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Partitioning net ecosystem carbon exchange and the carbon isotopic disequilibrium in a subalpine forest

JOHN M. ZOBITZ*, SEAN P. BURNS†, MARKUS REICHSTEIN‡ and DAVID R. BOWLING§

**Department of Mathematics, University of Utah, 155 S 1400 E, Salt Lake City, UT 84112, USA, †Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309-0334, USA, ‡Biogeochemical Model-Data Integration Group, Max Planck Institute for Biogeochemistry, Hans Knöll Street 10, 07745 Jena, Germany, §Department of Biology, University of Utah, 257 S 1400 E, Salt Lake City, UT 84112, USA*

Abstract

We investigate the utility of an improved isotopic method to partition the net ecosystem exchange of CO_2 (F) into net photosynthesis (F_A) and nonfoliar respiration (F_R). Measurements of F and the carbon isotopic content in air at a high-elevation coniferous forest (the Niwot Ridge AmeriFlux site) were used to partition F into F_A and F_R. Isotopically partitioned fluxes were then compared with an independent flux partitioning method that estimated gross photosynthesis (GEE) and total ecosystem respiration (TER) based on statistical regressions of night-time F and air temperature. We compared the estimates of F_A and F_R with expected canopy physiological relationships with light (photosynthetically active radiation) and air temperature. Estimates of F_A and GEE were dependent on light as expected, and TER, but not F_R, exhibited the expected dependence on temperature. Estimates of the isotopic disequilibrium D, or the difference between the isotopic signatures of net photosynthesis (δ_A, mean value −24.6‰) and ecosystem respiration (δ_R, mean value −25.1‰) were generally positive ($\delta_A > \delta_R$). The sign of D observed here is inconsistent with many other studies. The key parameters of the improved isotopic flux partitioning method presented here are ecosystem scale mesophyll conductance (g_m) and maximal vegetative stomatal conductance (g_{cmax}). The sensitivity analyses of F_A, F_R, and D to g_{cmax} indicated a critical value of g_{cmax} ($0.15\,mol\,m^{-2}\,s^{-1}$) above which estimates of F_A and F_R became larger in magnitude relative to GEE and TER. The value of D decreased with increasing values of g_m and g_{cmax}, but was still positive across all values of g_m and g_{cmax}. We conclude that the characterization of canopy-scale mesophyll and stomatal conductances are important for further progress with the isotope partitioning method, and to confirm our anomalous isotopic disequilibrium findings.

Keywords: carbon isotope discrimination, flux partitioning, mesophyll conductance, respiration, whole-ecosystem photosynthesis

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Introduction

Terrestrial ecosystems worldwide are currently sequestering 1–2 $Gt\,C\,yr^{-1}$ (Prentice *et al.*, 2001). The strength of the global terrestrial carbon sink is ultimately an integration of processes regulating the assimilation of carbon dioxide (CO_2) into sugars and the production of CO_2 through respiration by all organisms. Understanding how leaf-level processes and parameters affect the estimates of the net CO_2 exchange at large spatial scales will help to constrain uncertainties on the estimates of the global carbon sink (Schimel *et al.*, 2001).

Measurements of the $^{13}C/^{12}C$ ratio in atmospheric CO_2 can be used to infer the terrestrial carbon sink strength (Lloyd & Farquhar, 1994; Ciais *et al.*, 1995b; Fung *et al.*, 1997; Randerson *et al.*, 2002; Scholze *et al.*, 2003). Through the process of photosynthesis, plants discriminate against ^{13}C and leave the atmosphere more ^{13}C-enriched. At the leaf level, the measure of

Correspondence: Present address: J. M. Zobitz, Department of Mathematics, Augsburg College, 2211 Riverside Avenue, Minneapolis, MN 55454, USA, fax +1 612 330 1393, e-mail: zobitz@augsburg.edu

the deviation between the $^{13}C/^{12}C$ ratio of the plant compared to the atmosphere is defined as photosynthetic discrimination, represented in general by Δ (Farquhar *et al.*, 1989) (a complete list of symbols is given in Table 1). At the ecosystem scale, the analogous discrimination is denoted by Δ_{canopy} (Lloyd *et al.*, 1996; Bowling *et al.*, 2001, 2003c) and represents the flux-weighted average discrimination by the entire vegetation canopy. Randerson *et al.* (2002) showed that a 0.2‰ change in the global scale analogy of Δ_{canopy} changed the magnitude of the inferred terrestrial carbon sink by 25% (0.5 Pg C yr^{-1}), provided the change was correlated with a change in gross photosynthesis (GEE). Hence, reduction in uncertainty of Δ at a variety of spatial scales would be very helpful in reducing the uncertainty on the magnitude of the terrestrial carbon sink.

Uncertainties of global patterns of GEE, total ecosystem respiration (TER), and Δ can be constrained with records of these parameters at the ecosystem scale. The net CO_2 exchange between terrestrial ecosystems and the atmosphere (NEE, noted F here for convenience) is the sum of GEE and TER: $F = \mathrm{GEE} + \mathrm{TER}$. F is observed from flux towers at locations around the globe (Baldocchi *et al.*, 2001; Ciais *et al.*, 2005) using the eddy covariance technique (Wofsy *et al.*, 1993). Sign conventions in the micrometerological literature (and here) typically define all nonradiative fluxes as positive when directed to the atmosphere, so the GEE flux is negative.

Separating daytime F into its respective fluxes of carbon uptake and respiration is called *flux partitioning*. (Partitioning is not required at night in most ecosystems because there generally is no CO_2 fixation and $F = \mathrm{TER}$). A variety of flux partitioning techniques have been used to separate F into GEE and TER. These include statistical parameter estimation using F and climatic variables (e.g. temperature, light, moisture) as covariates (Huxman *et al.*, 2003; Yi *et al.*, 2004; Reichstein *et al.*, 2005a; Stoy *et al.*, 2006); estimating components of F with process-based biophysical models (Aber *et al.*, 1996; Baldocchi, 1997; Ogée *et al.*, 2003a; Braswell *et al.*, 2005; Sacks *et al.*, 2006, 2007); and scaling leaf, stem, and soil chamber measurements to the ecosystem (Lavigne *et al.*, 1997; Law *et al.*, 1999).

Another flux partitioning approach utilizes stable carbon isotopes (Yakir & Wang, 1996; Bowling *et al.*, 2001; Lai *et al.*, 2003, 2004; Ogée *et al.*, 2003a, b; Griffis *et al.*, 2005; Knohl & Buchmann, 2005; Zhang *et al.*, 2006). The uptake and release fluxes obtained from the isotope method differ from the other methods in the attribution of foliar respiration. Because of the formulation of equations used for stable isotope partitioning [see 'Isotope flux partitioning', also Lloyd *et al.* (1996) and Bowling *et al.* (2003c) for the derivation], this method provides estimates of the net photosynthetic flux

Table 1 List of symbols used in text

Symbol	Units	Explanation
ρ_a	mol air m^{-3}	Molar air density
z_h	m	Reference height (21.5 m)
w'	m s^{-1}	Turbulent component of vertical wind velocity
C'_a	μmol m^{-3}	Turbulent component of CO_2 molar density
c'_a	ppm	Turbulent component of [CO_2] mixing ratio
C_a	μmol m^{-3}	CO_2 molar density
F	μmol m^{-2} s^{-1}	Net ecosystem exchange of CO_2, see Eqn (1)
F_{eddy}	μmol m^{-2} s^{-1}	Turbulent eddy CO_2 flux, defined by $\overline{w'C'_a}$
$F_{storage}$	μmol m^{-2} s^{-1}	Storage CO_2 flux, see Eqn (1)
GEE	μmol m^{-2} s^{-1}	Gross primary production flux
TER	μmol m^{-2} s^{-1}	Total ecosystem respiration flux
F_A	μmol m^{-2} s^{-1}	Net photosynthetic flux (GEE – leaf respiration)
F_R	μmol m^{-2} s^{-1}	Nonfoliar respiration flux
F_L	μmol m^{-2} s^{-1}	Foliar respiration flux
Δ	‰	Leaf-level photosynthetic discrimination of $^{13}CO_2$
Δ_{canopy}	‰	Whole canopy ecosystem-scale photosynthetic discrimination of $^{13}CO_2$ [see Eqn (9)]
$c_a(z)$	ppm	[CO_2] mixing ratio at height z
c_c	ppm	Chloroplast [CO_2] mixing ratio
$\delta_a(z)$	‰	Isotope ratio at height z
δ_A	‰	Isotopic signature of net photosynthesis flux
δ_R	‰	Isotopic signature of total ecosystem respiration flux
$\boldsymbol{D}$	‰	Isotopic disequilibrium between net photosynthesis and respiration, defined as $\boldsymbol{D} = \delta_A - \delta_R$
$\bar{c}_a$	ppm	Average [CO_2] mixing ratio of 5, 7, 9, 11 m canopy air
$\bar{\delta}_a$	‰	Average isotope ratio of 5, 7, 9, 11 m canopy air
g_a	mol m^{-2} s^{-1}	Aerodynamic conductance to [CO_2]
g_s	mol m^{-2} s^{-1}	Vegetative surface conductance to [CO_2]
g_m	mol m^{-2} s^{-1}	Mesophyll conductance to [CO_2] (0.125 mol m^{-2} s^{-1})
g_c	mol m^{-2} s^{-1}	Bulk canopy conductance to [CO_2]
g_{cmax}	mol m^{-2} s^{-1}	Maximum canopy conductance to [CO_2], see Eqn (8)
$\bar{g}$	mol m^{-2} s^{-1}	Overall conductance to [CO_2], see Eqn (5)

Continued

Table 1. (*Contd.*)

Symbol	Units	Explanation
a_b	‰	Leaf boundary layer fractionation (2.9‰)
a	‰	Diffusion fractionation (4.4‰)
$a_s(T)$	‰	Water dissolution fractionation (1.1‰)
a_1	‰	Mesophyll diffusion fractionation (0.7‰)
$\bar{a}$	‰	Overall fractionation, see Eqn (10)
b_R	‰	Photosynthetic enzymatic fractionation of $^{13}CO_2$ (27.5‰)
$F_{\text{isostorage}}$	µmol m^{-2} s^{-1}‰	Isotopic storage flux, expressed in permil notation [see Eqn (4)]
δ_N	‰	Isotopic signature of daytime CO_2 flux, calculated as the intercept of a daytime Keeling plot.
$F_{\text{eddy-isoflux}}$	µmol m^{-2} s^{-1}‰	Eddy isoflux, expressed in permil notation, equal to $\delta_N F_{\text{eddy}}$
F_δ	µmol m^{-2} s^{-1}‰	Net ecosystem exchange of $^{13}CO_2$, expressed in permil notation [see Eqn (2)]
R_{ref}	µmol m^{-2} s^{-1}	Temperature-independent respiration rate [see Eqn (15)]
E_0	°C	Activation energy [see Eqn (15)]
T	°C	Air temperature
T_{ref}	°C	Reference temperature for Eqn (2) (10 °C)
T_0	°C	Base temperature in Eqn (2) (−46.02 °C)
Q_P	µmol m^{-2} s^{-1}	Photosynthetically active radiation
α	µmol m^{-2} s^{-1}	Half-saturation constant in Eqn (8)
c_Q	Unitless	Photosynthetically active radiation attenuation coefficient (0.6)
c_R	Unitless	Radiation attenuation coefficient (0.6)
R_n	W m^{-2}	Net radiation
$R_{n,c}$	W m^{-2}	Net radiation available to canopy
$R_{n,s}$	W m^{-2}	Net radiation available to soil
LE	W m^{-2}	Latent heat flux
LE_c	W m^{-2}	Canopy latent heat flux
LE_s	W m^{-2}	Soil latent heat flux
g'_a	mol m^{-2} s^{-1}	Aerodynamic conductance for water vapor
g''_a	mol m^{-2} s^{-1}	Aerodynamic conductance for heat
c_p	J kg^{-1} J^{-1}	Specific heat capacity of air (1012 J kg^{-1} J^{-1})
VPD	kPa	Vapor pressure deficit
LAI	m^2 leaf area m^{-2} ground area	Leaf area index of the forest (4.2)
γ	kPa K^{-1}	Psychrometric constant (0.0661 kPa K^{-1})

Continued

(F_A = GEE + F_L, where GEE < 0 and F_L represents foliar respiration), and nonfoliar respiration (F_R = TER−F_L).

There are advantages and disadvantages in all flux partitioning methods. These have been reviewed by Reichstein *et al.* (2005a) and Stoy *et al.* (2006) for the nonisotopic methods. An advantage to isotope flux partitioning is that in addition to estimates of net photosynthetic and respiratory fluxes, Δ_{canopy} is directly estimated from the routine, which can provide additional information about ecosystem physiology and the relations between carbon and water vapor fluxes. A fundamental requirement of isotope flux partitioning is that the isotopic signature (δ_R) of the nonfoliar respiration flux must be distinct from the isotopic signature (δ_A) of the net photosynthesis flux, otherwise there is no unique information contained in $^{13}CO_2$ fluxes (Bowling *et al.*, 2001). The parameter δ_A is typically approximated as the difference between the isotope ratio of canopy air ($\bar{\delta}_a$) and Δ_{canopy} (Lloyd *et al.*, 1996; Bowling *et al.*, 2003c). The difference between δ_A and δ_R is termed as the isotopic disequilibrium, $\boldsymbol{D} = \delta_A - \delta_R$.

Global scale estimates of GEE, TER, Δ_{canopy}, and $\boldsymbol{D}$ will ultimately reflect processes occurring at smaller spatial scales, but there can be a mismatch between global and local determinations of these quantities. Estimates of $\boldsymbol{D}$ from carbon cycle modeling studies at ecosystem, regional, or global scales have generally found $\boldsymbol{D}$ to be negative [i.e. δ_A more depleted than δ_R (Ciais *et al.*, 1995a; Fung *et al.*, 1997; Randerson *et al.*, 2002; Baldocchi & Bowling, 2003; Scholze *et al.*, 2003; Suits *et al.*, 2005)]. In contrast, observational and modeling studies at the ecosystem scale have frequently produced estimates of $\boldsymbol{D} > 0$ (Bowling *et al.*, 2001, 2003c; Ogée *et al.*, 2003b; Knohl & Buchmann, 2005; Aranibar *et al.*, 2006; Zhang *et al.*, 2006; Zobitz *et al.*, 2007). Differences in $\boldsymbol{D}$ from different studies, if correct, have major implications for our understanding of the terrestrial carbon sink, and thus need to be resolved. Positive $\boldsymbol{D}$ is inconsistent in sign with the widely accepted concept of isotope disequilibrium between terrestrial photosynthesis and respiration (Yakir, 2004). The $\delta^{13}C$ of atmospheric CO_2 has become more negative over the last two centuries due to the ^{13}C Suess effect (Francey *et al.*, 1999). Because a large component of the respiratory flux consists of carbon that resides in the biosphere for many decades (Trumbore, 2000), δ^{13}C of the photosynthetic flux is expected to be more negative than the respiratory flux (e.g. $\boldsymbol{D}<0$) (Fung *et al.*, 1997; Yakir, 2004), given a fairly constant photosynthetic discrimination at a particular site through time. There are many factors influencing the isotope content of respiration (Bowling *et al.*, 2008), so this Suess-effect argument is perhaps oversimplified in the current literature and more research is needed. Other processes such as CO_2-dependent methanogenesis may also influence the value of $\boldsymbol{D}$ (Han *et al.*, 2007).

The value of δ_R in the isotope flux partitioning method is determined from measurements of [CO_2] and δ^{13}C and represents an integrated estimate of the isotopic ratio of all respiratory sources (foliar as well as nonfoliar). For isotope partitioning, this value is typically measured at night (when photosynthesis is inactive) and then applied and held constant the following day. This approach may not be appropriate for several reasons. (a) As described above, δ_R should actually be derived from measurements of the isotopic signature of the nonfoliar respiratory flux (F_R). In practice, δ_R measurements typically reflect the total nocturnal respiratory flux (TER). The possibility exists for a constant offset between δ_R of F_R and δ_R of TER, therefore, biasing the values of $\boldsymbol{D}$. (b) The δ_R of F_R and TER may exhibit different diurnal patterns. Hymus *et al.* (2005) and Prater *et al.* (2006) showed that foliar needle respiration exhibited diurnal variation; and because the soil respiration flux is dynamically related to recent carbon uptake [e.g. Hogberg *et al.* (2001) and Ekblad *et al.* (2005)] we would expect nonfoliar respiration to show diurnal variation as well. However, Betson *et al.* (2007) reported that there was no diurnal variation observed in the isotopic composition of soil respiration in a boreal forest. Diurnal variation in δ_R of F_R would require daytime determinations of δ_R rather than determining δ_R from night-time measurements. (c) A growing body of research supports the presence of postphotosynthetic fractionations in various plant and ecosystem processes [see review by Bowling *et al.* (2008)]. If these processes were better understood then the simple respiration formulation that we use in the isotope-partitioning equations could be better represented.

Scaling leaf-level quantities of g_m or Δ to the canopy incorrectly in the isotope-partitioning model may also bias $\boldsymbol{D}$ to be more positive. Isotope flux partitioning models include equations to describe the overall pathway of CO_2 from the atmosphere to the sites of carboxylation (Yakir & Wang, 1996; Bowling *et al.*, 2001; Lai *et al.*, 2003, 2004; Ogée *et al.*, 2003a, b; Griffis *et al.*, 2005; Knohl & Buchmann, 2005; Zhang *et al.*, 2006). In addition, Ogée *et al.* (2003b) and Knohl & Buchmann (2005) considered mesophyll effects on F_A by including a mesophyll conductance term (g_m) that describes photosynthetic drawdown between internal CO_2 concentration (c_i) and CO_2 at the sites of carboxylation (c_c). Consideration of mesophyll effects in isotope partitioning is probably important, because studies at the leaf level have shown that the photosynthetic drawdown between c_c and c_i is significant (Warren, 2006; Warren & Adams, 2006). Ecosystem models that ignore mesophyll effects could potentially bias the estimates of F, GEE, TER, and Δ_{canopy} and hence, $\boldsymbol{D}$. Given a leaf-level value of g_m, the appropriate ecosystem-scale analogue of mesophyll conductance to use in isotope flux partitioning must be determined.

This study made use of continuous records of F, [CO_2], and δ^{13}C at a high-elevation forest (the Niwot Ridge Ameriflux site) during late summer 2003. These data were used to generate isotope flux partitioning estimates of F_A and F_R, as well as statistical flux partitioning estimates of GEE and TER (Reichstein *et al.*, 2005a). Three objectives of this study are as follows.

1. Comparison of isotope partitioning estimates of F_A, F_R, and Δ_{canopy} with statistical flux partitioning estimates of GEE and TER, as well as with expected functional relationships (ecosystem-scale light and temperature response curves).
2. Derivation of $\boldsymbol{D}$ for its discussion in the context of studies measuring postphotosynthetic fractionation of carbon pools.
3. Examination of isotope partitioning model parameters through a sensitivity analysis of F_A, F_R, and $\boldsymbol{D}$ to these parameters.

Site description and measurements

This study was conducted at the Niwot Ridge AmeriFlux site (40°1′58″N, 105°32′46″W), a subalpine forest at 3050 m elevation west of Boulder, Colorado, USA. The forest stand is a century old, recovering from early 20th century logging, and contains subalpine fir (*Abies lasiocarpa*), Engelmann spruce (*Picea engelmannii*), and lodgepole pine (*Pinus contorta*). Canopy height is 11–12 m. Mean annual precipitation averages 800 mm and the mean annual temperature is 1.5 °C (Monson *et al.*, 2002). For this study, we focus on a time period over 3 summer months (4 July–15 October 2003). For additional information about the site and other studies conducted there see Bowling *et al.* (2005), Monson *et al.* (2002, 2005, 2006a, b), Sacks *et al.* (2006, 2007), Scott-Denton *et al.* (2003, 2006), and Turnipseed *et al.* (2003, 2004).

Average daytime temperatures during the study period were 12 °C, with minimum and maximum values ranging from −6 to 26 °C. Average vapor pressure deficit was 1 kPa but ranged from minimum and maximum values of 0–2.6 kPa. The study period was characterized by frequent late-afternoon rain events with small amounts of precipitation. The largest rain event (20 mm precipitation) during the study period occurred on August 30. The maximum period between rain events for the study period lasted 2 weeks from September 18 to October 1.

Atmospheric [CO$_2$] and δ^{13}C measurements

The CO$_2$ mixing ratios and the carbon isotope ratios (δ^{13}C) were measured by tunable diode laser absorption spectrometry (TDL) as described in detail by Bowling *et al.* (2005). Measurements at nine canopy heights (0.1, 0.5, 1, 2, 5, 7, 9, 11, and 21.5 m) were made every 6 min. For the isotope partitioning method we average midday measurements (11:00–12:00 hours local standard time) of CO$_2$ mixing ratio (denoted $\bar{c}_a$) and δ^{13}C (denoted $\bar{\delta}_a$) made in the canopy (5–11 m).

Flux and meteorological measurements

Net ecosystem exchange of CO$_2$ (F), sensible, and latent heat fluxes were measured via the eddy covariance technique. Details about the meteorological and flux measurements at Niwot Ridge can be found in Monson *et al.* (2002). Meteorological data used in this analysis included photosynthetically active radiation (Q_P), air temperature, net radiation, ground heat flux, and vapor pressure deficit of air (VPD).

Instrument failure and a requirement that there be no gap-filled or nonstationary data reduced the number of available measurements. Half-hourly periods were defined as stationary if the ratio of the 5 min covariance of vertical wind speed and temperature to the half-hourly covariance of vertical wind speed and temperature was between 0.75 and 1.25 (Foken & Wichura, 1996). There was a 20-day gap from days 240 to 260 where F was completely gap-filled and, therefore, excluded from the isotope flux partitioning. Conservation of CO$_2$ with the eddy covariance technique can be represented as follows:

$$\overline{w'C'_a} + \int_0^{z_h} \frac{\partial}{\partial t}(C_a(z))\mathrm{d}z = F_{\mathrm{eddy}} + F_{\mathrm{storage}} = F, \qquad (1)$$

where the term F_{eddy} ($\overline{w'C'_a}$) is the covariance between fluctuations in vertical wind velocity (w', in m s^{-1}) and CO$_2$ molar density (C'_a, in μmol m^{-3}) and the term F_{storage} represents time-dependent changes in CO$_2$ molar density through the canopy.

Theory

Isotope flux partitioning

The fundamental equations for isotopic flux partitioning were derived by Bowling *et al.* (2003c) and expanded by Ogée *et al.* (2003b) and Knohl & Buchmann (2005). The isotope partitioning approach for this study generates one daily value of F_A, F_R, and Δ_{canopy} derived from midday flux, meteorological, atmospheric [CO$_2$] and δ^{13}C measurements. Midday is defined to be 11:00–13:00 hours local standard time. Using mass balance of ^{13}CO$_2$, one can derive an equation that represents net ecosystem exchange of ^{13}CO$_2$ [Eqn (2)]. The isotopic signature of photosynthetic products, δ_A, is expressed as the difference between the average isotope ratio of canopy air (5–11 m heights) ($\bar{\delta}_a$) and whole canopy photosynthetic discrimination Δ_{canopy} (details of how Δ_{canopy} is calculated are provided here).

$$\begin{aligned}\delta_A F_A + \delta_R F_R &= (\bar{\delta}_a - \Delta_{\mathrm{canopy}})F_A + \delta_R F_R \\ &= F_{\mathrm{eddy-isoflux}} + F_{\mathrm{isostorage}} = F_\delta. \qquad (2)\end{aligned}$$

The isotopic content of total ecosystem respiration (δ_R) was derived from night-time [CO$_2$] and δ^{13}C measurements using a Keeling plot (Keeling, 1958). The parameter δ_R was calculated as an ordinary least squares (OLS, type I) intercept of an isotopic mixing line between 1/[CO$_2$] and δ^{13}C using 6-min data from all measurement heights (0.1–21.5 m) from 21:00 to 3:00 hours local standard time. This value was computed each night and applied the following day. OLS regression was used to avoid the inherent bias introduced by the geometric mean or other type II regressions (Zobitz *et al.*, 2006).

The eddy isoflux, $F_{\mathrm{eddy\text{-}isoflux}}$ cannot at present be measured directly via eddy covariance due to instrument limitations (Saleska *et al.*, 2006). For this study, we approximate $F_{\mathrm{eddy\text{-}isoflux}}$ by δ_N F_{eddy}, where δ_N is the intercept from a Keeling plot using all heights (0.1–21 m) during midday periods (11:00–13:00 hours). Six-minute measurement pairs of [CO$_2$] and δ^{13}C were used to calculate δ_N with OLS regression. $F_{\mathrm{isostorage}}$ was calculated first by averaging 6 min [CO$_2$] and δ^{13}C measurements for a given half hour and then numerically determining the following quantity:

$$F_{\mathrm{isostorage}} = \rho_a \int_0^{z_h} \frac{\partial}{\partial t}[\delta_a(z) \times c_a(z)]\mathrm{d}z, \qquad (3)$$

where $c_a(z)$ (in ppm) and $\delta_a(z)$ represent a half-hourly average of [CO$_2$] or δ^{13}C made at height z, respectively, and ρ_a is the air density (in mol m^{-3}).

Net photosynthesis F_A is related to the [CO$_2$] mixing ratio in the chloroplast (c_c) via Fick's law.

$$F_A = \bar{g}(c_c - \bar{c}_a), \qquad (4)$$

where the overall conductance ($\bar{g}$) is composed of an aerodynamic conductance (g_a), a vegetative surface conductance (g_s), and g_m (all conductance units in $\mathrm{mol\,m^{-2}\,s^{-1}}$):

$$\frac{1}{\bar{g}} = \frac{1}{g_a} + \frac{1}{g_s} + \frac{1}{g_m}. \tag{5}$$

The aerodynamic conductance depends on windspeed and other parameters and was calculated following Ogée *et al.* (2003b) [see their Eqn (11)] and Knohl & Buchmann (2005). There were no published values of mesophyll conductance for the conifer species in this study (Warren & Adams, 2006). The mesophyll conductance for the conifers in this study was fixed and assumed constant at $0.125\,\mathrm{mol\,m^{-2}\,s^{-1}}$ (William K. Smith, personal communication). In 'Sensitivity of isotope flux partitioning to g_m, g_{cmax}, and c_A' we examine the sensitivity of our isotope partitioning results to g_m.

For this study, we determined surface conductance using a two-box model similar to Kelliher *et al.* (1995) that separates evaporative influences from the canopy and soil. The model assumes that the total latent evaporation (LE) from the forest is the sum of latent evaporation from the canopy ($\mathrm{LE_c}$) and soil ($\mathrm{LE_s}$). Similarly, the net radiation available to the forest (R_n) is split between the canopy ($R_{n,c}$) and the soil ($R_{n,s}$). A bulk canopy conductance for the forest can be given by the inversion of Penman–Monteith equation: (Grace *et al.*, 1995).

$$\frac{1}{1.6 g_c} = \frac{s(R_{n,c} - \mathrm{LE_c})/g''_a + \rho_a c_p \mathrm{VPD}}{\gamma \times \mathrm{LE_c}} - \frac{1}{g'_a}, \tag{6}$$

where s is the slope of the saturation vapor pressure curve ($\mathrm{kPa\,K^{-1}}$), $R_{n,c}$ the net radiation available to the canopy ($\mathrm{W\,m^{-2}}$), $\mathrm{LE_c}$ the vegetative latent heat flux ($\mathrm{W\,m^{-2}}$), c_p the specific heat capacity of air ($\mathrm{J\,kg^{-1}\,K^{-1}}$), VPD the vapor pressure deficit, and γ is the psychrometric constant ($\mathrm{kPa\,K^{-1}}$). The conductances g'_a and g''_a are the aerodynamic conductances for water vapor and heat, respectively [see Eqn (10) in Ogée *et al.* (2003b)].

In theory, a bulk vegetative surface conductance (g_s) for the entire forest can be determined with Eqn (6) by replacing $\mathrm{LE_c}$ with LE, and $R_{n,c}$ with R_n and g_c with g_s. This process is called Penman–Monteith inversion and has been used in previous isotope partitioning studies (Bowling *et al.*, 2001; Ogée *et al.*, 2003b; Knohl & Buchmann, 2005). Knohl & Buchmann (2005) noted that direct Penman–Monteith inversion following rain events overestimated g_s because measured values of the latent heat flux include contributions due to evaporation from soils and wet surfaces in the forest.

For this study, three key assumptions are made to determine g_s. First, soil evaporation is assumed to occur at the following equilibrium rate (Priestley & Taylor, 1972; Kelliher *et al.*, 1995):

$$LE_s = \frac{\gamma \times s}{\gamma \times s + 1} R_{n,s}. \tag{7}$$

Second, the net radiation incident on the soil surface ($R_{n,s}$) is assumed to equal $R_n e^{-c_R \mathrm{LAI}}$, where LAI is the leaf area density ($\mathrm{m^2}$ leaf area $\mathrm{m^{-2}}$ ground area) and c_R is an attenuation coefficient. Third, we assume that bulk canopy conductance, g_c, is a saturating function of photosynthetically active radiation Q_P ($\mathrm{\mu mol\,m^{-2}\,s^{-1}}$) (Jarvis, 1976):

$$g_c = \left(\frac{g_{cmax} Q_P}{\alpha + Q_P}\right)(1 - e^{-c_Q \mathrm{LAI}}), \tag{8}$$

where g_{cmax} is the maximal canopy conductance ($\mathrm{mol\,m^{-2}\,s^{-1}}$), c_Q the coefficient of attenuation for Q_P, and α is the half-saturation constant ($\mathrm{\mu mol\,m^{-2}\,s^{-1}}$) (Kelliher *et al.*, 1995). The term $1 - e^{-c_Q \mathrm{LAI}}$ represents the fraction of photosynthetically active radiation absorbed by the canopy. For simplicity, we assume that $c_R = c_Q$ (Kelliher *et al.*, 1995). LAI at Niwot Ridge is assumed to be $4.2\,\mathrm{m^2\,m^{-2}}$ (Monson *et al.*, 2002).

With these assumptions, $R_{n,c}$ equals $R_n - R_{n,s} = R_n (1 - e^{-c_Q \times \mathrm{LAI})}$. Measurements of R_n and Q_p determine $R_{n,c}$ and g_c [Eqn (8)]. Eqn (6) can then be solved to obtain values of $\mathrm{LE_c}$. Since $\mathrm{LE} = \mathrm{LE_c} + \mathrm{LE_s}$, with $\mathrm{LE_s}$ specified by Eqn (7), Penman–Monteith inversion can be done to estimate g_s. For this study we chose g_{cmax} to equal $0.15\,\mathrm{mol\,m^{-2}\,s^{-1}}$, α to equal $500\,\mathrm{\mu mol\,m^{-2}\,s^{-1}}$, c_Q, and c_r to equal 0.6. Sensitivity analysis for these parameters is conducted in 'Sensitivity of isotope flux partitioning to g_m, g_{cmax}, and c_A.'

The parameter Δ_{canopy} describes the total isotope fractionation when CO_2 (a) is transported through the leaf boundary layer, (b) diffuses into the stomatal cavity, (c) enters solution, (d) diffuses through the mesophyll to the sites of carboxylation, and (e) is reduced via photosynthesis. Eqns (9) and (10) are a simplified form of the model used for a more complete model of isotope fractionation that includes additional processes such as photorespiration (Vogel, 1980; Farquhar & Sharkey, 1982; Farquhar *et al.*, 1989; Farquhar & Lloyd, 1993):

$$\Delta_{canopy} = \bar{a} + (b_R - \bar{a}) \frac{c_c}{\bar{c}_a} \tag{9}$$

$$\bar{a} = \frac{g_s g_m a_b + g_a g_m a + [a_s(T) + a_1] g_s g_a}{g_s g_m + g_a g_m + g_s g_a}. \tag{10}$$

In Eqn (9), b_R is the photosynthetic enzymatic fractionation of $^{13}CO_2$ [constant at 27.5‰, Farquhar *et al.* (1982)]. By the model formulation of Farquhar *et al.* (1982), Δ_{canopy} is understood to be a net photosynthetic discrimination (photosynthesis less leaf respiration). Because of this necessary formulation, the appropriate

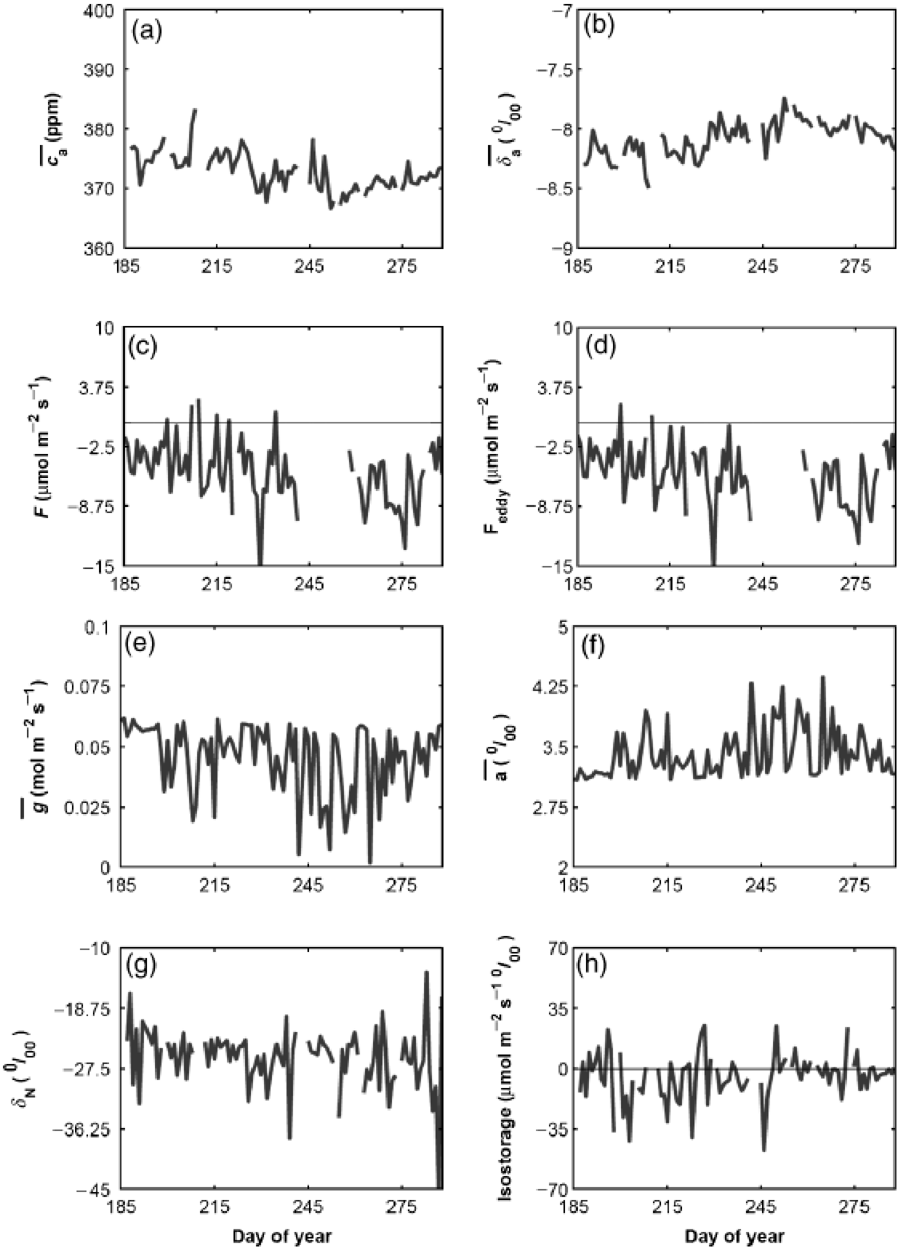

Fig. 1 Midday (11:00–13:00 hours local standard time) averages for each of the parameters used in isotope flux partitioning [see Eqns (11)–(14)]. Values of $\bar{c}_a$(panel a) and $\bar{\delta}_a$ (panel b) were found by averaging 6 min TDL data [see 'Atmospheric [CO$_2$] and δ^{13}C measurements']. Values of F (panel c) and F_{eddy} (panel d) were derived from eddy covariance measurements (see 'Flux and meteorological measurements'). Values of $\bar{g}$ (panel e) were derived from Eqn (5). Values of $\bar{a}$ (panel f) were derived from Eqn (10). Values of δ_N (panel g) were generated from a Keeling plot of CO$_2$ and δ^{13}C TDL measurements at all measurement heights. The isostorage (panel h) is numerically calculated from Eqn (3).

uptake flux for isotope flux partitioning must be F_A, not GEE (Lloyd *et al.*, 1996; Bowling *et al.*, 2003c).

Half-hourly measurements (F, F_{eddy}, $\bar{c}_a$, $\bar{\delta}_a$) or derived parameters (δ_N, $\bar{a}$, $\bar{g}$, and $F_{\text{isostorage}}$) between the hours of 11:00 and 13:00 local standard time were subsequently averaged into one representative value for the day. Time series of these measurements and derived parameters are shown in Fig. 1. From this averaging, all measurements (F, F_{eddy}, $\bar{c}_a$, $\bar{\delta}_a$) or derived parameters (δ_N, $\bar{a}$, $\bar{g}$, $F_{\text{isostorage}}$, and additionally δ_R) were on the same timescale.

Combination of the following equations generates an isotope flux partitioning estimate of F_A, F_R, Δ_{canopy}, and c_c:

$$F_A + F_R = F \tag{11}$$

$$(\bar{\delta}_a - \Delta_{canopy})F_A + \delta_R F_R = F_\delta \tag{12}$$

$$F_A = \bar{g}(c_c - \bar{c}_a) \tag{13}$$

$$\Delta_{canopy} = \bar{a} + (b_R - \bar{a})\frac{c_c}{\bar{c}_a}. \tag{14}$$

The Appendix in Bowling *et al.* (2001) shows how the combination of Eqns (11)–(14) can yield a solution for F_A by solving a quadratic equation for F_A. We present the derivation of the solution for F_A in the Appendix for two reasons: (a) errors were present in Eqn (A5) in Bowling *et al.* (2001) and (b) Bowling *et al.* (2001) claimed that the solution of this quadratic equation yielded a unique solution for F_A. As we demonstrate in the Appendix, under certain conditions the isotope-partitioning model can yield no unique, biologically realistic solutions for F_A.

Temperature-based statistical flux partitioning

Reviews of temperature-based partitioning methods were provided by Reichstein *et al.* (2005a) and Stoy *et al.* (2006). We briefly describe the method used here and refer to the method as 'statistical flux partitioning.' At night, GEE = 0, implying F = TER. Night-time regressions of F and air temperature (T) were calculated using an exponential regression model (Lloyd & Taylor, 1994):

$$\mathrm{TER} = R_{ref} \times e^{E_0\left(\frac{1}{T_{ref}-T_0}-\frac{1}{T-T_0}\right)}, \tag{15}$$

where $T_0 = -46.02°$, $T_{ref} = 10\,°C$, and R_{ref}, the temperature-independent respiration rate, and temperature sensitivity, E_0, were free parameters. The temperature sensitivity E_0 was estimated on a 15-day time scale, but then averaged and assumed to be constant in time. Then a temporally varying estimate of R_{ref} was generated; hence, linking variation in F between shorter and longer time scales. Once $R_{ref}(t)$ and E_0 are determined, half-hourly daytime TER was modeled with Eqn (11) from measured air temperature, and GEE was calculated as the difference between F and TER. The uncertainty in a TER estimate from using the expected values plus or minus the standard errors of $R_{ref}(t)$ and E_0 led to an intrinsic uncertainty in TER of 0.7 μmol m^{-2} s^{-1}. The half-hourly values of GEE and TER between the 11:00 and 13:00 hours local standard time were subsequently averaged into one representative value for the day.

Results

Estimates of F_A, F_R, Δ_{canopy}, c_c, GEE, and TER were generated using each of the partitioning routines described in 'Theory.' Time series of F, F_A, F_R, GEE, and TER are shown in Fig. 2.

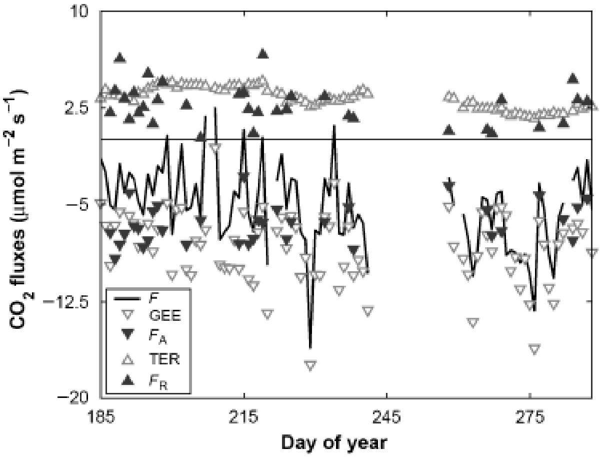

Fig. 2 Time series of isotope partitioned and statistical flux partitioned photosynthetic and respiratory fluxes. Values of F_A and F_R were generated from the solution of Eqns (11)–(14) (see 'Isotope flux partitioning'). Values of TER and GEE were produced from the statistical flux partitioning routine described in 'Temperature-based statistical flux partitioning.'

We compared the estimates of F_A and F_R and GEE and TER with expected physiological relationships, including light-response curves (F_A and GEE vs. photosynthetically active radiation, Q_P) and temperature-response curves (F_R and TER vs. air temperature). These results are shown in Fig. 3. To emphasize the underlying relationships, the fluxes are binned with respect to Q_P or air temperature (Greco & Baldocchi, 1996). In general, the sensitivity of F_A to Q_P was similar to the sensitivity of GEE to Q_P (Fig. 3a), whereas sensitivities of F_R to air temperature and TER and air temperature were not similar (Fig. 3b).

Shown in Fig. 4 are time series of Δ_{canopy}, (Fig. 4a), δ_R, F_A-weighted δ_A (Fig. 4b), and F_A-weighted isotopic disequilibrium $\boldsymbol{D}$ (Fig. 4c). Values of δ_A are generally more enriched than δ_R, which leads to a positive $\boldsymbol{D}$ ($= \delta_A - \delta_R$).

Figure 5 shows the results of the sensitivity analyses of F_A, F_R, and $\boldsymbol{D}$ to either g_m, g_{cmax}, or c_Q. In this analysis, the fluxes were isotopically partitioned using incremental values of these parameters. For example, Fig. 5a and b show mean midday estimates of F_R and F_A for a given value of g_m. Figure 5c shows mean midday values of $\boldsymbol{D}$ weighted by F_A for a given value of g_m. The solid line in Fig. 5a represents a midday mean value of daytime TER. The dashed line in Fig. 5b represents a midday mean value of daytime GEE. Because mesophyll conductance is not a parameter used to derive statistical flux partitioning estimates, there is no sensitivity of GEE and TER to g_m. A sensitivity analysis of F_A, F_R, and $\boldsymbol{D}$ to α [see Eqn (8)] showed no sensitivity (results not shown).

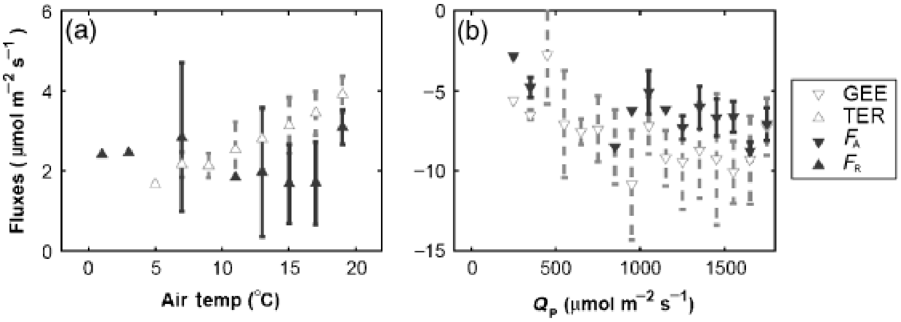

Fig. 3 Binned comparison of air temperature and F_R (or TER), or photosynthetically active radiation (Q_P) and F_A (or GEE) relationships for each of the partitioning routines. Periods with Q_P less than 100 μmol m^{-2} s^{-1} were excluded from the averaging. Q_P was binned in 100 μmol m^{-2} s^{-1} increments, and air temperature was binned in 2 °C increments.

Discussion

Comparison of flux partitioning estimates at different time scales

Estimates of F_A and F_R were more variable than statistical flux partitioned estimates of GEE and TER (Fig. 2). These differences may arise from a variety of factors related to measurement errors. First, the turbulent flux F_{eddy} may not be measured correctly in times of strong atmospheric stability (Goulden *et al.*, 1996). Recent work at Niwot Ridge has shown that both horizontal and vertical advection can be a significant flux at Niwot Ridge (Turnipseed *et al.*, 2003, 2004; Yi *et al.*, 2005). Studies of F via the eddy covariance technique (Baldocchi *et al.*, 1988) generally do not consider vertical advection. Second, the isostorage has been estimated with standard numerical integration techniques; however, Finnigan (2006) has shown that such techniques may underestimate storage (and by extension isostorage) as much as 50%. A detailed analysis into the calculation of isostorage is beyond the scope of this study, but the focus on midday time periods should minimize problems with storage fluxes. Bowling *et al.* (2003c) compared different measurement-based techniques to estimate F_δ and found convergent results, yet there still is nonnegligible uncertainty in the measurements of F_δ. Direct measurements of the $^{12}CO_2$ and $^{13}CO_2$ fluxes (Saleska *et al.*, 2006) are needed to reduce the uncertainty associated with F_δ.

The failure to produce expected F_R–air temperature relationships (Fig. 3) has been observed in previous isotopic partitioning studies (Bowling *et al.*, 2001; Knohl & Buchmann, 2005; Zobitz *et al.*, 2007). The isotope partitioning method makes no assumption of the temperature influence of respiration. Ecosystem respiration is expected to vary with temperature, but also with other environmental factors such as moisture or substrate availability (Giardina & Ryan, 2000; Reichstein *et al.*, 2002, 2003, 2005a, b; Davidson *et al.*, 2006). Studies at Niwot Ridge by Scott-Denton *et al.* (2003, 2006) from soil chambers reported that soil respiration is sensitive to these factors. Hence, the inability in this study to derive robust relationships of F_R with temperature may be the result of the assumption that the diurnal cycle of respiration is only sensitive to temperature. If this is true, a simple temperature dependence for diurnal respiration as in the statistical flux partitioning method may not be applicable for this site. Additional reasons for this failure to reproduce F_R–air temperature relationships include poor correlations between air and soil temperature and possibly limitations of the isotope partitioning method for reasons described in 'Comparison of Δ_{canopy} and D with other studies.'

*Comparison of Δ_{canopy} and **D** with other studies*

Estimates of $\boldsymbol{D}$ from modeling and measurement-based studies at different spatial scales sometimes conflict in their sign and magnitude. Estimates of Δ_{canopy} from carbon cycle modeling studies at ecosystem, regional, or global scales generally have found $\Delta_{\text{canopy}} = 17$–19‰ (Lloyd & Farquhar, 1994; Ciais *et al.*, 1995b; Fung *et al.*, 1997; Randerson *et al.*, 2002; Baldocchi & Bowling, 2003; Miller *et al.*, 2003; Scholze *et al.*, 2003; Suits *et al.*, 2005), indicating that $\delta_A \approx \bar{\delta}_a - \Delta_{\text{canopy}} \approx -8-18‰ \approx -25‰$ to $-27‰$, or that $\delta_A < \delta_R$ (mean value of $\delta_R \approx -25.1‰$ implies for this study $\boldsymbol{D} < 0$). Negative values of $\boldsymbol{D}$ are consistent with expectations of isotope disequilibrium between terrestrial photosynthesis and respiration based on the long-term change in $\delta^{13}C$ of atmospheric CO_2 associated with the ^{13}C Suess effect. Other processes in addition to the ^{13}C Suess effect lead to positive $\boldsymbol{D}$. A recent study by Han *et al.* (2007) showed that methane oxidation produced more enriched δ_R (2–5‰) relative leaf bulk isotopic composition in a Japanese rice paddy.

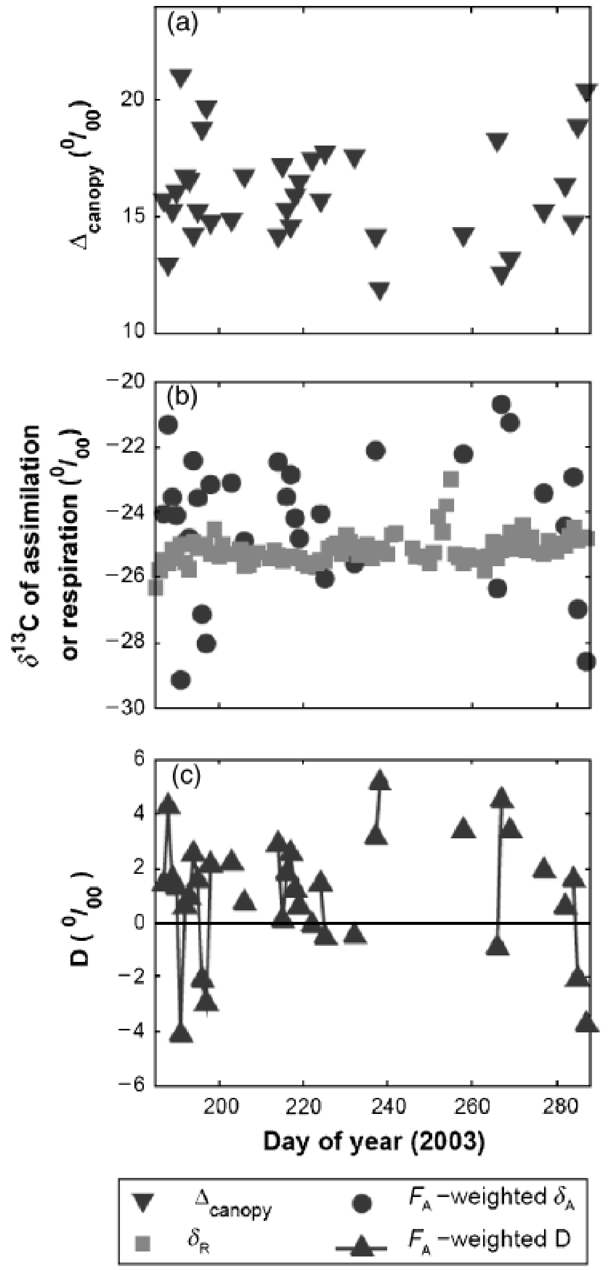

Fig. 4 (a) Time series of Δ_{canopy} from isotope flux partitioning. (b) Time series of F_A-weighted mean value of δ_A (circles) compared with δ_R from the previous night (squares). (c) Isotopic disequilibrium ($\boldsymbol{D} = \delta_A - \delta_R$) calculated from panel (b).

Isotope flux partitioning studies (Fig. 4) frequently generate short-term (hours to days) estimates of $\delta_A > \delta_R$ (Bowling *et al.*, 2001, 2003c; Ogée *et al.*, 2003b; Knohl & Buchmann, 2005; Zhang *et al.*, 2006). Using the biophysical model ISOLSM to simulate carbon fluxes of CO_2 and $^{13}CO_2$, Aranibar *et al.* (2006) found $\delta_A > \delta_R$. Furthermore, Scartazza *et al.* (2004) measured phloem sugars over the course of the growing season and consistently found these were more enriched than δ_R; however, no significant difference between these two was found in a similar study by Barbour *et al.* (2005). As phloem sugars are thought to be representative of recent photosynthate, this suggests that possibly $\delta_A > \delta_R$. The sign of $\boldsymbol{D}$ is potentially very important for carbon cycle studies, and the lack of agreement between the studies needs to be resolved.

There are three potential complications that may require reevaluation of the hypothesis that δ_A is consistently more negative than δ_R. The first complication is that there is a growing body of evidence that suggests there is apparent fractionation associated with respiration (Ghashghaie *et al.*, 2003; Bowling *et al.*, 2008). It is well established that the difference between the isotopic composition of air and plant matter reflects photosynthetic discrimination, Δ_{canopy} (Farquhar *et al.*, 1989). Bulk leaf organic matter is more depleted in ^{13}C than primary photosynthetic products such as sucrose or starch (Brugnoli & Farquhar, 2000; Scartazza *et al.*, 2004; Xu *et al.*, 2004; Barbour *et al.*, 2005; Hymus *et al.*, 2005; Prater *et al.*, 2006; Bowling *et al.*, 2008). Badeck *et al.* (2005) compiled data from published studies and showed a significant difference between sucrose and organic matter, with sucrose being more enriched than organic matter. A study by Hobbie & Werner (2004) found apparent fractionation of between leaf and phloem sucrose due to transport processes; however, studies by Gessler *et al.* (2004), Barbour *et al.* (2005), and Keitel *et al.* (2003) suggest that the differences may be due to variation in Q_P, air temperature, moisture, and stomatal conductance.

A second complication is the variation in respiratory substrates and respiratory fractionations of ^{13}C and may affect isotopic signatures of respiration, and hence, the value of $\boldsymbol{D}$. The value of δ_R represents the isotopic signature of the nonfoliar respiratory flux (F_R). In controlled studies, isotopic signatures of respiration have been shown to vary between different plant organs, with leaf respiration being more ^{13}C-enriched than possible substrates (Brugnoli & Farquhar, 2000; Ghashghaie *et al.*, 2003; Tcherkez *et al.*, 2003; Scartazza *et al.*, 2004; Xu *et al.*, 2004; Badeck *et al.*, 2005; Barbour *et al.*, 2005; Hymus *et al.*, 2005; Prater *et al.*, 2006), root respiration more ^{13}C-depleted than respiratory substrates, and whole-shoot respiration more enriched than respiratory substrates (Klumpp *et al.*, 2005; Schnyder & Lattanzi, 2005). Diel variation in leaf δ_R has also been found in the field (Hymus *et al.*, 2005; Knohl *et al.*, 2005; Prater *et al.*, 2006), with late afternoon values of leaf δ_R tending to becoming more enriched in ^{13}C. At Niwot Ridge, Bowling *et al.* (2005) found night-time canopy δ_R to be more enriched than below-canopy δ_R, and Schaeffer *et al.* (2008) showed that this pattern of enrichment is consistent in summer at the Niwot Ridge

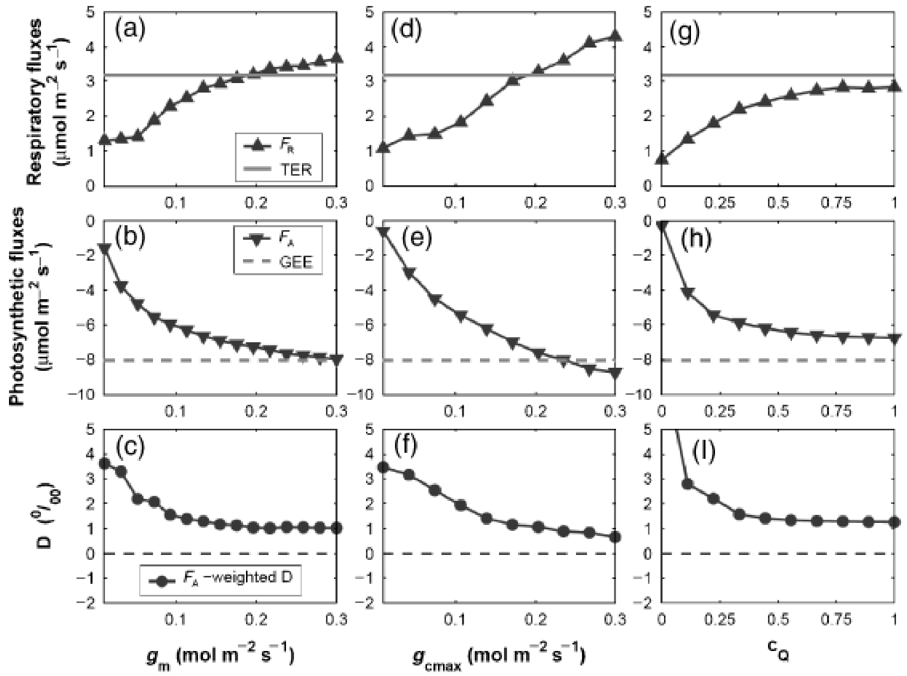

Fig. 5 Sensitivity of F_R, F_A, and D to mesophyll conductance [g_m, panels (a)–(c)], maximum canopy conductance [g_{cmax}, panels (d)–(f)], and attenuation coefficient [c_Q, panels (g)–(i)]. The same legend applies across each row. The isotope flux partitioning equations [Eqns (11)–(15)] were applied for each value of g_m, g_{cmax}, or c_Q. The mean midday values of TER (solid line) and GEE (dashed line) are shown for comparison. For panels (c), (f), and (i), the midday F_A-weighted mean value of D is shown. Note that the temperature-based partitioning method ('Temperature-based statistical flux partitioning') from which GEE and TER are calculated does not provide an estimate of D for comparison. Default values for the parameters were 0.125 mol m^{-2} s^{-1}, for g_m, 0.15 mol m^{-2} s^{-1} for g_{cmax}, and 0.6 for c_Q.

forest. Determining the apparent fractionation effects of leaf, root, shoot, and soil respiration is an active area of research; it is unknown how these different fractionation effects are manifested at the ecosystem scale. In practice, δ_R is derived from total night-time respiration. Correct determination of δ_R as the isotopic signature of nonfoliar respiration is considerably more complicated given these considerations (Bowling *et al*., 2008; Cai *et al*., 2008). By association, our understanding of D is weak at present.

The third complication is that D may vary on different spatial and time scales. Measurement-based studies and models of terrestrial discrimination estimate D ranging from 6-hourly to yearly values (Lloyd & Farquhar, 1994; Fung *et al*., 1997; Randerson *et al*., 2002; Miller *et al*., 2003; Suits *et al*., 2005). The present study estimates generate a single daily value of D. Patterns of carbon isotope disequilibrium could potentially be quite dynamic in time over a season. The present study found $D > 0$ during the late summer, but of course it is possible that the sign of D changes temporally throughout the season in response to moisture, light, temperature, plant phenology, or other factors. Monson *et al*. (2005) found the duration and timing of snow melt strongly influenced the length of summer net carbon uptake. The largest periods of carbon uptake at Niwot Ridge occur in the early growing season; late in the growing season, carbon uptake is reduced due to moisture limitations (Monson *et al*., 2002). The 2003 snow melt period lasted 28 days, beginning around day 130 (Monson *et al*., 2005). The present study period was generally warm with dry soils; measurements began after the strongest carbon uptake and wettest soils [see Fig. 7 in Bowling *et al*. (2005)]. Hence, D could change from positive to negative on seasonal time scales in relationship to periods of stronger net carbon uptake. Negative values of D early in the growing season may transition to positive late in the growing season in response to moisture limitations. Annual mean D could be consistent in sign and magnitude with estimates of other studies [e.g. Fung *et al*. (1997)] if this is true.

Isotopic linkages between different carbon pools should be addressed in future applications of the isotope partitioning method. Correlative isotopic linkages between photosynthesis and respiration have been well established in previous studies (Ekblad & Hogberg, 2001; Hogberg *et al*., 2001, 2008; Bowling *et al*., 2003a, b; Ekblad *et al.*, 2005; Knohl *et al.*, 2005), and models of the isotopic content of respiration are now beginning to take them into account (Aranibar *et al.*, 2006; Cai *et al.*, 2008). Describing these linkages in the isotope flux partitioning equations may potentially improve our ability to predict half-hourly fluxes of F_A and F_R and perhaps lead to more robust relationships of isotope flux-partitioned estimates of F_R with temperature.

Sensitivity of isotope flux partitioning to g_m, g_{cmax}, *and* c_A

The value of g_m used in this study was $0.125\,\mathrm{mol\,m^{-2}\,s^{-1}}$ (William K. Smith, personal communication). There are relatively few published studies on g_m for a given species, much less diurnal measurements of g_m (Loreto *et al.*, 1992; Warren *et al.*, 2003; Manter & Kerrigan, 2004; Singsaas *et al.*, 2004; Grassi & Magnani, 2005; Tissue *et al.*, 2005). Furthermore, for use in partitioning of net ecosystem exchange, leaf-level estimates of g_m need to be scaled to the canopy as done in Ogée *et al.* (2003b) and Knohl *et al.* (2005).

The value of g_m directly influences $\overline{g}$ [Eqn (5)], which in turn influences the estimates of F_R and F_A [Eqns (11)–(14)]. TER and GEE estimates (solid and dashed lines in Fig. 5a and b) provide a baseline to determine values of ecosystem-level estimates of g_m that would produce unreasonable estimates F_R and F_A relative to TER and GEE. The magnitude of F_A should be smaller than GEE, as F_A is GEE less foliar respiration. Similarly, F_R should be smaller in magnitude than TER because the daytime foliar respiration flux is in principle included in F_A. Mesophyll conductance is not a parameter used to derive statistical flux partitioning estimates; hence, there is no sensitivity of GEE and TER to g_m. Over the range considered, there were no values of g_m that produced unrealistic estimates relative to the mean values of TER and GEE.

Determinations of $\boldsymbol{D}$ by previous studies vary in their consideration of mesophyll effects, which may account for their differences. Values of $\boldsymbol{D}$ decreased in our study with increasing values of g_m (Fig. 5c). Note, the sign of $\boldsymbol{D}$ was still positive over the range of all tested values of g_m. Fung *et al.* (1997) did not consider mesophyll effects in the model-based formulation of Δ_{canopy}. Similar studies by Miller *et al.* (2003) and Suits *et al.* (2005) found more enriched values of $\delta_A = (\overline{\delta}_a - \Delta_{\mathrm{canopy}})$ in northern latitudes than Fung *et al.* (1997). Suits *et al.* (2005) included mesophyll effects in their model whereas Fung *et al.* (1997) did not; we hypothesize that these model differences led to more enriched δ_A (decreased Δ_{canopy}) in Suits *et al.* (2005). Baldocchi & Bowling (2003) did not include mesophyll fractionation effects in the model CANISOTOPE applied to a deciduous forest, which may account for the higher values of Δ_{canopy} than those found with isotope partitioning by Bowling *et al.* (2001). Because mesophyll conductance directly influences Δ_{canopy} [Eqns (9) and (10)], and not the calculation of δ_R, we can infer from Fig. 5c that increasing values of g_m increase Δ_{canopy} and subsequently make δ_A more depleted (decreasing the value of $\boldsymbol{D}$), assuming that g_c and g_m do not covary.

The sensitivity analysis of F_A, F_R, Δ_{canopy} to g_{cmax} suggests values of g_{cmax} greatly above $0.15\,\mathrm{mol\,air\,m^{-2}\,s^{-1}}$ generate biologically higher values of F_R and F_A relative to TER and GEE (Fig. 5d and e). Kelliher *et al.* (1995) reported maximal stomatal conductances in coniferous forests to range from $5.7 \pm 2.4\,\mathrm{mm\,s^{-1}}$ ($0.24 \pm 0.10\,\mathrm{mol\,m^{-2}\,s^{-1}}$). The g_{cmax} value of $0.15\,\mathrm{mol\,air\,m^{-2}\,s^{-1}}$ is at the lower end of this range; however, a value of $0.15\,\mathrm{mol\,air\,m^{-2}\,s^{-1}}$ is reasonable based on previously published studies in coniferous forests (Ogée *et al.*, 2003b) or by direct experimentation (Loreto *et al.*, 1992). The sensitivity analyses suggest that the values of F_A, F_R, and Δ_{canopy} are more sensitive to the values of g_{cmax} than to g_m (Fig. 5d–f). While F_A, F_R, and Δ_{canopy} were strongly sensitive to lower values of the canopy attenuation coefficient c_Q (Fig. 5g and h), F_A, F_R, and Δ_{canopy} were not as sensitive at typically expected values of c_Q [0.5–0.7, Kelliher *et al.* (1995)]. Values of $\boldsymbol{D}$ generally decreased for increasing values of g_{cmax} and c_Q, but did not change sign (Fig. 5f and i). The stomatal conductance model presented here is a simplified version of the one presented in Kelliher *et al.* (1995), which also considered the parallel sum of stomatal conductances of individual leaves through the entire canopy.

Conclusions

In this study, we evaluated an improved isotope flux partitioning method using measurements of F, $[\mathrm{CO_2}]$, and δ^{13}C over a 3-month period at a high-elevation coniferous forest. Comparison of the independent statistical flux partitioning and isotope flux partitioning methods showed agreement. Variation in measured and derived quantities strongly influences estimates of net photosynthesis (F_A) and nonfoliar respiration (F_R) in the isotope partitioning method. Additionally, this variability complicates correlative relationships with environmental variables and the partitioned fluxes (especially between F_R and air temperature).

During the late summer period of this study, we found a consistent positive isotopic disequilibrium ($\boldsymbol{D}$) ranging from 1‰ to 2‰. Positive values of $\boldsymbol{D}$ may reflect postphotosynthetic fractionation of ecosystem carbon pools or ecosystem-scale responses due to seasonal environmental variation and moisture stresses.

Future success of the isotope partitioning method requires further investigation of (a) the linkages between estimates of F_A, F_R, and $\boldsymbol{D}$ at a variety of spatial and temporal scales, (b) better characterization of mesophyll and stomatal conductances at the ecosystem scale, and (c) accurate determination of the isotopic signature of the components of ecosystem respiration, particularly their temporal and spatial variability.

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Supplementary material

The following material is available for this article online:
Appendix S1. Appendix: solution of flux equations.

This material is available as part of the online article from http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-2486.2008.01609.x