the shortened growing season. Increases in leaf N with declines in growing season length have been observed along gradients in altitude and latitude (Körner 1989) and along natural snow depth gradients (Kudo et al. 1999). Alternatively, increasing leaf N from Xeric to Mesic to Hydric may reflect increases in N availability (Chapin 1980).

We were surprised to find that increases in  $g_s$  and leaf N from N-Mesic to N-Hydric did not lead to a corresponding increase in  $A_{\text{max}}$ . Increasing  $g_s$  should relieve  $\text{CO}_2$  limitations, while increasing leaf N should provide for raw materials to relieve enzymatic limitations to photosynthesis. Consequently, strong positive correlations between  $g_s$ , leaf N and  $A_{\text{max}}$  are generally observed (e.g., Field et al. 1983). Our observations suggest photosynthesis of *S. arctica* in N-Hydric may be limited by the regeneration of RuBP, which is ultimately limited by the rate of electron transport. Reductions in the rate of electron transport are generally linked to some form of stress. Unfortunately, our data are insufficient to conclusively implicate reductions in electron transport or to identify a potential stressor.

#### $\delta^{18}\text{O}$ of precipitation and stem waters

There was a difference of  $8.8\text{‰}$  between  $\delta^{18}\text{O}$  of snow cores collected in early April 2004 and amount-weighted precipitation collected throughout the 2004 growing season. Stem water was enriched relative to both winter snow and summer rain, with a range of  $1.9\text{‰}$  across the eight sites. Enrichment of stem water above winter snow and summer rain may reflect near-surface soil water evaporative enrichment, or the importance of fog as a water source for plants (Dawson 1998) in the maritime High Arctic. Fog water was highly enriched relative to rain and snow water, with a  $\delta^{18}\text{O}$  signature of  $-7.7\text{‰}$ , over three collection dates during the 2004 growing season.

The Hydric sites at the base of the hill slopes have winter snow packs that are much deeper than any of the other sites in this study. Previous studies have suggested that snowmelt water can be an important water source for Arctic plants (Welker et al. 1995,

2005). We were, therefore, surprised to find stem water  $\delta^{18}\text{O}$  values that were no different to those for upland sites at N-Hydric and that tended to be more enriched than upland sites at S-Hydric. While the Hydric sites have standing water for much of the growing season, the hummocks extend above the water table by as much as 30 cm, and those at S-Hydric tend to be taller than hummocks at N-Hydric. *Salix arctica* colonizes the tops and, to a limited extent, the sides of the hummocks, which are composed of low-density structured organic matter. Source water in the raised hummocks may have been more subject to evaporative enrichment than source water at more upland sites, with hummock height and aspect contributing to the magnitude of enrichment. This hypothesis is supported by our observation that hummock soil temperatures in the S-Hydric were warmer than those in the drier, upland S-Xeric. Similar observations have been made in northern Alaska, where soils in tussocks of *Eriophorum vaginatum* are warmer than intertussock soils (Chapin et al. 1979). Our observation that stem water  $\delta^{18}\text{O}$  was not a faithful indicator of snowmelt water inputs suggests that  $\delta^{18}\text{O}$  of precipitation would not have been a good proxy for source water  $\delta^{18}\text{O}$  in calculations of leaf tissue  $\Delta^{18}\text{O}$ , limiting the potential for reconstructions of historical leaf tissue  $\Delta^{18}\text{O}$  at our site.

#### Correlations among leaf $\alpha$ -cellulose $\delta^{18}\text{O}$ , bulk leaf tissue $\delta^{18}\text{O}$ and stem water $\delta^{18}\text{O}$

Simple linear regression of leaf  $\alpha$ -cellulose  $\delta^{18}\text{O}$  on bulk leaf tissue  $\delta^{18}\text{O}$  revealed a positive correlation, with a slope estimate of 0.45. Leaf  $\alpha$ -cellulose  $\delta^{18}\text{O}$  was an average of  $4.2\text{‰}$  enriched relative to bulk leaf tissue  $\delta^{18}\text{O}$ . The average offset between leaf  $\alpha$ -cellulose  $\delta^{18}\text{O}$  and bulk leaf tissue  $\delta^{18}\text{O}$  observed in our study is within the range observed in previous studies ( $2.8$ – $11.2\text{‰}$ ) (Barbour and Farquhar 2000; Barbour et al. 2000; Cernusak et al. 2004).

There is reason to expect variation in the offset between bulk leaf tissue and leaf  $\alpha$ -cellulose  $\delta^{18}\text{O}$ . Cernusak et al. (2002) found substantial diurnal variation in *Lupinus angustifolius* bulk leaf tissue  $\delta^{18}\text{O}$ . When sampled in the early afternoon, bulk leaf tissue  $\delta^{18}\text{O}$  was approximately  $4\text{‰}$  enriched relative to samples collected during the night, reflecting enrichment of leaf water and nonstructural carbohydrates (Cernusak et al. 2004). Leaf  $\alpha$ -cellulose  $\delta^{18}\text{O}$ , in contrast, is presumed to show limited diurnal variation.

We were surprised to find such a shallow slope between leaf  $\alpha$ -cellulose  $\delta^{18}\text{O}$  and bulk leaf tissue  $\delta^{18}\text{O}$ , as previous studies have found slopes close to unity (Barbour and Farquhar 2000; Barbour et al. 2001). We

hypothesize the shallow slope reflects differences in the extent to which leaf  $\alpha$ -cellulose and bulk leaf tissue integrate over time. Leaf  $\alpha$ -cellulose is a structural constituent that is laid down predominantly early in the life of the leaf, while bulk leaf tissue is a composite of constituents that have a wide range of turnover times, from months to hours. The highest coefficients of variation for soil water content, stem water  $\delta^{18}\text{O}$  and many leaf gas exchange variables were found in early to mid-July of both 2003 and 2004, when examined across sites. If leaf  $\alpha$ -cellulose tends to record predominantly early season conditions (i.e., mid-late June), a reduced range of  $\delta^{18}\text{O}$  would be expected, relative to bulk leaf tissue  $\delta^{18}\text{O}$ . Additionally, leaf tissue samples were collected near the solar maximum, when the effects of leaf physiology on bulk leaf tissue  $\delta^{18}\text{O}$  are expected to be greatest.

Simple linear regression of leaf  $\alpha$ -cellulose  $\delta^{18}\text{O}$  on stem water  $\delta^{18}\text{O}$  revealed a positive correlation across sites. The presence, or assumed presence, of this correlation is the basis of the notion that plant tissue  $\delta^{18}\text{O}$  may be used as an indicator of source water  $\delta^{18}\text{O}$  which, in turn, may be related to the temperature of droplet formation (e.g., Libby et al. 1976). Across our eight sites, stem water  $\delta^{18}\text{O}$  explained approximately 50% of the variation in leaf  $\alpha$ -cellulose  $\delta^{18}\text{O}$ , with a 1‰ change in stem water  $\delta^{18}\text{O}$  balanced by a 1‰ change in leaf  $\alpha$ -cellulose  $\delta^{18}\text{O}$ . Much of the remaining variation observed in leaf  $\alpha$ -cellulose  $\delta^{18}\text{O}$  can probably be attributed to subtle variations in relative humidity and substantial variations in leaf physiology across the sites, as discussed below.

In contrast with results for leaf  $\alpha$ -cellulose, we found no evidence of a correlation between bulk leaf tissue  $\delta^{18}\text{O}$  and stem water  $\delta^{18}\text{O}$ . We hypothesize the effects of variation in relative humidity and leaf physiology on bulk leaf tissue  $\delta^{18}\text{O}$  were sufficient to swamp the source water signal, as bulk leaf tissue was collected during a point in the diurnal cycle when the effects of leaf physiology on bulk leaf tissue  $\delta^{18}\text{O}$  are expected to be greatest and because bulk leaf tissue integrated over a period in the growing season when differences in leaf physiology were greatest across the sites. This observation has important implications for studies that attempt to extract a paleoclimate signal from plant material in the Pituffik landscape. Our results suggest that leaf  $\alpha$ -cellulose in *S. arctica* may provide a “coarse” depiction of variation in source water  $\delta^{18}\text{O}$ , while bulk leaf tissue in *S. arctica* will provide little to no information on source water  $\delta^{18}\text{O}$ . It is important to note that source water  $\delta^{18}\text{O}$  and precipitation  $\delta^{18}\text{O}$  were probably not closely correlated at our sites.

## Comparing instantaneous gas exchange measurements with integrative isotope data

Comparison of instantaneous leaf-level gas exchange measurements with inferences made using stable isotopes is inherently flawed, as the time-scales of the two sources of information do not correspond. Midday gas exchange measurements were taken on three dates, evenly dispersed during July of 2004, when *S. arctica* leaves are most physiologically active. The gas exchange measurements do not account for diurnal variation, variation between sampling dates; nor do they account for the construction of the current year's leaves using reserves stored during the preceding growing season. The temporal disconnect between instantaneous measurements of leaf-level gas exchange and the inferences made using stable isotopes must be noted. In many cases, the actual relationships between leaf physiology and stable isotopic composition are probably stronger than our analyses would suggest.

## $\Delta^{18}\text{O}$ versus $g_s$

We found strong negative correlations between  $\Delta^{18}\text{O}$  and  $g_s$  for bulk leaf tissue and leaf  $\alpha$ -cellulose, consistent with theory and results in greenhouse (Barbour and Farquhar 2000) and agricultural settings (Barbour et al. 2000). Bulk leaf tissue provided a steeper slope, while leaf  $\alpha$ -cellulose provided a closer fit. Differences in slope, again, probably reflect differences in the extent to which bulk leaf tissue and leaf  $\alpha$ -cellulose integrate over time, with bulk leaf tissue integrating over a period of the growing season and a time in the diurnal cycle when differences across sites are expected to be greatest.

As mentioned previously, bulk leaf tissue is a composite of many compounds, at least some of which are known to have different  $\delta^{18}\text{O}$  values. Lignin, for instance, was found to be 6–7‰ depleted relative to  $\alpha$ -cellulose in *Quercus* and *Pinus* wood samples from around the world (Barbour et al. 2001). Subtle variation in leaf composition may, therefore, create additional scatter in the  $\Delta^{18}\text{O}:g_s$  correlation for bulk leaf tissue.

## $\Delta^{13}\text{C}$ versus iWUE

We found negative correlations between  $\Delta^{13}\text{C}$  and iWUE for bulk leaf tissue and leaf  $\alpha$ -cellulose, consistent with results from many previous studies in the greenhouse and in the wild (e.g., Rundel and Sharifi 1993). Bulk leaf tissue provided a steeper slope and a closer fit than leaf  $\alpha$ -cellulose. Differences in slope,

again, probably reflect differences in the extent to which bulk leaf tissue and leaf  $\alpha$ -cellulose integrate over time.

Variability in  $A_{\max}/g_s$  and corresponding variability in cellulose  $\Delta^{18}\text{O}/\Delta^{13}\text{C}$

Theory predicts that when variation in  $\Delta^{13}\text{C}$  is driven by changes in  $g_s$ , a negative relationship between  $\Delta^{13}\text{C}$  and  $\Delta^{18}\text{O}$  will emerge because, for instance, rising  $g_s$  increases  $c_i/c_a$  and reduces leaf water enrichment. In contrast, when variation in  $\Delta^{13}\text{C}$  is driven by changes in  $A_{\max}$ , there should be no relationship between  $\Delta^{13}\text{C}$  and  $\Delta^{18}\text{O}$ , as  $A_{\max}$  does not affect  $\Delta^{18}\text{O}$  (Yakir and Israeli 1995). When variation in  $\Delta^{13}\text{C}$  is driven by changes in both  $g_s$  and  $A_{\max}$ , the change in  $\Delta^{18}\text{O}$  for each unit change in  $\Delta^{13}\text{C}$  should increase, as changes in  $A_{\max}$  typically counteract effects of  $g_s$  on  $c_i/c_a$  (Barbour et al. 2002).

Comparison of S-Hydric with N-Hydric revealed an increase in  $g_s$ , coincident with a nonsignificant reduction in  $A_{\max}$ . Increasing  $g_s$  led to an increase in  $c_i/c_a$ , as expected. Corresponding isotope measurements in leaf  $\alpha$ -cellulose revealed an increase in  $\Delta^{13}\text{C}$  and a slight reduction in  $\Delta^{18}\text{O}$ , with a slope of  $-1.135$ . Isotope measurements made on bulk leaf tissue revealed an increase in  $\Delta^{13}\text{C}$  and a slight reduction in  $\Delta^{18}\text{O}$ , with a slope of  $-0.664$ . The observed negative slopes are consistent with dual isotope theory and the results of previous studies (Sternberg et al. 1989; Saurer et al. 1997; Barbour et al. 2000, 2002; Barbour and Farquhar 2000; Cernusak et al. 2005). It should be noted that a negative slope between  $\Delta^{18}\text{O}$  and  $\Delta^{13}\text{C}$  is equivalent to a positive slope between  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$ .

Comparison of S-Mesic and N-Mesic revealed an increase in  $g_s$ , coincident with an increase in  $A_{\max}$ , yielding a slope estimate of  $44.86 \mu\text{mol CO}_2/\text{mol H}_2\text{O}$ . In this case, the increase in  $g_s$  led to a proportionately smaller increase in  $c_i/c_a$  and a steeper slope in the plot of  $g_s$  on  $c_i/c_a$ . This observation is consistent with expectations, as increases in  $A_{\max}$  should negate some of the effect of increasing  $g_s$  on  $c_i$ . Corresponding isotope measurements in leaf  $\alpha$ -cellulose revealed an increase in  $\Delta^{13}\text{C}$  and a reduction in  $\Delta^{18}\text{O}$ , with a slope of  $-1.296$ . Isotope measurements made on bulk leaf tissue revealed an increase in  $\Delta^{13}\text{C}$  and a reduction in  $\Delta^{18}\text{O}$ , with a slope of  $-2.307$ . The slopes were steeper than those observed when  $g_s$  alone changed. This result is, again, consistent with dual isotope theory, as rising  $A_{\max}$  counteracts the effect of rising  $g_s$  on  $c_i/c_a$ , producing a greater change in  $\Delta^{18}\text{O}$  per unit change in  $\Delta^{13}\text{C}$ .

## Modeling variation in leaf $\alpha$ -cellulose $\Delta^{18}\text{O}$

We found limited variation in air temperature and relative humidity, but substantial variation in soil temperature and soil water content across the eight sites. Variations in air temperature and relative humidity affect leaf water enrichment and  $\Delta^{18}\text{O}$  of plant material, in the absence of variation in leaf gas exchange physiology. Application of the Barbour et al. (2004) model suggests that increases in atmospheric vapor pressure from the south- to the north-facing hill slope can account for approximately 0.3–0.4‰ of the observed declines in leaf  $\alpha$ -cellulose  $\Delta^{18}\text{O}$ . This amounts to approximately 25% of the observed declines in leaf  $\alpha$ -cellulose  $\Delta^{18}\text{O}$ . Leaf  $\alpha$ -cellulose  $\Delta^{18}\text{O}$  declined by a further 0.4–0.5‰ when leaf physiology was allowed to vary as observed in the Barbour et al. (2004) model. This effect of leaf physiology does not, however, incorporate the effects of  $E$  on leaf temperatures. When leaf temperatures were cooled by  $1^\circ\text{C}$ , leaf  $\alpha$ -cellulose  $\Delta^{18}\text{O}$  declined by a further 1.1–1.2‰. Application of the Barbour et al. (2004) model, using data collected in our study, revealed the following: (1) the magnitude of change in leaf  $\alpha$ -cellulose  $\Delta^{18}\text{O}$  from the south- to the north-facing hill slope is consistent with expectations; (2) “most” of the observed effects on leaf  $\alpha$ -cellulose  $\Delta^{18}\text{O}$  were a consequence of changes in  $E$ ; (3) changes in  $E$  probably had their greatest effect on leaf  $\alpha$ -cellulose  $\Delta^{18}\text{O}$  through changes in leaf temperature.

Our results suggest that the dual isotope approach is capable of revealing the qualitative contributions of  $g_s$  and  $A_{\max}$  to  $\Delta^{13}\text{C}$  at the site level when differences are large and significant. The dual isotope approach may fail to detect more subtle changes, as the approach relies upon five measurements or assumptions of isotopic composition ( $\delta^{13}\text{C}$  of atmospheric  $\text{CO}_2$ ,  $\delta^{18}\text{O}$  of atmospheric water vapor, leaf  $\delta^{13}\text{C}$ , leaf  $\delta^{18}\text{O}$  and stem water  $\delta^{18}\text{O}$ ), all of which contain “noise” and measurement error. Bulk leaf tissue provided a steeper slope in  $g_s$ – $\Delta^{18}\text{O}$  and  $i\text{WUE}$ – $\Delta^{13}\text{C}$  correlations and a greater difference in  $\Delta^{18}\text{O}/\Delta^{13}\text{C}$  with changes in the source of variation in  $\Delta^{13}\text{C}$ . In our study, bulk leaf tissue was a better medium for application of the dual isotope approach than leaf  $\alpha$ -cellulose.

**Acknowledgments** This project was supported by grant no. 0221606 from the National Science Foundation. We thank D. Ruess and D. Sjoström for assistance with stable isotope analyses, M. Smith for field assistance and S. Arens for help in the laboratory. D. Yakir and three anonymous reviewers made constructive comments that substantially improved this manuscript.

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