High-frequency field measurements of diurnal carbon isotope discrimination and internal conductance in a semi-arid species, *Juniperus monosperma*

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ABSTRACT

We present field observations of carbon isotope discrimination (Δ) and internal conductance of CO2 (g) collected using tunable diode laser spectroscopy (TDL). Δ ranged from 12.0 to 27.4‰ over diurnal periods with daily means from 16.3 ± 0.2‰ during drought to 19.0 ± 0.5‰ during monsoon conditions. We observed a large range in g, with most estimates between 0.04 and 4.0 µmol m⁻² s⁻¹ Pa⁻¹. We tested the comprehensive Farquhar, O'Leary and Berry model of Δ (Δcomp), a simplified form of Δcomp (Δsimple) and a recently suggested amendment (Δrev). Sensitivity analyses demonstrated that varying g had a substantial effect on Δcomp, resulting in mean differences between observed Δ (Δobs) and Δcomp ranging from 0.04 to 9.6‰. First-order regressions adequately described the relationship between Δ and the ratio of substomatal to atmospheric CO2 partial pressure (p_i/p_a) on all 3 d, but second-order models better described the relationship in July and August. The three tested models each best predicted Δobs on different days. In June, Δsimple outperformed Δcomp and Δrev, but incorporating g and all non-photosynthetic fractionations improved model predictions in July and August.

Key-words: decarboxylation; Farquhar model; mesophyll conductance; p_i/p_a.

INTRODUCTION

Stable carbon isotope analyses have a long history in plant biology that includes differentiation of photosynthetic pathways (Smith & Epstein 1971), development of physiological theory of carbon isotope fractionation (O’Leary 1981; Farquhar, O’Leary & Berry 1982), crop improvement (Farquhar & Richards 1984), ecological studies (Ehleringer 1993; Brooks et al. 1997), ecosystem process studies (Bowling et al. 2002; McDowell et al. 2004) and biosphere–atmosphere interactions (Yakir 2003; Randerson et al. 2006). The biological and physical discrimination against the ¹³C¹⁶O₂ isotopologue during diffusion and carboxylation is a strong regulator of the isotopic signature of ecosystem exchange with the atmosphere as it largely determines the ¹³C composition of the substrate pool, which supplies respiratory activity (Barbour et al. 2005; Knolh et al. 2005; Bowling, Pataki & Randerson 2008). The transfer of this signature throughout the ecosystem provides a useful signal to partition components of ecosystem carbon exchange and aids in carbon cycle modelling (Ciais et al. 1995; Tu & Dawson 2005; McDowell et al. 2008a).

A substantial body of literature describing a linear relationship between leaf carbon isotope discrimination (Δ) and the ratio of internal to atmospheric CO2 partial pressure (p_i/p_a) has accumulated in the last three decades (Farquhar et al. 1982b; Brugnoli et al. 1988; Farquhar, Ehleringer & Hubick 1989; Ehleringer, Phillips & Comstock 1992; Brugnoli & Farquhar 2000). The p_i/p_a ratio is useful because it succinctly describes the dominant physical and biochemical constraints to photosynthesis. Similarly, the linear relationship between Δ and p_i/p_a observed in previous studies emphasizes the importance of stomatal conductance and biochemistry in Δ. The full model of Δ developed by Farquhar et al. (1982) also accounts for other factors such as internal conductance of CO2 from stomatal cavities to sites of carboxylation (g_i) and apparent isotopic fractionations associated with the decarboxylation processes of day respiration and photorespiration (Δr), as well as other diffusion-related fractionations. Recent evidence suggests that g_i and Δr are sensitive to environmental factors that vary diurnally (Bernacchi et al. 2002; Ghashghaie et al. 2003; Warren, Livingston & Turpin 2004), but their role in the variation in Δ observed in a field setting remains poorly understood.

Temperature and water stress have been shown to impact g_i. Bernacchi et al. (2002) found that temperature regulated g_i within the biologically significant range of 10–40 °C in tobacco, a finding supported in work presented by Yamori et al. (2006) and Warren & Dreyer (2006) using different species. Water stress also reduces g_i, as demonstrated experimentally in *Pseudotsuga* seedlings (Warren et al. 2004) and *Olea* (Diaz-Espejo, Nicolás & Fernandez 2007) and in a comprehensive field study using Quercus and Fraxinus (Grassi & Magnani 2005). Recently, a strong
linkage between aquaporin function and $g_\mu$ was established (Flexas et al. 2006; Uehlein et al. 2008), providing a possible mechanism for rapid variation in $g_\mu$ in response to a multitude of environmental factors, as has been demonstrated in response to CO$_2$ concentration (Flexas et al. 2007). While seasonal changes in $g_\mu$ have been documented in a field setting (Grassi & Magnani 2005; Diaz-Espejo et al. 2007), diurnal variation in $g_\mu$ has not yet been reported.

The influence of environmental factors on $\Delta_e$ is less well known. Temperature and light have been shown to influence day respiration and photorespiration, both of which affect CO$_2$ evolution within a leaf (Brooks & Farquhar 1985; Kozaki & Takeba 1996; Atkin et al. 2000, 2005). The apparent fractionation associated with day respiration $(e)$ and photorespiration $(f)$ are each the result of biochemical reactions that may be subject to environmental control (Ghashghaie et al. 2003). A consistent enrichment of 6‰ in the dark respired $^{13}$C/$^{12}$C ratio $(\delta ^{13}C_{resp})$ of CO$_2$ compared to sucrose of droughted and control Phaseolus leaves has been observed (Duranceau et al. 1999). Such respiratory enrichment has been shown to depend on species and on plant water status (Ghashghaie et al. 2001), temperature (Tcherkez et al. 2003), and light exposure (Barbour et al. 2007a).

Estimates of $e$ have largely been inferred from studies of dark respiration, but recent evidence suggests these dark respiration fractionations may not be representative of day respiratory fractionation (Tcherkez et al. 2008). Field observations of the diurnal patterns of the cumulative fractionation associated with respiratory and photorespiratory processes, estimated here in $\Delta_e$, may allow us to better understand the influence of environmental factors on this component of $\Delta$.

In recent years, advances in optical systems utilizing tunable diode laser spectroscopy (TDL) have simplified high-frequency measurements of the abundance of individual isotopologues $^{13}$C$^{16}$O$_2$, $^{12}$C$^{16}$O$_2$ and $^{12}$C$^{18}$O$^{16}$O in ecosystem studies (Bowling et al. 2003; Griffis et al. 2004; McDowell et al. 2008a) and leaf-scale studies in greenhouse settings (Barbour et al. 2007a,b). Similar TDL leaf-scale measurements can now be attempted in a field setting. The objectives of this study were to (1) examine the temporal variation in $\Delta$, $\delta ^{13}C_{resp}$, $g_\mu$ and $\Delta_e$ under ambient field conditions; (2) test the hypothesis that $g_\mu$ varies across the day; (3) test the hypothesis that $\Delta$ varies linearly in response to shifts in $p_\mu/p$ under field conditions; (4) test the influence of $g_\mu$ in a comprehensive leaf model of $\Delta$; and (5) test the predictive capabilities of three models: the comprehensive Farquhar et al. (1982) model of $\Delta$ ($\Delta_{comp}$), a recently suggested amendment to $\Delta_{comp}$ ($\Delta_{comp}$; Wingate et al. 2007) and the simplified form of the comprehensive model ($\Delta_{simp}$). We used a combined TDL-infrared gas analyser (IRGA) system to obtain high-frequency field measurements of leaf gas exchange synchronized with online isotopic measurements, similar to those used in previous greenhouse studies (Barbour et al. 2007a). Previous work has demonstrated substantial diurnal variation in leaf discrimination in diverse field settings including tropical forest (Harwood et al. 1998) and mesic conifer forest (Wingate et al. 2007). We report ~20 $\Delta$ measurements per hour over diurnal periods during both dry and wet seasons from a semi-arid woodland.

**METHODS**

The field site was located on Mesita Del Buey in Los Alamos, New Mexico, USA (35°50′N, 106°16′W; elevation 2140 m) in a piñon-juniper woodland (Pinus edulis Engelm. and Juniperus monosperma Engelm. respectively) dominated primarily by juniper and understorey grasses and forbs (Breshears 2008; McDowell et al. 2008b). This semi-arid region typically has a bimodal precipitation regime, with substantial winter snowfall (October–April), followed by a dry period (May–June) and monsoonal precipitation from July through early September (Breshears 2008). Precipitation at our site in 2006 totaled 119 mm in winter and 224 mm in summer. Soils on the site are Typic Haplustalfs and Typic Ustochrepts (Davenport, Wilcox & Breshears 1996).

**Leaf gas exchange measurements**

We measured diurnal (0600–1900 h) leaf gas exchange from the bottom third of the canopy on two juniper trees on 12 June 2006, two different juniper trees on 11 July 2006 and a single juniper on 14 August 2006. We coupled a TDL (TGA100A; Campbell Scientific Inc., Logan, UT, USA) to a portable photosynthesis system (Li-Cor 6400; Li-Cor Biosciences, Lincoln, NE, USA) fitted with a conifer chamber (Li-Cor 6400-05) to quantify the concentration of CO$_2$ and its isotopologues $^{13}$C$^{16}$O$_2$ and $^{12}$C$^{18}$O$^{16}$O in gas entering and exiting the leaf chamber, herein referred to as the reference and sample gas streams (i.e. Barbour et al. 2007a). We supplied atmospheric air via a 50 L buffer volume to the Li-Cor 6400, which recorded the CO$_2$ and water vapour concentration of the reference and sample gas every 10 s. These gas streams were dried to a constant low humidity and plumbed directly into the TDL using ultra-low porosity tubing (Synflex type 1300 1/4 in. diameter; Saint Gobain Performance Plastics, Northboro, MA, USA) wherein the TDL measured the CO$_2$ isotopologues $^{13}$C$^{16}$O$^{16}$O and $^{12}$C$^{18}$O$^{16}$O at a rate of 500 Hz. These 500 Hz data were then averaged down to 10 Hz, and all means were calculated from the 10 Hz data. Our 3 min TDL measurement cycle consisted of two reference tanks and the reference and sample gas streams, each measured for 45 s, from which we calculated means of isotopologue concentrations over the last 15 s of each inlet cycle. We combined these TDL data with IRGA-generated data after incorporating the 33 s lag between the two instruments.

We used a Li-Cor conifer chamber to maximize leaf area and allow natural light interception on the scalelike juniper foliage, regulating the chamber flow rate between 250 and 500 $\mu$mol s$^{-1}$ to maintain a sufficient CO$_2$ drawdown and control chamber humidity. We attempted to maintain CO$_2$ drawdown $\approx$40 $\mu$mol CO$_2$ mol$^{-1}$ air within the leaf chamber. Under moderate conditions, chamber temperature was unregulated, but under conditions of high ambient
air temperature (>35 °C) and solar radiation, the IRGA block temperature control was engaged to control leaf temperature below 35 °C, as measured by energy balance. On 12 June, we collected data from six leaf areas diurnally and from two leaf areas at night. On 11 July, we collected data from five leaf areas diurnally and two leaf areas during dark measurements. In both June and July, each leaf area was measured for 30 min to an hour and leaves were typically measured more than once each day. Finally, on 14 August, we collected all data from one leaf area diurnally during a 7 h period, and one leaf area during dark measurements.

The isotopic signature of nocturnal respiration ($\delta^{13}C_{\text{resp}}$) was measured immediately following daylight measurements and beginning when ambient photosynthetic photon flux density (PPFD) fell below 30 μmol photons m$^{-2}$ s$^{-1}$ and foliage exhibited net CO$_2$ efflux. To achieve a true dark measurement, we applied a heavy shade cloth over the leaf chamber to reduce PPFD to zero and waited for stable chamber conditions (e.g. leaf temperature and respiration rate), which occurred within 5 min after the shade cloth was applied. We also determined the carboxylation capacity of these juniper trees on 22 June and 23 July 2007 using assimilation ($A$) responses to changes in substomatal CO$_2$ concentration ($A/p_n$). We collected these data using a Li-Cor 6400 fitted with a chamber light source (Li-Cor 6400-02B). We measured pre-dawn and midday xylem water potential ($\psi_o$) on 5 to 10 nearby juniper trees on each measurement day using a Scholander-type pressure bomb (PMS Instruments Co., Corvallis, OR, USA; McDowell et al. 2008b).

The working standard (WS) calibration tanks used during our diurnal measurements were calibrated against World Meteorological Organization (WMO)-certified standard tanks (541.67 μmol CO$_2$ mol$^{-1}$ air, $\delta^{13}C = -16.16\%o$ and 350.34 μmol CO$_2$ mol$^{-1}$ air, $\delta^{13}C = -8.39\%o$) within 24 h of each measurement campaign. The intertank calibration between WMO and WS tanks typically required 2 h to complete. Molar mixing ratios of $^{12}$CO$_2$: $^{13}$CO$_2$ in the WS tanks used in the June campaign were 354.04 ± 0.27:3.82 ± 0.003 μmol CO$_2$ mol$^{-1}$ air (mean ± standard error; $n = 11$ inter-tank calibrations) and 563.85 ± 0.27:6.09 ± 0.003 μmol CO$_2$ mol$^{-1}$ air ($n = 11$). Molar mixing ratios of $^{12}$CO$_2$: $^{13}$CO$_2$ in the WS tanks used in the July and August campaigns were 340.46 ± 0.29:3.67 ± 0.003 μmol CO$_2$ mol$^{-1}$ air ($n = 10$) and 518.71 ± 0.08:5.60 ± 0.001 μmol CO$_2$ mol$^{-1}$ air ($n = 6$). The WMO-certified tanks were filled and calibrated at the Stable Isotope Lab (SIL) of the Institute for Arctic and Alpine Research, a cooperating agency of the Climate Monitoring division of the National Oceanic and Atmospheric Administration’s Earth Research Laboratory. Measurement variation in the $\delta^{13}C$ of a known tank in the TDL measurement mode we used exhibited an SD of 0.06% across an hour and 0.20% across the day. To account for diurnal instrument drift, the TDL measured the high and low WS tanks during each 3 min cycle, and we calculated the deviation between the measured values and the known values to determine a gain and offset for each isotopologue in each tank being measured (Bowling et al. 2003). These gain and offset values were then applied to all data. The TDL measures the absolute concentration of each isotopologue, so the range of $^{12}$CO$_2$ and $^{13}$CO$_2$ in each WS tank should span the measurement range. During the three measurement days, our measurements occasionally exceeded the lower end of the total [CO$_2$] in our WS tanks (maximum deviation: 45.7 μmol mol$^{-1}$). To test that the calibration was valid below the lower tank, we used a WMO traceable standard tank (total [CO$_2$] = 142.86 μmol mol$^{-1}$, $\delta^{4}C = -7.96\%o$) and an additional unknown tank that had a target total [CO$_2$] of 250 μmol mol$^{-1}$. We measured these two tanks and two WS tanks (344.88 μmol mol$^{-1}$, -8.16%o and 548.16 μmol mol$^{-1}$, -16.42%o) in series. We calculated the total [CO$_2$] and isotope ratio of the unknown tank by calculating the gain and offset values in two ways: (1) using the span between the 142.86 μmol mol$^{-1}$ tank and the 344.88 μmol mol$^{-1}$ tank and (2) using the span between the 344.86 μmol mol$^{-1}$ tank and the 548.16 μmol mol$^{-1}$ tank measurements. The unknown tank was calculated to have a total [CO$_2$] of 247.44 μmol mol$^{-1}$ and a $\delta^{13}C$ of −20.45‰ using the lower calibration span (#1), and a total [CO$_2$] of 247.43 μmol mol$^{-1}$ and a $\delta^{13}C$ of −20.45‰ using the higher calibration span (#2), a net difference of 0.01 μmol mol$^{-1}$ and 0.00‰. We also determined the [CO$_2$] and $\delta^{13}C$ of the 142.86 μmol mol$^{-1}$ WMO tank using gain and offset values calculated using the higher calibration span (#2). The result was a total [CO$_2$] of 142.66 μmol mol$^{-1}$ and a $\delta^{13}C$ of −7.88‰, a net difference of 0.20 μmol mol$^{-1}$ and 0.08‰ from SIL-certified values. Based on this assessment, we conclude our TDL has a linear response that extends beyond the lowest CO$_2$ range we measured in this study.

The IRGA was calibrated the morning of each measurement day, and the reference and sample gas analysers of the IRGA were frequently matched to the same gas stream, while disconnected from the TDL inlet tubes. After reconnecting the TDL inlet tubes with the IRGA, the system was leak tested by gently blowing around the chamber, all connections and the pressure-equilibrating vent tube located on the sample line to the TDL. The TDL was also used to measure the reference and sample gas streams with an empty leaf chamber, and differences were lower than instrument precision (data not shown).

### $\Delta$ and $\delta^{4}C_{\text{resp}}$ calculations

We calculated $\Delta_{\text{obs}}$ in the chamber following Evans et al. (1986):

$$\Delta_{\text{obs}} = \frac{\xi(\delta_c - \delta_o)}{1 + \xi(\delta_c - \delta_o)}$$

(1)

where $\xi = c_o/(c_c - c_o)$ is the ratio of the reference CO$_2$ concentration entering the chamber ($c_c$) relative to the sample CO$_2$ concentration outgoing from the chamber ($c_o$), and $\delta_c$ and $\delta_o$ are the $\delta^{4}C$ of the reference and sample gas, respectively. All variables incorporated in $\Delta_{\text{obs}}$ and $\delta^{4}C_{\text{resp}}$ (below) are derived from TDL measurements of $^{12}$CO$_2$ and $^{13}$CO$_2$, removing interinstrument variability. Mixing ratios of total [CO$_2$] were calculated following Barbour et al.
Because the TDL measures the concentration of each isotopologue, δ and δ are calculated from the ratio of the molar abundance of each isotopologue and then presented in ratio to the Vienna Pee Dee belemnite (VPDB) standard, that is, \( \delta = R/R_{\text{VPDB}} - 1 \), where \( \delta \) represents either \( \delta \) or \( \delta \), and \( R \) and \( R_{\text{VPDB}} \) represent the carbon isotope ratio of the sample and VPDB standard, respectively. We determined \( \delta^{13}C_{\text{resp}} \) following Barbour et al. (2007a):

\[
\delta^{13}C_{\text{resp}} = \frac{\delta - \delta(1 - p)}{p}
\]

where \( p \) equals \((c_a - c_c)/c_c\). We estimated the \( \delta^{13}C \) of assimilated sugars (\( \delta^{13}C_s \)) based on Farquhar et al. (1989), where \( \delta^{13}C_s = (\delta - \delta_{\text{obs}})/(\delta_{\text{obs}} + 1) \). All other reported gas exchange values are calculated by the Li-6400 software following the methods of Farquhar, Caemmerer & Berry (1980), after correcting for leaf area. We determined the projected leaf area using a calibrated leaf area metre (Li-3100; Li-Cor Biosciences), and all gas exchange calculations are reported on a projected leaf area basis.

### Model parameterization

We incorporated our data into the comprehensive model of leaf \( \Delta \) (Farquhar et al. 1982; Farquhar & Richards 1984):

\[
\Delta_{\text{comp}} = a_0 \frac{p_a - p_i}{p_a} + a \frac{p_c - p_i}{p_a} + (b + a_u) \frac{p_i - p_c}{p_a} + b \frac{p_a - p_i}{p_a} + \frac{eR_*}{k} + f \frac{\Gamma *}{p_a}
\]

where \( a_0, a, a_u, b \) and \( p \) are the fractionation factors associated with CO\(_2\) diffusion through the leaf boundary layer (2.9%), stomata (4.4%), water (0.7%), fractionation attributed with CO\(_2\) entering solution (11.1%), and the net fractionation attributed to phosphoenolpyruvate carboxylase and ribulose-1,5-bisphosphate carboxylase/oxygenase activity (estimated at 29%; Roeske & O’Leary 1984), respectively. The variables \( p_a, p_i, p_c \) represent the partial pressure (Pa) of CO\(_2\) in the atmosphere surrounding the leaf, at the leaf surface, in the intercellular spaces and at the sites of carboxylation, respectively. The variables \( \Gamma \), \( R_0 \), \( k \), \( f \) and \( e \) represent the CO\(_2\) compensation point (Pa) in the absence of day respiration, day respiration rate (\( \mu \text{mol m}^{-2} \text{s}^{-1} \)), carboxylation efficiency (\( \mu \text{mol m}^{-2} \text{s}^{-1} \text{Pa}^{-1} \)), and fractionations associated with photorespiration and day respiration (\%o; see Table 1 for values), respectively. We calculated \( p_a, p_i, p_c \) and \( p \) by incorporating mole fraction measurements of [CO\(_2\)] with atmospheric pressure in Los Alamos (mean = 79 kPa), and estimated \( p_* \) following Farquhar & Sharkey (1982):

\[
p_* = p_i - \Delta / g_i
\]

where \( g_i \) is internal conductance to CO\(_2\) (\( \mu \text{mol m}^{-2} \text{s}^{-1} \text{Pa}^{-1} \)). We chose a moderate \( g_i \) of 1.5 \( \mu \text{mol m}^{-2} \text{s}^{-1} \text{Pa}^{-1} \) based on the range of \( g_i \) values observed over the study period. Prevaling theory suggests \( \Gamma \) is highly conserved among C\(_3\) species, and previous work has demonstrated a strong temperature dependence of the CO\(_2\) photocompensation point (Jordan & Ogren 1984; Brooks & Farquhar 1985), on which we based our calculations of \( \Delta_{\text{obs}} \). Our \( \Gamma \) calculations accounted for the reduced atmospheric pressure in Los Alamos, and we confirmed our estimates of \( \Gamma \) with those calculated using the Sharkey et al. (2007) \( \Gamma / p_0 \) estimating utility (Table 1). Strictly, \( k \), the carboxylation efficiency, is \( A/p_* \); we used the initial slope of \( A/p_0 \), response curves \((n = 10)\) as a surrogate estimate and confirmed these slope-based results with calculations presented in Ku & Edwards (1977) and Wingate et al. (2007) (Table 1). Much work has demonstrated an inhibitory effect of light on respiration rate, even at an irradiance as low as 12 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) (Atkin et al. 2000; Tcherkez et al. 2005, 2008). To facilitate estimation of \( R_0 \), we measured nocturnal respiration rate (PPFD = 0) on all 3 d for approximately 120 min after cessation of daytime measurements (see Results) and used these data to calculate an estimated \( R_0 \) value for each day, where \( R_0 = 0.5R \) (Tcherkez et al. 2005) and \( R \) equals steady-state respiration rate 30–120 min post-illumination (Table 1). We parameterized the decarboxylation component of \( \Delta_{\text{comp}} \) using constant \( f (8\%) \) (Roeke 1988; Tcherkez 2006) and \( e (6\%) \) (Ghashghaei et al. 2003) values. Parameterizing \( e \) based on \( \delta^{13}C_{\text{resp}} \) (typically estimated at \(-6\%)\) may be problematic because of shifts in respiratory biochemistry under illuminated conditions (Tcherkez et al. 2008). We assessed the magnitude of uncertainty introduced at high and low \( A \) when varying \( e \) by comparing \((R_0 / A) \times (p_a / p_i)\) multiplied by values of \( e = -6 \) and \(-1\%), and calculating the resulting variation in the \( \Delta_{\text{ct}} \) term (see Eqn 11).

We also ran model simulations following the recent revisions to the comprehensive model (Eqn 3) put forward by Wingate et al. (2007):

\[
\Delta_{\text{revised}} = a_0 \frac{p_* - p_i}{p_a} + a \frac{p_c - p_i}{p_a} + (b + a_u) \frac{p_i - p_c}{p_a} + b \frac{p_a - p_i}{p_a} + \frac{(e + e^*)R_*}{k} + f \frac{\Gamma *}{p_a}
\]

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### Table 1. Parameters used in model simulations of observed discrimination using the comprehensive model (\( \Delta_{\text{comp}} \)) and the revised model (\( \Delta_{\text{revised}} \)). The fractionation factors associated with day respiration, e, and photorespiration, f, were assumed based on literature values while all the other terms are derived from our data.

<table>
<thead>
<tr>
<th>Day</th>
<th>( k )</th>
<th>( R_0 )</th>
<th>( \Gamma * )</th>
<th>( e )</th>
<th>f</th>
<th>g_i</th>
<th>( e^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 June</td>
<td>0.38</td>
<td>1.23</td>
<td>2.86–5.23</td>
<td>-6</td>
<td>8</td>
<td>1.5</td>
<td>-11.5 to -1.6</td>
</tr>
<tr>
<td>11 July</td>
<td>0.40</td>
<td>2.2</td>
<td>3.17–5.17</td>
<td>-6</td>
<td>8</td>
<td>1.5</td>
<td>-12.5 to -0.9</td>
</tr>
<tr>
<td>14 August</td>
<td>0.40</td>
<td>1.83</td>
<td>2.43–4.29</td>
<td>-6</td>
<td>8</td>
<td>1.5</td>
<td>-10.5 to 1.2</td>
</tr>
</tbody>
</table>
where $e^*$ represents apparent fractionation for day respiration expressing the difference between the isotopic composition of the respiratory substrate and photosynthetic assimilates at a given time (Table 1). We calculated an $e^*$ value for each three minute isotopic measurement using the following equation:

$$
e^* = \delta^{13}p_a - \Delta_{\text{simple}} - \delta^{13}C_{\text{mean}}$$

(6)

where $\delta^{13}p_a$ is the carbon isotope ratio of atmospheric air in the leaf chamber, and $\delta^{13}C_{\text{mean}}$ equals the mean calculated from the $\delta^{13}C_{\text{resp}}$ measurements for each measurement date (see Results). In $\Delta_{\text{recved}}$, we used a constant $e$, $f$, $R_a$, $g$, and $k$ and a temperature-dependent $\Gamma^*$ (Table 1).

Lastly, we modelled $\Delta$ for comparison to $\Delta_{\text{obs}}$ using the most simplified form of the Farquhar et al. (1982) model ($\Delta_{\text{simple}}$), which eliminates boundary layer, $g$, and decarboxylation contributions to CO$_2$ flux and their associated fractionation factors:

$$\Delta_{\text{simple}} = a + (b - a) \frac{p_i}{p_a}$$

(7)

where $b = 27\%$ (Gessler et al. 2008). All modelling was performed in Microsoft Excel XP Professional.

### Estimation of $g_i$ and $\Delta_{\text{ef}}$

We estimated $g_i$ following the slope-based approach ($g_{\text{a}}$) in Evans et al. (1986):

$$g_{\text{a}} = (b - b_i - a_e) \frac{1}{r_i}$$

(8)

where $r_i$ is the internal resistance to CO$_2$ transfer estimated as the slope of predicted $^{13}$C discrimination minus $\Delta_{\text{obs}}$ versus $A/p_a$. In this application, predicted discrimination ($\Delta$) was determined using Eqn 3 calculated with infinite $g_i$, i.e. $p_i = p_a$. In this study, variation in $A/p_a$ was the result of natural variation in the leaf environment. We calculated slopes for each time period where new leaf material was enclosed in the leaf chamber, and tested each slope using a simple linear regression. All negative slopes were rejected because negative slopes result in negative $g_{\text{a}}$ estimates. All regression analyses were performed using JMP 5.0.1 (SAS Institute Inc., Cary, NC, USA). We used significant ($P \leq 0.10$) slope values to estimate $g_{\text{a}}$ for each foliage measurement, and determined the viability of each $g_{\text{a}}$ estimate by comparing them to $A$ across the entire measurement period. If the $g_{\text{a}}$ estimate was too low to facilitate observed $A$ during any portion of the measurement period, we deemed that estimate to be erroneous. Finally, based on the theory developed by Evans et al. (1986) and Caemmerer & Evans (1991), we used the y-intercept of significant $g_{\text{a}}$ plots to estimate $\Delta_{\text{ef}}$.

We also estimated $g_i$ using the point-based method ($g_{\text{p}}$, Evans et al. 1986):

$$g_{\text{p}} = \frac{(b - b_i - a_e) A/p_a}{\Delta_{\text{pred}} - \Delta_{\text{obs}} - \Delta_{\text{ef}}}$$

(9)

where $\Delta_{\text{pred}}$ represents a simplified predictive model of leaf $\Delta$:

$$\Delta_{\text{pred}} = a_o \frac{p_a - p_i}{p_a} + a \frac{p_a - p_i}{p_a} + b \frac{p_i}{p_a}$$

(10)

and $\Delta_{\text{ef}}$ is calculated as:

$$\Delta_{\text{ef}} = \frac{eR_i}{k} + f\Gamma^*$$

(11)

where all factors are the same as described in $\Delta_{\text{comp}}$ (Eqn 3).

### Statistical analysis

We assessed the sensitivity of $\Delta_{\text{comp}}$ to changes in $g_i$ by holding all parameters listed in Table 1 constant and by varying the $g_i$ value used to calculate $p_i$ over each day. We used $g_i$ values ranging from 0.5 to 2.5 μmol m$^{-2}$ s$^{-1}$ Pa$^{-1}$, and applied each value uniformly across each measurement day.

We estimated the error in $\Delta_{\text{obs}}$ and $\delta^{13}C_{\text{resp}}$ by implementing the parametric bootstrap (Davidson & Hinkley 1997); we describe the procedure for $\Delta_{\text{obs}}$, but $\delta^{13}C_{\text{resp}}$ can be substituted in the description. For each measurement cycle, we used the sample mean and SEs of the concentrations of $^{12}$CO$_2$ and $^{13}$CO$_2$ for the high WS tank, low WS tank, reference gas and sample gas to define eight normal distributions. We drew eight random deviates of $[^{12}\text{CO}_2]$ and $[^{13}\text{CO}_2]$ from these distributions, calculated a bootstrap replicate of $\Delta_{\text{obs}}$, and repeated this 10 000 times to provide a bootstrap sampling distribution of $\Delta_{\text{obs}}$. This insured that the variance measured with each isotopologue was propagated into each calculation of $c_x$, $c_o$, $\xi$, $\delta$, and $\Delta$, and, therefore, into $\Delta_{\text{obs}}$ and $\delta^{13}C_{\text{resp}}$. The SE of the bootstrap replicates provides an estimate of the SE of $\Delta_{\text{obs}}$. We observed that the bootstrap sampling distributions of $\Delta_{\text{obs}}$ were roughly normal, so the estimated SE characterizes the variation in $\Delta_{\text{obs}}$. All bootstrap analyses were performed in R (R Core Development Team 2008).

For both $g_{\text{a}}$ and $g_{\text{p}}$, the $g_i$ estimate is a reciprocal transformation of a normally distributed random variable. While the SEs describe the normal distributions well, they are not easily interpretable for the skewed distributions associated with $g_{\text{a}}$ and $g_{\text{p}}$. $g_{\text{a}}$ is the reciprocal of $r_i$, estimated using the normally distributed regression slope (Table 2). For the slope-based $g_i$, we calculated $r_i$ and $r_i \pm 1$ SE, and transformed these three values to the $g_i$ scale (Eqn 8) to generate $g_i$ and an estimate of its error. Similarly, for the point-based $g_i$, we calculated the roughly normally distributed bootstrap mean $\Delta_{\text{obs}} \pm 1$ SE and transformed these to the $g_i$ scale.
(Eqn 9). For these data, 1 SE on the \( r_i \) or \( D_{\text{obs}} \) scale is asymmetric on the \( g_i \) scale with the upper SE being roughly twice the lower SE.

To assess model performance, we first used least squares regression analysis of predicted and observed values but found that the residual analysis of data in all months and models exhibited a non-random distribution. Additionally, both the slope and intercept terms were significantly different from one and zero, respectively, and substantially different from one another, making model comparisons difficult to evaluate. We then modified the computation of the residuals so that all models conformed to a slope of one and an intercept of zero (i.e. residuals \( = \) model prediction – observed data), and calculated the SD of the residuals. These SD values represented the square root of the sum of the variance and squared model bias, or the root mean square error (RMSE), for each month and model, and facilitated a direct comparison of the predictive performance between models within each month.

**RESULTS**

**Diurnal \( D_{\text{obs}} \)**

Juniper \( D_{\text{obs}} \) averaged (mean \( \pm \) SE) 16.3 \( \pm \) 0.2‰ in June, 17.2 \( \pm \) 0.2‰ in July and 19.0 \( \pm \) 0.5‰ in August \((P \leq 0.0002 \) between each). Leaf \( D_{\text{obs}} \) tended to be highest in the early morning in all three months, followed by midmorning variability and a decline through much of the afternoon (Fig. 1). The seasonal \( D_{\text{obs}} \) trend tracked the transition from low (June) to high (August) soil, leaf and atmospheric water content (Table 3, Fig. 2d–f). Similarly, the diurnal trend towards lower \( D_{\text{obs}} \) in the afternoon reflects the transition from relatively high morning leaf \( \psi_w \) to lower midday \( \psi_w \) (Table 3). On July and August measurement days, the variation in leaf \( D_{\text{obs}} \) reflects the stability of the light environment, with a relatively stable PPFD in July concurrent with a stable \( D_{\text{obs}} \) and a heterogeneous light environment in August resulting in fluctuating \( D_{\text{obs}} \) (Fig. 2). On 14 August, we lack reliable isotopic data after 1300 h because of low ambient light (PPFD < 100 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)), preventing \( A \) rates high enough to sustain reliable isotopic measurements. We found a weak but significant correlation between leaf vapour pressure deficit (VPD) and \( D_{\text{obs}} \) \((r^2 = 0.20, \) Table 2.

<table>
<thead>
<tr>
<th>Campaign</th>
<th>Time (h)</th>
<th>Slope</th>
<th>SE</th>
<th>( P )</th>
<th>( \Delta_{\text{def}} )</th>
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Figure 1. Diurnal variation in carbon isotope discrimination (\( \bullet \): \( D_{\text{obs}} \)) on 12 June, 11 July and 14 August. Error bars represent 1 SE. Note the change of \( y \)-axis scaling in panels.
Table 3. Mean xylem water potential with SE on all three measurement days. Midday values from McDowell et al. (2008b)

<table>
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<td>0.03</td>
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<td>August</td>
<td>–0.58</td>
<td>0.04</td>
<td>–1.58</td>
<td>0.44</td>
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</table>

P < 0.0001; F = 110.22; Fig. 3). PPFD and Δobs (r² = 0.20, P < 0.0001; F = 114.11), and A and Δobs (r² = 0.11, P < 0.0001; F = 54.97; Fig. 3) using data pooled across all 3 d. Excluding the seven very high Δobs values in the early August morning, there was a significant relationship between stomatal conductance (gₚ) and Δobs (r² = 0.03, P < 0.0001; F = 16.60; Fig. 3).

Nocturnal δ¹³C.resp

The isotopic composition of nocturnal respiration was similar in June (mean = –22.6 ± 0.2‰) and July (mean = –22.7 ± 0.2‰; P = 0.70) (Fig. 4), while respiration rates were dissimilar (2.6 ± 0.04 and 4.8 ± 0.1 μmol m⁻² s⁻¹, respectively; P < 0.0001). In August, mean Δ³⁴C.resp was more depleted (mean = –23.5 ± 0.1‰) than values measured in June (P < 0.0001) and July (P < 0.0001), while respiration rate (mean = 3.7 ± 0.004 μmol m⁻² s⁻¹) was higher than that observed in June (P < 0.0001) and lower than that observed in July (P < 0.0001). These Δ³⁴C.resp values were enriched compared with estimates of the composition of recently assimilated sugars, which were –24.66 ± 0.20‰ in June, –25.19 ± 0.17‰ in July and –25.97 ± 0.30‰ in August. The step change in Δ³⁴C.resp observed approximately 50 min post-illumination in June and July was due to cessation of measurement on one group of foliage and the movement to new foliage.

Temporal variation in gₚ and Δₜ

We tested 32 slopes and found that 17 were significant across the 3 d. These produced 14 viable gₚ and Δₜ estimates based on comparisons to A, including two in June, six in July and six in August (Fig. 5; Table 2). We also found three slopes in the August morning, which failed our criteria for having a significant slope (P ≤ 0.1), but whose estimates of gₚ fit the observed trend and are included in Fig. 5 (Table 2). Other gₚ estimates failed to support observed A or displayed negative slope relationships between Δₜ–Δobs and A/pᵣ. a were excluded from the analysis. Estimates of gₚ produced non-viable values when Δobs was larger than Δₜ, in bootstrap resamples, resulting in negative gₚ estimates. These 98 negative values, representing 22% of all gₚ estimates, were excluded from the analysis.

Internal conductance calculated from slope-based measurements ranged from 0.04 to 2.14 μmol m⁻² s⁻¹ Pa⁻¹ (mean ± SE = 1.06 ± 0.17 μmol m⁻² s⁻¹ Pa⁻¹) across the 3 d. The 14 August gₚ, measurements were obtained from one leaf area across the morning and early afternoon, and demonstrated an increase in gₚ from 0.04 to 2.14 μmol m⁻² s⁻¹ Pa⁻¹ (Fig. 5c). We observed a lower range of variability in July gₚ, with afternoon values ranging between 0.92 and 1.3 μmol m⁻² s⁻¹ Pa⁻¹. We did not find a significant relationship between leaf temperature (Tᵢ) and gₚ (r² = 0.03, P = 0.87; F = 0.028). Estimates of gₚ ranged between 0.05 and 8.53 μmol m⁻² s⁻¹ Pa⁻¹ (mean ± SE = 1.89 ± 0.07 μmol m⁻² s⁻¹ Pa⁻¹) across the three measurement days (Fig. 5). Sensitivity analysis demonstrated a significant increase (P < 0.0001) in gₚ estimates when varying e = –6‰ and f = 8‰ (mean ± SE = 1.60 ± 0.04 μmol m⁻² s⁻¹ Pa⁻¹) to e = –1‰ and f = 11‰ (3.31 ± 0.14 μmol m⁻² s⁻¹ Pa⁻¹). There was a small but significant relationship between gₚ and Tᵢ (r² = 0.03, P = 0.0003; F = 13.168).

Δₜ also exhibited diurnal variation, ranging between –21.3 and +1.34‰. In August, we observed a low Δₜ value of –21.3‰ in the early morning, later morning values that were not significantly different from zero (P ≤ 0.10), and afternoon values near –2.5‰ (Table 2). The morning value in July was not significantly different from zero, whereas the afternoon Δₜ values were between –4.9 and –3.5‰. Our single significant Δₜ value in June was –10.56 ± 5.3‰. The non-zero values of Δₜ occur at early morning, midday or late afternoon, when fluxes are small and errors are likely to be greatest (Table 2).

Δobs and p/pᵣ

First-order linear relationships between Δobs and p/pᵣ were significant in June (r² = 0.25, P < 0.0001; F = 58.31; Fig. 6a), July (r² = 0.51, P < 0.0001; F = 182.61) and August (r² = 0.72, P < 0.0001; F = 248.99); however, second-order polynomials described the relationships with greater predictive power in July (r² = 0.64, P < 0.0001; F = 151.90) and August (r² = 0.88, P < 0.0001; F = 334.27; Fig. 6b,c). The curvilinear relationship between Δobs and p/pᵣ was most pronounced in the p/pᵣ range between 0.75 and 0.85.

gₚ sensitivity analysis

Incorporation of variable gₚ into Δcomp over diurnal periods produced variation in predictions of Δcomp. Sensitivity analysis demonstrated using low gₚ (0.5 μmol m⁻² s⁻¹ Pa⁻¹) in Δcomp resulted in a mean 6.9‰ underestimate of Δobs, while relatively high gₚ (2.5 μmol m⁻² s⁻¹ Pa⁻¹) resulted in a 0.7‰ overestimate of Δobs (Table 4). Pairwise comparisons of the residuals (Δobs–Δcomp) resulting from Δcomp predictions incorporating a gₚ value of 0.5 μmol m⁻² s⁻¹ Pa⁻¹ were significantly different from the residuals produced when using gₚ values of 1.0, 1.5, 2.0 and 2.5 μmol m⁻² s⁻¹ Pa⁻¹ in Δcomp (P ≤ 0.05; Tukey’s Honestly Significant Differences (HSD)) within and across all 3 d. Similarly, all other gₚ inputs into Δcomp (1.0, 1.5, 2.0 and 2.5 μmol m⁻² s⁻¹ Pa⁻¹) produced significantly different residuals from one another within each day and across all 3 d (Table 4). The RMSE, a measure of the variance and squared bias associated with the residuals, largely followed the trend observed in the pairwise residual
comparisons and was lower when residual differences were smaller; this demonstrates the importance of an accurate estimate of $g_i$ for model fit. Internal conductance values of 1.5 and 2.0 mmol m$^{-2}$ s$^{-1}$ Pa$^{-1}$ produced the best predictions, as determined by the lowest pairwise residual differences and RMSE, when applied uniformly across each measurement day (Table 4).

**Model predictions: $\Delta_{\text{comp}}$, $\Delta_{\text{revised}}$ and $\Delta_{\text{simple}}$**

Model performance varied across the three measurement days (Fig. 7). Assessing the error between model predictions and $\Delta_{\text{obs}}$ in each month showed that $\Delta_{\text{simple}}$ had the lowest RMSE, 2.11‰, in June, $\Delta_{\text{comp}}$ had the lowest error in July (RMSE = 1.50‰), and $\Delta_{\text{revised}}$ exhibited the lowest error in August (RMSE = 3.15‰; Table 5). Substituting $b = 25\%$ into $\Delta_{\text{simple}}$, reduced model prediction bias (mean = 0.31 ± 0.12‰) but resulted in higher RMSE (mean = 2.65‰ versus 2.42‰ for $b = 27\%$) on all 3 d compared with using $b = 27\%$. The estimated model prediction bias between $\Delta_{\text{comp}}$, $\Delta_{\text{revised}}$ and $\Delta_{\text{simple}}$ and observed discrimination across all three dates was (mean ± SE) –0.62 ± 0.18‰, –0.28 ± 0.19‰ and 1.63 ± 0.18‰, respectively. However, error assessment revealed that the apparent close simulations suggested by the small model prediction bias between modelled and observed values masked substantial variance in all models’ predictions of $\Delta_{\text{obs}}$ (Table 5). At high $A$, defined here as >4.0 mmol m$^{-2}$ s$^{-1}$, uncertainty introduced into $\Delta_{\text{obs}}$ by utilizing $e = -6\%$ versus $-1\%$ was equal to 2.21 ± 0.01‰, while at low $A$, defined here as <2.0 mmol m$^{-2}$ s$^{-1}$, the same uncertainty increased to 9.40 ± 1.51‰ (Table 6).

**DISCUSSION**

The objectives of this study were to (1) examine the temporal variation in $\Delta$, $\delta^{13}\text{C}_{\text{resp}}$, $g_i$ and $\Delta_{\text{obs}}$ under ambient field conditions; (2) test the hypothesis that $g_i$ varies across the day; (3) test the hypothesis that $\Delta$ varies linearly in response to shifts in $p_i/p_a$ under field conditions; (4) test the influence of $g_i$ in a comprehensive leaf model of $\Delta$; and (5) test the predictive capabilities of three models: the comprehensive Farquhar et al. (1982) model of $\Delta$ ($\Delta_{\text{comp}}$), a recently suggested amendment to $\Delta_{\text{comp}}$ ($\Delta_{\text{revised}}$: Wingate et al. 2007) and the simplified form of the comprehensive model ($\Delta_{\text{simple}}$). We observed a large range of variation in $\Delta$, $g_i$ and $\Delta_{\text{obs}}$ over diurnal time periods and across the season. Seasonally, $\delta^{13}\text{C}_{\text{resp}}$ decreased as water availability increased. We found that $g_i$ varied across the day in August and that $g_i$ exerted

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**Figure 2.** Environmental parameters on each measurement day. Panels a–c depict incident photosynthetic photon flux density (PPFD) trends across each measurement day. Panels d–f show leaf temperature, as measured by energy balance (e) and vapour pressure deficit (VPD; V) across each measurement day.
substantial influence on Δ predictions. We found Δ was varied in a linear fashion in response to \( p_i/p_a \) in June, but second-order expressions better described the relationship in July and August. Finally, we found all models reasonably predicted Δ, but Δ best predicted Δ in June, Δ best predicted Δ in July, and Δ best predicted Δ in August.

### Diurnal Δ and nocturnal δ\(^\delta\)C\(_{\text{resp}}\)

Diurnal Δ in our juniper woodland varied between 12.0 and 27.4‰, which was similar in trend and magnitude to Δ observed in a tropical forest (Harwood et al. 1998) and in a mesic Picea stand (Wingate et al. 2007) (Fig. 1). Variation in Δ was generally related to environmental drivers such as PPFD and VPD (Figs 1–3). An inverse relationship between VPD and Δ (Figs 1–3). A cumulative relationship between VPD and Δ was similar to those seen in modelled canopy Δ (Baldocchi & Bowling 2003; Chen & Chen 2007). We also observed several high, but transient, discrimination values in all 3 months including midday values of 31.4‰ in June and 36.9‰ in July, and observations ranging from 29.7 to 44.9‰ in the early morning in August. These Δ values were associated with greater uncertainty, but were similar to values observed in Piper and Picea (Harwood et al. 1998; Wingate et al. 2007).

Nocturnal δ\(^\delta\)C\(_{\text{resp}}\) for the juniper trees in our study ranged from −24 to −21‰, and was moderately enriched compared with most observations in the literature (Bowling et al. 2002; Hymus et al. 2005; Prater, Mortazavi & Chanton 2005). δ\(^\delta\)C\(_{\text{resp}}\) values were similar in June and July, and were more enriched in 13C compared with the values in August (Fig. 4). The consistent 2–3‰ enrichment of δ\(^\delta\)C\(_{\text{resp}}\)
compared to estimates of recently assimilated carbohydrate is consistent with previous reports (Duranceau et al. 1999; Ghashghaie et al. 2001) and may reflect respiratory fractionation, possibly combined with diverse respiratory substrate utilization (Tcherkez et al. 2003). This $^{13}$C$_{res}$ pattern is consistent with the temporal transition period from drought in June through the onset of summer monsoon in July to the strong monsoon in August.

**Temporal variation in $g_i$ and $\Delta_{ef}$**

We observed a diurnal increase in $g_i$ occurring in one leaf area across the August morning and early afternoon, and a range of variation in $g_i$ across the 3 months (Fig. 5). The physiological drivers of this variation in $g_i$ are unknown, but likely involved changes in protein activity facilitating the transfer of CO$_2$ across cell or chloroplast membranes (Flexas et al. 2006; Hanba et al. 2006; Uehlein et al. 2008). Previous work has demonstrated variability in $g_i$ in response to environmental variables such as temperature (Bernacchi et al. 2002; Warren & Dreyer 2006; Yamori et al. 2006) and water availability (Warren et al. 2004; Grassi & Magnani 2005; Galmés, Medrano & Flexas 2007; Diaz-Espejo et al. 2007), both of which fluctuate in a field setting. We did not find a significant correlation between $T_l$ and $g_i$, but we did find a significant relationship between $T_l$ and $g_i$. It is possible that variable irradiance over each measurement period may have confounded any temperature effect on $g_i$, but the higher temporal frequency of $g_i$ was closer to the frequency $T_l$ was changing diurnally. Juniper displays anisohydric leaf hydraulic behaviour, and concurrent $\psi_w$ measurements (Table 3) demonstrated a seasonal increase and diurnal decrease in xylem $\psi_w$. The seasonal $\psi_w$ pattern paralleled our seasonal $g_i$ measurements, suggesting a linkage between leaf water status and the $g_i$ patterns we observed, but are confounded by the increase in both $g_i$ and $g_i$ in the August morning when $\psi_w$ was decreasing. Notably, there was a distinct decrease in $g_i$ in the upward morning trend that coincides with extended cloud cover (mean

**Figure 5.** Diurnal variation in internal conductance of CO$_2$ estimated using sloped-based methods ($g_i$) and point-based methods ($g_{ip}$) on 12 June (a), 11 July (b) and 14 August (c). Internal conductance values derived from non-significant slopes ($P \geq 0.10$) on 14 August are also represented; all $g_i$ estimates from 14 August were measured on one leaf area. Error bars represent 1 SE and are presented with grey ($g_{ip}$) and black ($g_i$) lines.

**Figure 6.** The relationship between observed discrimination ($\Delta_{obs}$) and $p_i/p_a$. First-order linear relationships were observed in June (a; $r^2 = 0.25, P < 0.0001$), July (b; $r^2 = 0.51, P < 0.0001$) and August (c; $r^2 = 0.72, P < 0.0001$) although second-order polynomial relationships better described the data in July ($r^2 = 0.64, P < 0.0001$) and August ($r^2 = 0.88, P < 0.0001$).
Table 4. Results from a sensitivity analysis utilizing variable $g_i$ values within $\Delta_{\text{comp}}$ and applied across each measurement day. $\Delta_{\text{obs}} - \Delta_{\text{comp}}$ represents the pairwise residual difference (%) between observed discrimination ($\Delta_{\text{obs}}$) and model predictions ($\Delta_{\text{comp}}$). $\Delta_{\text{comp}}$ predictions using each of the $g_i$ values produced residuals significantly different from one another within each day and across days. As determined by the lowest root mean square error (RMSE; %) and pairwise residual difference, $g_i$ of 1.5 and 2.0 $\mu$mol m$^{-2}$ s$^{-1}$ Pa$^{-1}$ performed best in predicting $\Delta_{\text{obs}}$.

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</tbody>
</table>

Figure 7. The relationship between observed discrimination ($\Delta_{\text{obs}}$) and discrimination values predicted using $\Delta_{\text{comp}}$ (Δ) and $\Delta_{\text{simple}}$ (■) relative to the 1:1 $\Delta_{\text{obs}}$ line (solid line). Note: axes are unequal among panels to enhance resolution. $\Delta_{\text{predicted}}$ and $\Delta_{\text{comp}}$ utilized a $b = 0.95$, while $\Delta_{\text{simple}}$ was fit with a $b = 0.7$; other parameters are listed in Table 1. $\Delta_{\text{simple}}$ exhibited the lowest overall error in predicting $\Delta_{\text{obs}}$ in June, $\Delta_{\text{comp}}$ exhibited the lowest error in July, and $\Delta_{\text{predicted}}$ exhibited the lowest error in August.

PPFD = 266 ± 46 $\mu$mol m$^{-2}$ s$^{-1}$). We speculate that the large and prolonged drop in incident light played a regulatory role in the lower $g_i$, similar to observations of other environmental regulators of $g_i$ in controlled studies (Delfine et al. 1999; Bernacchi et al. 2002; Flexas et al. 2007). The July data exhibit modest variation in diurnal $g_i$, but may reflect natural variation among branches. Given that our measurements were collected under ambient environmental conditions, an accurate assessment of the factors driving the variation in $g_i$ we observed is not possible and should be addressed in controlled studies.

The variation in $g_i$ is potentially problematic for the slope-based method because it assumes that $g_i$ is constant over the period the slope data are collected. While rapid variation in $g_i$ has been demonstrated in response to [CO$_2$] (Flexas et al. 2007), the rate and magnitude of diurnal shifts in $g_i$ under field conditions have not been previously reported. Our 30–45 min $g_i$ measurement periods may have spanned too long and allowed time for $g_i$ to change in response to the environment. However, aside from periods where $\Delta_{\text{obs}}$ was highly variable, such as the July midday period, $g_i$ values were generally stable around each $g_i$ value and show that variation was low enough to provide valid $g_i$ estimates. Slope-based estimates of $g_i$ tended to be lower than $g_{ip}$ in June and July, but both trended together in August (Fig. 5). $g_{ip}$ is sensitive to the parameterization of $e$ and $f$, and errors in estimating these values may have resulted in over- or underestimation of $g_i$.

Most of our $g_i$ estimates agree with values reported in other woody species (Lloyd et al. 1992; De Lucia, Whitehead & Clearwater 2003; Warren et al. 2003; Ethier et al. 2006), but we also found low $g_i$ estimates in the early morning and relatively high $g_{ip}$ estimates when $\Delta_{\text{obs}}$ was highly variable. We found a low $g_i$ estimate (0.03 $\mu$mol m$^{-2}$ s$^{-1}$ Pa$^{-1}$) in the August early morning transition period from respiration to net $A$, where net CO$_2$ drawdown was between 6 and 10 $\mu$mol mol$^{-1}$, uncertainty in $\Delta_{\text{obs}}$ was higher, and measurements may have been more strongly influenced by the isotopic signature of CO$_2$ evolved during concurrent day respiration processes. Although low, model simulations demonstrated that the 0.03 $\mu$mol m$^{-2}$ s$^{-1}$ Pa$^{-1}$ conductance estimate was high enough to allow observed $A$ across the measurement period. Estimates from $g_{ip}$ during this period...
show consistently negative estimates of $g_e$ (data not shown). High and variable $g_m$ estimates ranged between 4 and 8 $\mu$mol m$^{-2}$ s$^{-1}$ Pa$^{-1}$ during the midday period in July, driven by higher uncertainty in $\Delta_{obs}$ over this period.

Our measurements of $\Delta_r$ suggest that fractionations attributed to decarboxylation activity may not be negligible at dawn and in the afternoon when rates of either respiration or photorespiration may be high (Table 2). Our early morning August measurement occurred during a time of low $A/p_e$ and generated a very negative $\Delta_r$ value. If respiration had not fully deactivated to its daytime rate, then it may have had an unusually large impact during that time period (Gillon & Griffiths 1997). By midmorning in July and August, $A$ and $g_e$ had reached a plateau, and $\Delta_r$ was not significantly different from zero. However, in the June and July afternoons, high temperature and PPFD created conditions conducive to higher photorespiration rates that may have contributed to greater variation in the afternoon $\Delta_r$ values. Further, compared with other C$_3$ species, juniper exhibits high $R$, from which we estimated $R_e$, and thus the respiratory component of $\Delta_r$ would have a larger impact on net $\Delta$ than would be expected for other species. Carefully controlled studies partitioning different components of the net flux will be necessary to elucidate the contribution of each component.

$\Delta_{obs}$ and $p/p_e$

We observed significant first-order linear relationships between $\Delta$ and $p/p_e$ in all months, but found that second-order models better described the curvilinear relationship between $\Delta$ and $p/p_e$ in July and August (Fig. 6). We propose that the curvilinear relationship is related to the increasing dominance of respiration and associated isotopic signatures on leaf-exchanged CO$_2$ at high $p/p_e$ values. Previous work and theory have demonstrated a linear relationship between $\Delta$ and $p/p_e$ in C$_4$ plants (Farquhar et al. 1982b, 1989; Evans et al. 1986; Brugnoli et al. 1988), but unlike our study, these data were collected in controlled settings under steady-state conditions. In both July and August, the curvilinear trend between $\Delta$ and $p/p_e$ was driven by high $\Delta$ values. These high $\Delta$ values correspond with conditions conducive to high respiratory and photorespiratory flux, notably the early morning and midday periods, and may reflect the isotopic signature of a highly enriched substrate.

**g$_e$ sensitivity analysis**

Incorporating variable internal CO$_2$ conductance into $\Delta_{comp}$ demonstrated that $g_e$ exerted substantial influence on predictions of diurnal discrimination. Average-observed $g_e$ was near 1.5 $\mu$mol m$^{-2}$ s$^{-1}$ Pa$^{-1}$, and our sensitivity analysis showed that relatively low (0.5 $\mu$mol m$^{-2}$ s$^{-1}$ Pa$^{-1}$) and high (2.5 $\mu$mol m$^{-2}$ s$^{-1}$ Pa$^{-1}$) values resulted in large deviations between model predictions and $\Delta_{obs}$ (Table 4). However, we have shown that $g_e$ can vary in a leaf over several hours, and it is likely that incorporating this diurnal variability into leaf and ecosystem models would improve discrimination predictions (McDowell et al. 2008a). Future studies should focus on assessing the diurnal variability in $g_e$ independently and on testing whether variable diurnal $g_e$ significantly improves the accuracy and precision of predictions of $\Delta$ in leaf models.

**Model predictions: $\Delta_{comp}$, $\Delta_{revised}$ and $\Delta_{simple}$**

Our study supports the use of the more comprehensive models, $\Delta_{comp}$ and $\Delta_{revised}$, that incorporate fractionations associated with the diffusion pathway and decarboxylation activity, to describe leaf $\Delta$ in our semi-arid system. The limitations of these models are that they require assumptions of the true value of fractionation during carboxylation and decarboxylation, in addition to an accurate estimate of $g_e$. Our sensitivity analysis showed that variation in $e$ at low $A$ resulted in $\sim$9% variation in $\Delta_{tr}$, emphasizing the importance of $e$ in plants, such as juniper, that exhibit relatively
high $R$ compared with $A$. Our estimate of $e$ was based on the dark respiration fractionation, and we may have over- or underestimated the true value of $e$ or $R_d$ and introduced model error. However, we have shown that both models produced similar errors in their predictions of $\Delta$.

The importance of decarboxylation activity in juniper $\Delta$ is reflected both in the $e^*$ values we calculated and the $\Delta_{air}$ estimates obtained from $g_i$ plots. We calculated $e^*$ values ranging from $-1.25$ to $+1.2\%_o$, values that suggest the isotopic disequilibria between recent photosynthate and the respiratory substrate being utilized was, at times, substantial. Further, our $\Delta_{air}$ estimates were mostly between $-6.9$ and $0\%_o$, whereas previous observations were close to $0\%_o$ (Evans et al. 1986). It is also possible that other factors, such as stomatal patchiness, may not be fully captured in our estimates of $p_i$, which could alter the $p_i/p_a$ ratio important to all of the $\Delta$ models (Farquhar 1989).

Despite lacking decarboxylation and $g_i$ components, $\Delta_{simple}$ outperformed the more comprehensive models in June. Further, $\Delta_{simple}$ exhibited modest error in predicting $\Delta_{obs}$ compared with $\Delta_{comp}$ and $\Delta_{revised}$ in July and August, but consistently overestimated $\Delta_{obs}$, predicting $\Delta$ values whose mean difference were $>1.0\%_o$ above $\Delta_{obs}$ in all 3 months. This may represent a larger systematic bias than that exists in the other models, although utilizing a lower $b$ value reduced model bias while moderately increasing error. However, all of the models exhibited non-trivial RMSE, ranging from 1.5 to 3.2$\%_o$, suggesting that a significant amount of variability remains to be captured. Future field studies should aim to independently estimate the variability in diurnal $\Delta$ and $g_i$ to ascertain their impacts on diurnal leaf isotopic exchange. Similarly, future controlled studies should partition the net flux to assess $g_i$ and $\Delta_{air}$ as well as the regulatory influence of environmental variables, such as temperature and PPFD, on these components of carbon discrimination.

CONCLUSIONS

Our study demonstrates that the diurnal variation in $\Delta$ in our semi-arid conifer ecosystem was of similar trend and magnitude to that observed in ecosystems as diverse as tropical forest and mesic conifer forest. Additionally, we demonstrated that $\Delta$ varies rapidly in response to shifts in environmental conditions, and that the comprehensive Farquhar et al. (1982) model and its descendents are capable of capturing a wide range of diurnal variation in leaf $\Delta$. Our observations are consistent with previous results showing low $\Delta$ during conditions of low soil water availability and elevated VPD and PPFD, and higher $\Delta$ when soil water was more abundant, PPFD was variable, and VPD was low. We observed a linear relationship between $\Delta$ and $p_i/p_a$ in June, but found a strong curvilinear relationship in July and August. Future studies might be strengthened by testing this relationship in other species over a wide range of $p_i/p_a$ and environmental conditions. Our findings support the inclusion of $g_i$ and decarboxylation activity to attain the most accurate and precise predictions of $\Delta$ from leaf models, and evolving technologies, such as TDL, make these improvements more easily achievable. Lastly, the magnitude of diurnal variation in $g_i$ of other $C_3$ species needs to be quantified, as do the environmental and physiological drivers of this variation, so that $g_i$ can be more accurately parameterized in future ecosystem process models.

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