Revisiting the Partitioning of Net Ecosystem Exchange of CO₂ into Photosynthesis and
Respiration with Simultaneous Flux Measurements of ¹³CO₂ and CO₂, Soil Respiration and a
Biophysical Model, CANVEG

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Abstract

The partitioning of net ecosystem exchange of CO₂ (NEE) into photosynthesis and respiration can be challenging and is often associated with assumptions that yield unknown amounts of uncertainty, thereby hindering model development. This occurs because we are inferring two pieces of information from one equation and measurement, NEE. While there are multiple methods for partitioning NEE, each has unique limitations that are difficult to evaluate as these techniques are often not implemented simultaneously. Here we present an analysis of multiple partitioning methods (a non-linear regression model known as the Reichstein method, artificial neural networks (ANN), stable carbon isotopes, and soil respiration (R_{soil} measurements) under ideal field conditions. We measured ecosystem-scale fluxes of stable C isotopes via a new quantum cascade laser (QCL) spectrometer and paired those measurements with a biophysical model CANVEG in order to close a system of equations and solve for gross primary productivity (GPP) and ecosystem respiration (R_{eco}). Isotope-partitioned GPP and R_{eco} were on average 10-13% lower than Reichstein- and ANN-partitioned GPP and R_{eco} . These results suggest that the Reichstein and ANN approaches, which use nighttime NEE to infer daytime R_{eco} , may be overestimating R_{eco} and GPP during the day. This may be attributed to higher plant respiration rates at night compared to the day, otherwise known as the Kok effect. As isotope measurements and theory become more accessible to diverse ecosystems it will grow to be more than a benchmarking technique and into a valuable means of improving understanding of carbon cycling. Future studies are encouraged to evaluate these techniques in increasingly complex ecosystems to determine when significant differences appear between partitioning methods and how those differences influence modeling of terrestrial carbon budgets.

1. Introduction

With a growing number of eddy covariance towers worldwide, net ecosystem exchange measurements of CO₂ (NEE) are playing a critical role in the advancement of climate change science across multiple scales (Baldocchi, 2008, Baldocchi *et al.*, 2001). One of the struggles with using these data to inform models is that NEE is the net signal between offsetting fluxes associated with photosynthesis and respiration processes (Wohlfahrt & Gu, 2015). To interpret the controls on NEE we need to understand its partitioning with greater accuracy. This is especially important as the global budget for gross primary productivity (GPP) is highly uncertain with values ranging between 110-150 Pg-C yr⁻¹ (Beer *et al.*, 2010, Jung *et al.*, 2011). There are multiple measurement and modeling approaches that are used to partition NEE into respiration and photosynthesis-driven CO₂ exchange, each of which has its advantages and flaws (Desai *et al.*, 2008).

Perhaps the most widely used method for partitioning NEE is the Reichstein approach which uses the temperature sensitivity of nighttime NEE to predict R_{eco} during the day (Reichstein *et al.*, 2005). GPP is then calculated by subtracting R_{eco} from NEE. This non-linear regression modeling method is valuable because it is easy to implement, requiring only direct measurements of NEE during day and night and air or soil temperature. The Reichstein method and other non-linear regression modeling approaches have been shown to be robust across diverse sites with small amounts of bias (Desai *et al.*, 2008). While temperature is a strong driver of respiration, there are multiple factors that influence the plant and soil components of R_{eco} differentially, including water and substrate availability and disturbances such as re-wetting events and, in managed landscapes, irrigation and harvest events (Jenerette & Chatterjee, 2012, Tang *et al.*, 2005). The Reichstein approach accounts for these additional factors by assessing the

temperature sensitivity of R_{eco} over short time periods (~15 days), thereby adjusting this relationship to account for changes in substrate and water availability over time. This approach allows for an algorithm that is easy to apply across diverse ecosystems (Reichstein *et al.*, 2005). The Reichstein approach may lead to systematic biases in partitioning as nighttime measurements are used to inform daytime R_{eco} assuming that respiration processes are not inhibited in the light and that the temperature responses of R_{eco} behave similarly during the day and night. These two assumptions, however, are often violated. Nighttime leaf respiration is often higher than daytime leaf respiration (aka the Kok effect), leading to an overestimation of daytime R_{eco} and GPP (Amthor, 1995, reviewed by Heskell *et al.*, 2013, Kok, 1949, Wehr *et al.*, 2016, Wohlfahrt *et al.*, 2005, Wohlfahrt & Gu, 2015). The size of this error may also depend on the fraction of leaf to soil respiration which will vary with changes in leaf area index, below-ground root respiration and soil carbon pools (Giasson *et al.*, 2013). In both forest and agricultural settings, changing availability of substrate for below-ground respiration can make it difficult to predict daytime R_{soil} from nighttime R_{soil} rates (Oikawa *et al.*, 2014, Tang *et al.*, 2005, Vargas *et al.*, 2011) and sudden disturbances such as harvest events are difficult to capture using non-linear regression modeling approaches. In addition, if nighttime data are noisy or filtered due to low turbulence, it can become difficult to rigorously assess temperature sensitivity of R_{eco} using nighttime data. It is therefore recommended that non-linear regression modeling techniques be used in conjunction with ancillary data such as R_{soil} measurements in order to avoid systematic errors.

Another modeling approach for partitioning fluxes is gaining popularity: artificial neural networks (ANN) (Moffat *et al.*, 2007). This is a machine learning approach that uses multiple driving variables to predict daytime R_{eco} from nighttime measurements. ANNs employ non-

linear regression models to estimate the interconnections between independent and dependent variables, resulting in a network of relationships resembling neural pathways. The main benefit to this approach is that it can account for the interactions among multiple variables regulating R_{eco} such as re-wetting or harvesting events without presupposing functional relationships, thereby making it less susceptible to model structural error. One of the major flaws is that it is computationally expensive and therefore difficult to run over multiple sites across multiple years. In addition, it suffers from the same drawbacks as the Reichstein method in that it assumes that nighttime R_{eco} behaves similarly to daytime R_{eco} , potentially leading to systematic biases. Finally, ANNs work best when the all significant driving variables are measured, yielding a method which is more data intensive compared to the Reichstein method.

Independent and direct measurements of R_{soil} can be valuable for constraining the partitioning of net carbon fluxes. Unlike the modeling approaches described above, these data avoid systematic errors stemming from the reliance on nighttime data to predict daytime respiration. R_{soil} measurements can also capture fast respiration responses to disturbances such as rain and irrigation events (Baldocchi *et al.*, 2006, Oikawa *et al.*, 2014). Importantly, these measurements are less likely to have as many gaps as NEE data due to independence from turbulence conditions. However, these measurements are not often used to partition NEE as R_{soil} does not equal R_{eco} . R_{soil} measurements can be combined with measurements of above-ground respiration and/or modeling to partition NEE (Lavigne *et al.*, 1997, Schrier-Uijl *et al.*, 2010). However, in most cases R_{soil} is used to inform and benchmark other partitioning efforts such as the Reichstein approach (Reichstein *et al.*, 2005). In addition, R_{soil} measurement systems, even if replicated, represent a small fraction of the soil surface vs. eddy covariance data that integrate over a much larger spatial region. There are also errors associated with using chambers and soil

profiles to estimate soil surface CO₂ fluxes due to chambers physically disrupting the diffusion of gas from the soil to the atmosphere and profiles requiring a modeled or measured diffusion rate of CO₂ through soil.

In addition to the partitioning methods described above there are multiple emerging partitioning methods such as the flux variance similarity method (FVS) and the carbonyl sulfide (COS) method which have recently gained attention. The FVS approach uses high-frequency boundary layer CO₂ concentration measurements with mean leaf-scale water use efficiency (WUE) to partition NEE (Scanlon & Kustas, 2010) and has recently been tested at the decadal time scale in a forest ecosystem (Sulman *et al.*, 2016). The FVS approach requires measurements and/or modeling of leaf-level WUE and is sensitive to errors in estimates of WUE especially in complex canopies and during dry periods (Sulman *et al.*, 2016). The COS method exploits the fact that carbonyl sulfide is taken up during photosynthesis and not emitted in significant amounts during respiration processes (Blonquist *et al.*, 2011). The COS method is complicated by COS uptake by soils as well as the non-constant ratio of CO₂ to COS assimilation (Commane *et al.*, 2015). As these alternative partitioning approaches are further refined and tested in diverse ecosystems it will become clearer where and when they are most appropriately applied, thereby supporting future efforts to constrain C budgets.

Fluxes and concentration measurements of stable isotopes of carbon have much promise for partitioning NEE (Bowling *et al.*, 1999, Bowling *et al.*, 2001, Wehr *et al.*, 2016, Wehr & Saleska, 2015). This approach is appealing as it solves the closure problem that is associated with using NEE to solve one equation with two unknowns (GPP and R_{eco}). Simultaneous flux measurements of ¹³CO₂ and CO₂ can yield a second equation describing GPP and R_{eco}, which can produce estimates of R_{eco} from daytime measurements, resulting in fewer gaps, and

integrating over large spatial areas using the eddy covariance technique. Previously, the biggest drawback to the isotope partitioning method was low sampling frequency and high maintenance of instrumentation in the field associated with flask samples of air analyzed on mass spectrometers (Bowling *et al.*, 1999, Bowling *et al.*, 2001). Recently, the development of quantum cascade laser spectrometers has significantly lowered maintenance requirements while also enabling higher sampling frequencies (Wehr *et al.*, 2013), which is conducive for application in eddy covariance systems. The new spectrometers still have high power requirements, making deployment at remote locations and long durations not yet feasible. Another important issue with the isotope partitioning method is the modeling requirements that are needed to partition fluxes which often rely on multiple un-measured parameters drawn from the literature and theory that requires un-tested assumptions, potentially leading to high model parameter and structural error. However, recent advances in partitioning theory have helped to identify and minimize error. For example, error associated with the fractionation of carbon during photorespiration and leaf dark respiration has been shown to be small (Wehr & Saleska, 2015). Error associated with the assumption that the fractionation of carbon during respiration is similar during the day and night is also suggested to be small based on measurements of the isotopic composition of soil respiration during the day and night in ecosystems where ecosystem respiration is dominated by below-ground sources (Bowling *et al.*, 2015, Bowling *et al.*, 2001, Wehr & Saleska, 2015). Canopy conductance can be difficult to estimate as the most commonly used modeling technique, the Penman-Monteith model (Monteith, 1965, Penman, 1948), provides an estimate of surface conductance (soil and canopy), not canopy stomatal conductance alone (see detailed description of the Penman-Monteith model below). However other modeling methods employing water and heat flux measurements during periods when evaporation is small

can help reduce this type of error (Wehr *et al.*, 2016, Wehr & Saleska, 2015). Previous research has also indicated that uncertainty in estimates of fractionation by Rubisco, which has only been measured in a few plant species, and estimates of leaf boundary layer conductance contribute the most to uncertainty in isotope-partitioned fluxes (Wehr & Saleska, 2015). Finally, isotope studies often treat the canopy as a big leaf which can lead to errors in complex canopy conditions. Uncertainty in flux partitioning due to this assumption has not been previously evaluated, however seasonal trends in the divergence between isotope-partitioned and standard-partitioned fluxes were similar under diffuse and direct light conditions in a temperate forest suggesting that big leaf assumptions may not lead to biases in isotope partitioning (Wehr *et al.*, 2016).

By employing quantum cascade laser spectrometers and recent advances in partitioning theory the isotope partitioning technique has recently been shown to be able to detect the Kok effect at the canopy scale during periods when leaf respiration is high (Wehr *et al.*, 2016). These results were in contrast with standard partitioning approaches (e.g. the Reichstein approach), which estimated much higher R_{eco} and GPP during the first half of the growing season relative to isotope-partitioned fluxes. Wehr *et al.* (2016) were also able to detect the saturating response of GPP to absorbed photosynthetically active radiation (APAR) using isotope partitioning, a response that was not detected using standard partitioning approaches. Due to the fact that standard-partitioned GPP has errors resulting from ignoring the Kok effect, it was shown to be unable to describe functional responses of photosynthesis to absorbed light. These results are compelling and raise the question of whether or not differences between isotope-partitioned and standard-partitioned fluxes are significant in sites beyond a temperate forest.

Due to the diverse limitations among the flux partitioning methods that were surveyed above, there is great need to evaluate and compare them simultaneously. There is also need to rigorously evaluate and compare the uncertainty associated with each method. Here we employ multiple partitioning approaches (i.e. Reichstein, ANN, continuous R_{soil} measurements, and isotope partitioning) in an agricultural field under ideal meteorological conditions that minimized errors associated with low nighttime turbulence, complex canopies, and heterogeneous landscapes. We also assess the uncertainty in each method due to data and model uncertainty. Finally, we improve upon previous isotope partitioning methods by employing a biophysical model CANVEG to estimate parameters at the canopy scale.

2. Theoretical background

Using stable isotopes of carbon, we partition NEE into its two components, R_{eco} and GPP, by employing the following two equations:

$$NEE = R_{eco} + GPP \quad (1)$$

$$isoflux = \delta^{13}C_r * R_{eco} + \left(\frac{\delta^{13}C_a - \Delta}{1 + \Delta} \right) * GPP \quad (2)$$

where NEE is the net ecosystem exchange of CO_2 ($\mu mol\ m^{-2}\ s^{-1}$), R_{eco} is ecosystem respiration (including both heterotrophic and autotrophic respiration (roots, stems and leaves); $\mu mol\ m^{-2}\ s^{-1}$), GPP is gross primary productivity or apparent photosynthesis (carboxylation minus photorespiration; $\mu mol\ m^{-2}\ s^{-1}$), $isoflux$ is the product of NEE and the $\delta^{13}C$ of NEE ($\mu mol\ m^{-2}\ s^{-1}\ \text{‰}$), $\delta^{13}C_r$ is the isotopic composition of respired CO_2 (‰), $\delta^{13}C_a$ is the isotope ratio of atmospheric CO_2 (‰), and Δ is the fractionation factor associated with GPP (carboxylation and

photorespiration; ‰). $\delta^{13}C_a$ is directly measured and $\delta^{13}C_r$ is estimated using a Keeling plot (Keeling, 1958) with nighttime data that were filtered by vertical wind velocity (rotated vertical wind velocity $\geq 0.05 \text{ m s}^{-1}$ sustained for $\geq 5 \text{ s}$) and by range in CO_2 concentration ($> 30 \text{ ppm}$). Only upwelling air masses were used as they contain information about the fractionated carbon sources from R_{eco} , including heterotrophic respiration and autotrophic respiration (roots, stems and leaves). The $\delta^{13}C$ of upwelling air includes the respiration isotopic signature diluted by the atmospheric signature. It is therefore important to include nights when a sufficient range of CO_2 concentrations are observed, including both the pure atmospheric signal ($\sim 400 \text{ ppm}$) and air enriched with CO_2 from respiration ($> 400 \text{ ppm}$). Without sufficient ranges in CO_2 concentration, the intercept of the Keeling plot will be poorly constrained (Zobitz *et al.*, 2006). Sufficient ranges of CO_2 were only observed during the first 5 nights of the campaign (max range = 400–440 ppm). Geometric mean regression was used to determine the intercept with a 95% confidence interval (Figure 1). Our approach assumes that the isotopic ratio of respired CO_2 is similar during the day and night. As foliar respiration is expected to be higher at night than during the day, this assumption may be violated. However, previous research on alfalfa has demonstrated that the $\delta^{13}C$ of whole plant biomass and $\delta^{13}C$ of whole plant dark respiration (including roots, stems and leaves) measured both during day and night are not significantly different from each other (Klumpp *et al.*, 2005). Klumpp *et al.* (2005) repeated experiments across a diversity of environmental conditions and found similar results. We therefore feel that this assumption is acceptable at least in the case of alfalfa. In addition, Wehr and Saleska (2015) previously demonstrated that isotope partitioned fluxes were insensitive to errors associated with fractionation of carbon due to foliar respiration.

Figure 1. Keeling plot of nighttime data filtered for updrafts (rotated vertical wind speed ≥ 0.05 m s⁻¹ sustained ≥ 5 s) and CO₂ range (>30 ppm per night). The intercept (-30.7‰) is used to estimate the isotopic composition of respired CO₂ ($\delta^{13}C_r$). 95% confidence intervals are based on geometric mean regression. Data are binned (10 measurements per bin).

Fractionation during photosynthesis and photorespiration is estimated following Farquhar et al. (1982):

$$\Delta = a + (b_R - a) \frac{C_i}{C_a} + \frac{f\Gamma^*}{C_a} \quad (3)$$

where a is the fractionation from diffusion of CO₂ from the atmosphere to the sites of carboxylation within the canopy (‰), b_R is the fractionation from Rubisco (‰), C_i is the intercellular concentration of CO₂ integrated across the canopy (ppm), C_a is the concentration of CO₂ in the atmosphere (ppm), f is the fractionation due to photorespiration (‰), and Γ^* is the photocompensation point (ppm). C_a is directly measured and b_R and f are assumed to be constant at 27.5‰ and 11‰, respectively (Farquhar & Richards, 1984, Wehr & Saleska, 2015). Γ^* depends on temperature following (Brooks & Farquhar, 1985):

$$\Gamma_* = 42.7 + 1.68(T_L - 25) + 0.0012(T_L - 25)^2 \quad (4)$$

where T_L is leaf temperature (°C) and Γ_* (ppm) is estimated at 21% O₂. Air temperature was used as a surrogate for leaf temperature. This model is based on measurements in spinach and predicts a Γ_* value of 30 ppm at 18°C for alfalfa, which follows theoretical expectations as it is lower than a previously measured CO₂ compensation point (Γ) in alfalfa (Γ =49 ppm; (Platt *et al.*, 1977)).

a is estimated at the canopy scale, adapted from Ogée *et al.* (2003):

$$a = \frac{a_b G_s + a_d G_b + (a_s(T) + a_l) G_s G_b G_m^{-1}}{G_s + G_{a,v} + G_s G_b G_m^{-1}} \quad (5)$$

where a_b is the fractionation of CO₂ due to diffusion through the boundary layer to the stomata (2.9‰) (Farquhar, 1983), (Farquhar *et al.*, 1989), G_s is the integrated canopy stomatal conductance (see below for different methods of estimating G_s ; mol m⁻² s⁻¹), a_d is the fractionation of CO₂ due to diffusion in air through the stomatal pore (4.4‰), G_b is the canopy-integrated boundary layer conductance for CO₂ (m s⁻¹), $a_s(T)$ is the temperature dependent fractionation of CO₂ dissolved into water ($a_s(T) = -1.18 + 0.0041 \cdot T$) (Vogel *et al.*, 1970), a_l is the fractionation of CO₂ due to diffusion within the cell (0.7‰) (O'Leary, 1984), and G_m is the conductance of CO₂ within the mesophyll (assumed to be 0.35 mol m⁻² s⁻¹ based on previously measured G_m in a wide variety of grasses and herbs (Flexas *et al.*, 2012)). G_b is calculated following Wehr and Saleska (2015) which employs a modified version of the McNaughton and Hurk (1995) model for estimating R_{bH} , the boundary layer resistance to heat transfer:

$$R_{bH} = \frac{150}{LAI} \sqrt{\frac{L}{u_h}} \int_0^1 \exp \left[\frac{\alpha}{2} (1 - \zeta) \right] \phi(\zeta) d\zeta \quad (6)$$

where LAI is the leaf area index, L is the leaf dimension (0.02 m for alfalfa), u_h is the mean wind speed at the top of the canopy, ζ is height at each level of the canopy divided by the height at the top of the canopy, $\phi(\zeta)$ is the vertical profile of the heat sources normalized so that $\int \phi(\zeta) d\zeta = 1$, and α is the extinction coefficient (1.8 for the alfalfa field). We make the assumption that $\phi(\zeta)$ can be estimated using a normalized profile of light absorption previously measured in a clover crop (Lantinga *et al.*, 1999). u_h is calculated following:

$$u_h = \frac{u_a}{\exp\left[\alpha\left(\frac{a}{h}-1\right)\right]} \quad (7)$$

where u_a is wind speed measured at the tower, a is the flux measurement height, and h is the height of the canopy. Finally, G_b is derived from R_{bH} following:

$$R_b = N \left(\frac{Sc}{Pr}\right)^n R_{bH} \quad (8)$$

$$G_b = 1/R_b \quad (9)$$

where $N = 1$ for amphistomatous leaves, n is an empirical exponent accounting for the transfer through the boundary layer that is turbulent ($n = 2/3$), Sc is the Schmidt number for CO₂ (1.05) and Pr is the Prandtl number for air (0.71).

We also employ the following equations to inform estimates of C_i at the canopy scale (Bowling *et al.*, 2001):

$$C_i = \frac{GPP}{\bar{G}} + C_a \quad (10)$$

$$\bar{G} = \frac{G_s G_b}{G_s + G_b + G_s G_b G_m^{-1}} \quad (11)$$

where C_i is a function of canopy integrated GPP, the total conductance of CO₂ at the canopy scale ($\bar{G}$; mol m⁻² s⁻¹), and atmospheric CO₂ concentration (C_a).

To help inform equations 3, 5, 9 and 10, we estimate G_s and C_i at the canopy scale by employing the CANVEG model (Baldocchi & Harley, 1995, Baldocchi & Meyers, 1998). CANVEG is a one-dimensional multilayer gas exchange model that predicts H_2O , CO_2 and sensible heat flux densities within a canopy. This model combines micrometeorological and ecophysiological modules to estimate stomatal conductance in addition to photosynthesis, transpiration, leaf energy balance, and auto and heterotrophic respiration. Leaf photosynthesis is estimated from the balance between supply and demand for CO_2 regulated by diffusion of CO_2 into the leaf, intercellular CO_2 concentration, and rates of carboxylation, oxygenation and dark respiration. Stomatal conductance is a function of photosynthesis, relative humidity and CO_2 concentration at the leaf's surface (Collatz *et al.*, 1991). G_s and C_i are scaled to the canopy using a radiative transfer model to compute the functions on the sunlit and shaded portions of discrete layers of leaf area throughout the canopy. Radiative transfer was considered for the visible, near infrared and infrared bands of the electromagnetic spectrum across 30 canopy layers. Turbulent transfer was computed with a Lagrangian random walk model, following the algorithm of Thomson, and the dispersion matrices of Raupach. The set of equations are shown in Baldocchi *et al.* (1999). Key model parameters included leaf area index (1.65), maximum carboxylation velocity (V_{cmax} ; $170 \mu\text{mol m}^{-2} \text{s}^{-1}$), maximum rate of electron transport (J_{max} ; $278 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Erice *et al.*, 2007), and dark respiration, R_d ($0.22 \mu\text{mol m}^{-2} \text{s}^{-1}$).

It is common to invert the Penman-Monteith equation and solve for the bulk surface conductance (G_c) as an estimate of G_s (Bowling *et al.*, 2001, Ogée *et al.*, 2003). We therefore also used this method for comparison purposes and to explore error in partitioning that results from different approaches for estimating canopy stomatal conductance. The inverted Penman-Monteith equation follows:

$$G_c = \frac{\gamma * G_{aH} * \lambda E}{s(R_{net} - G) + \rho * C_p * D * G_{aH} - \lambda E(s + \gamma)} \quad (12)$$

where γ is the psychrometric constant (Pa Kelvin⁻¹), G_{aH} is the aerodynamic conductance for heat, λE is the total latent heat flux density (J m⁻² s⁻¹), s is the slope of saturation vapor pressure versus air temperature (Pa Kelvin⁻¹), R_{net} is net radiation (J m⁻² s⁻¹), G is soil heat flux (J m⁻² s⁻¹), ρ is the dry air density (kg m⁻³), C_p is the isobaric specific heat of air (J kg⁻¹ Kelvin⁻¹), and D is vapor pressure deficit (Pa). G_{aH} is calculated following:

$$G_{aH} = 1/R_{aH} \quad (13)$$

$$R_{aH} = R_{aM} + R_{bH} \quad (14)$$

$$R_{aM} = \frac{u}{u_*^2} \quad (15)$$

where R_{aH} is the aerodynamic resistance for heat (m s⁻¹), R_{aM} is the aerodynamic resistance for momentum transfer (m s⁻¹), u is wind speed (m s⁻¹), and u_* is friction velocity (m s⁻¹).

We approached stable carbon isotope partitioning in three ways. First, we employed CANVEG modeled G_s and C_i to calculate Δ , leaving a system of two equations (equations 1, 2) and two unknowns (R_{eco} , GPP), allowing us to solve for the partitioned fluxes R_{eco} and GPP . Next, we only employed CANVEG modeled G_s , resulting in a system of four equations (equations 1, 2, 3, 9) and four unknowns (R_{eco} , GPP , Δ , C_i). Finally, we employed the Penman-Monteith derived estimate of G_c as a surrogate for G_s , again resulting in a system of four equations (equations 1, 2, 3, 9) and four unknowns (R_{eco} , GPP , Δ , C_i). We refer to the partitioning strategies as CANVEG- G_s - C_i , CANVEG- G_s , and PM- G_c for the three approaches, respectively.

3. Materials and methods

3.1. Site description

All data were collected from an alfalfa field (*Medicago sativa*, L.) on Twitchell Island in the Sacramento-San Joaquin River Delta, California, USA (latitude: 38.1159N; longitude: 121.64666W; elevation: -9 m;). The climate is Mediterranean with hot, dry summers and cool, rainy winters. The site is very flat, has extensive fetch and is nearly ideal for eddy covariance flux measurements and flux partitioning (Baldocchi *et al.*, 2015). During the summer growing season, winds are vigorous both day and night as air flows through the Carinquez straight from the cool coastal San Francisco Bay to the hot dry Central Valley. This 32 ha field has been under alfalfa cultivation for over 7 years where alfalfa is grown year-round and is typically harvested 7 times per year. The soil is composed of a layer of oxidized peat (20-40 cm) above a deep peat horizon that extends up to 6 m and is classified as euic, thermic, Typic Medisaprist (Miller, 2011). Soil bulk density was 0.83 g cm⁻³ at 0-12 cm with 7.5% C and 0.53 % N at 10-40 cm depth.

3.2. Eddy covariance and meteorological measurements

We used meteorological and eddy covariance flux measurements from June 29 to July 13 2015, totaling 15 days. Alfalfa is typically harvested 7 times per year on a 45 day cycle and had been harvested 25 days prior to the start of the campaign. Measurements were collected on scaffolding located at the eastern edge of the field with prevalent wind from the west. Half-hourly meteorological variables included net radiation (W m⁻²; NR01, Hukseflux, Delft, Netherlands), photosynthetically active radiation (PAR; $\mu\text{mol m}^{-2} \text{s}^{-1}$; PAR-LITE; Kipp and

Zonen, Delft, Netherlands), air temperature above the canopy ($^{\circ}\text{C}$; aspirated and shielded temperature sensor HMP45C; Vaisala, Vantaa, Finland), soil heat flux (W m^{-2} ; HFP01, Hukseflux, Delft, Netherlands), and relative humidity (%; HMP60, Vaisala, Helsinki, Finland). Leaf area index (LAI) was estimated by destructive sampling along a transect running east-west through the center of the field. At 5 locations along the transect all leaves within a 0.16 m^2 area were harvested. Leaf area was measured using a leaf area meter (LI-3100, Li-COR Biosciences, NE, USA).

The eddy covariance method was used to measure ecosystem exchange of water, energy, CO_2 and $^{13}\text{CO}_2$ (Baldocchi *et al.*, 2015). We measure wind using a 3D sonic anemometer (Gill instruments, Hampshire, UK) and CO_2 and H_2O vapor concentration using an open path infrared gas analyzer (LI-7500, Li-COR Biosciences, NE, USA), both sampled at 20 Hz at a height of 2.8 m. CO_2 isotopes were measured using a closed path sensor (CCIA-48, Los Gatos Research, CA, USA) sampled at 1 Hz at a height of 2.8 m. The CCIA-48 employs a continuous wave mid-infrared quantum cascade laser (QCL) to measure absorption of all major stable isotopologues of CO_2 ($^{12}\text{CO}_2$, $^{13}\text{CO}_2$, $^{18}\text{O}^{12}\text{C}^{16}\text{O}$ and $^{17}\text{O}^{12}\text{C}^{16}\text{O}$) in a single scan. The instrument uses off-axis integrated cavity output spectroscopy (OA-ICOS), weighs 50 kg and consumes 350 W of power. Measurement range is 150-2500 ppm for CO_2 . The 1 s precision of the instrument for $\delta^{13}\text{C}$ and $^{13}\text{CO}_2$ is 0.7‰ and 2 ppb, respectively at a continuous flow sampling rate of 1 Hz. The 100 s precision for $\delta^{13}\text{C}$ is 0.07‰, which is a more relevant metric for performance in eddy covariance applications as covariances are averaged over long times scales (usually 30min) (Saleska *et al.*, 2006). For reference, a high precision Isotope-Ratio Mass Spectrometer (IRMS) typically achieves a precision of 0.01‰ for $\delta^{13}\text{C}$ and 0.02‰ for $\delta^{18}\text{OCO}$.

In the lab, the CCIA-48 with a Multiport Inlet Unit (MIU, Los Gatos Research, CA, USA) was tested in a temperature-controlled chamber to evaluate performance of the analyzer under simulated field conditions. The temperature profile of the chamber was programmed to resemble a diel cycle in the field. The MIU was connected to two cylinders of ambient air with different CO₂ concentrations (400.5 and 411.2 ppm). One cylinder was treated as the reference gas, the other was treated as the sample. The reference gas was measured for 10 min every 2 hrs. The Allan Deviation of the sample gas measurement along with precision specifications is shown in Figure A1.

For field deployment, a reference gas with known CO₂ isotopic composition (Scott-Marín) was measured for 5 min once every 4 hours automatically using a MIU to allow for post-campaign analysis. The CO₂ isotope composition of the reference gas was independently analyzed by an IRMS. An external pump pulled sample air through the optical cavity at a rate of approximately 0.5 L min⁻¹. Water vapor and particulate matter were removed from the sampling air via Nafion dryer (backed by Drierite) and in-line filters. The spectra were averaged down to 1 Hz to improve signal to noise ratio. We used nonlinear least-squares fits to determine mixing ratios of CO₂ isotopologues (Figure A2). The instrument operated at 40 Torr and was temperature-stabilized for optimal performance. The QCL operates at room temperature and the detector is thermo-electrically cooled.

All flux calculations include standard corrections and are quality controlled using in-house software as described in Knox et al. (2015). First, data are filtered for spikes and diagnostic instrument values. Coordinate rotation was used to align the x-axis of the sonic anemometer into the main wind direction so that the mean lateral and vertical velocities equaled zero. Fluxes were calculated using the 30 min covariance of vertical wind speed and scalar. The

Webb-Pearman-Leuning correction was applied to open-path CO₂ and H₂O measurements to account for fluctuations in air density (Webb *et al.*, 1980). Fluxes were filtered for low friction velocity (u_*), at a threshold of $u_* = 0.2 \text{ m s}^{-1}$ below which nighttime NEE varied with increasing u_* .

Eddy covariance measurements suffer from systematic biases resulting from the separation between the sonic and gas analyzer, tubing attenuation and residence time in the cell of closed path analyzers, digital filters, and spectral cut-off for under sampling (sampling frequency < 10 Hz). These biases can lead to high-frequency losses. High frequency losses for the open path LI-7500 were found to be less than 5% and therefore within the accuracy of the flux measurement (Knox *et al.*, 2015). In order to assess the spectral performance of the CCIA-48, frequency-normalized cospectra for the covariance between vertical wind and CO₂ and ¹³CO₂ measured by the CCIA-48 were compared with LI-7500 cospectra. Under-sampling of CO₂ exchange by the CCIA-48 was corrected using the LI-7500 CO₂ exchange measurements following Detto *et al.* (2011). A correction factor (CF) was calculated as the ratio of the CO₂ flux computed from the LI-7500 (i.e. the ‘true’ flux rate) to that measured with the closed path sensor. The CF had no relationship with wind speed, therefore we did not add an extra correction for higher wind speeds (Figure 2).

Figure 2. (a) Normalized cospectra of CO₂ recorded between DOY 180-194, 2015 above an alfalfa field using an open-path LI-7500 at 20 Hz sampling rate and a closed path CCIA-48 (LGR) at 1 Hz sampling rate. Cospectra are normalized so the area beneath the curve is equal to one. (b) Linear correction applied to LGR NEE measurements using NEE measured by the LI-7500. This relationship was strong ($r^2 = 0.95$) and did not vary with wind speed. The one-to-one line is shown as a reference (dotted grey line).

We calculated *isoflux* as:

$$isoflux = \overline{w'(\delta^{13}C * C)'} \quad (16)$$

$$\delta^{13}C = \left(\frac{\left(\frac{^{13}C}{^{12}C} \right)_{sample}}{\left(\frac{^{13}C}{^{12}C} \right)_{standard}} - 1 \right) * 1000 \quad (17)$$

where w' is the fluctuation in vertical wind speed, C is the molar density of CO_2 in air, $\delta^{13}C$ (‰) is the stable isotopic composition of CO_2 in air relative to a standard (Vienna PeeDee Belemnite), and $(\delta^{13}C * C)'$ is the fluctuation in the product of C and $\delta^{13}C$. *Isoflux* units are expressed in ‰ $\mu mol\ m^{-2}\ s^{-1}$. All flux processing was performed in Matlab (Mathworks, MA, USA).

3.3. Partitioning NEE using ANNs and the Reichstein method

Open-path CO_2 fluxes were partitioned and gap-filled using artificial neural networks (ANN) (Baldocchi *et al.*, 2015, Dengel *et al.*, 2013, Knox *et al.*, 2015, Moffat *et al.*, 2007, Papale *et al.*, 2006). Neural network predictions from nighttime gap-filling were used to model R_{eco} during the day. Explanatory variables used to gap-fill daytime NEE were air temperature, PAR, vapor pressure deficit (VPD), soil moisture at 10 cm depth, friction velocity, cumulative days between harvest events, and sine and cosine functions to represent seasonal changes. Similar explanatory variables were used to gap-fill nighttime NEE except did not include PAR or VPD. The ANN was optimized for representativeness and generalizability. Explanatory data were divided into 8-15 clusters using a k-means clustering algorithm. Data used to train, test and validate the ANN were proportionately sampled from these clusters. For generalizability, the simplest ANN architecture with good performance (<5% gain in model accuracy for additional increases in architecture complexity) was chosen for 20 extractions of the training, test, and validation data. Within each extraction, each tested ANN architecture was reinitialized 10 times,

and the initialization with the lowest root mean square error (RMSE) was selected to avoid local minima.

In addition to ANN, CO₂ fluxes were partitioned following Reichstein (2005), which uses nighttime NEE to extrapolate R_{eco} from night to daytime using a temperature sensitivity model (Lloyd & Taylor, 1994):

$$R_{eco} = R_{ref} * e^{E_0(\frac{1}{T_{ref}-T_0} - \frac{1}{T-T_0})} \quad (18)$$

where R_{ref} is a reference respiration rate and a fitted parameter, T_{ref} and T_0 are constant (10 and -46.02°C, respectively), T is air temperature, and E_0 is a fitted parameter similar to an activation energy parameter in Michaelis-Menten functions. R_{ref} and E_0 were estimated using a Markov chain Monte Carlo optimization approach (see below).

3.4. Soil respiration measurements

Soil respiration measurements were conducted 3 m west of the eddy covariance tower within the tower's footprint. Three continuous measurement systems were in operation during the experiment. Two CO₂ gradient profiles were used, with CO₂ probes buried at 2, 8 and 16 cm depth (GMP221, Vaisala, Helsinki, Finland). Soil temperature by each CO₂ probe was measured using a thermocouple. Soil moisture was measured at 2, 8, and 16 cm depth using soil moisture sensors (ML3 ThetaProbe, Dynamax, Houston, TX, USA) buried between the two profiles. Soil surface CO₂ flux was estimated following Fick's law of diffusion (Tang *et al.*, 2003):

$$R_{soil} = -D_s \frac{dc}{dz} \quad (19)$$

where R_{soil} is the soil surface CO₂ flux rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), D_s is the diffusion coefficient through the soil ($\text{m}^2 \text{ s}^{-1}$), and dC/dz is the vertical gradient of CO₂ concentration. D_s is estimated as a function of soil moisture based on empirical relationships derived from diffusion measurements in the lab with intact soil cores from the alfalfa field. Gas diffusivity of undisturbed soil cores under a range of moisture contents was measured in the laboratory under steady-state conditions using oxygen as the diffusing gas. The empirical function gave better estimates of D_s compared to the Moldrup model (Moldrup *et al.*, 2003) (Figure A3).

The third continuous measurement system was a forced diffusion (FD) chamber deployed on the soil surface (Forced Diffusion Classic, Eosense, Dartmouth, Nova Scotia, Canada) also using CO₂ sensors (GMP221, Vaisala, Helsinki, Finland). The FD system consists of 2 chambers each with a diffusion membrane allowing the exchange of CO₂ at a known rate. One of the chambers is exposed to the soil and the other is sealed. Using the difference in CO₂ concentration between the two chambers and the known rate of diffusion through the membrane on the sides of the chamber, a soil surface CO₂ flux is estimated (Lavoie *et al.*, 2012, Risk *et al.*, 2011):

$$R_{soil} = G_{diff}(C_{chamb} - C_{atm}) \quad (20)$$

where R_{soil} is the soil surface CO₂ flux rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), G_{diff} is the diffusive term that describes the diffusive transport properties of the chamber system and is empirically measured (m s^{-1}), and C_{cham} and C_{atm} are the concentrations of CO₂ measured within the two chambers ($\mu\text{mol mol}^{-1}$).

Manual R_{soil} measurements were also collected during 4 days of the campaign using a portable soil chamber (LI-6400, Li-COR Biosciences, NE, USA) and soil collars (n=6) dispersed

around the profiles and FD chamber. A comparison of all R_{soil} methods relative to manual chamber measurements are shown in Figure A3.

3.5. Long-term data comparisons

Unlike the isotope measurements, R_{soil} and NEE are collected continuously at the site. Using the previous year of data (2014 DOY 259 - 2015 DOY 258), we explored environmental conditions when R_{soil} and R_{eco} significantly diverged. We also compared annual sums for R_{eco} partitioned by Reichstein and ANN with annual sums for R_{soil} . Small gaps in R_{soil} (<1 day) were filled using linear interpolation in order to estimate an annual sum. Large gaps (1-8 days) were filled using an empirical temperature function. Cumulative gaps in R_{soil} data were 18 days or 5% of the annual record.

3.6. Uncertainty in data and partitioning approaches

Random error in eddy covariance and soil respiration measurements was estimated using the daily differencing approach, where error followed a Laplace distribution for all fluxes (Lavoie *et al.*, 2015, Richardson *et al.*, 2006). This approach uses the difference between fluxes made on two successive days at one location under similar environmental conditions to estimate random error in the flux measurement. Soil respiration fluxes were constrained to days that did not differ by more than 0.5°C soil temperature or 0.01 cm³ cm⁻³ soil moisture at 0-10 cm depth. Eddy covariance fluxes (including NEE and *isoflux*) were constrained to PAR within 75 $\mu\text{mol m}^{-2} \text{s}^{-1}$, air temperature within 3°C, wind direction within 10 degrees, and wind speed within 1 m s⁻¹.

1.

Sampling error for soil respiration measurements was estimated by taking the standard error of the mean flux across the 3 locations. Total error for soil respiration measurements was estimated conservatively by adding the random and sampling errors linearly (Richardson *et al.*, 2010).

Error in ANN partitioned fluxes was estimated using variation across the 20 ANN predictions (Knox *et al.*, 2015). Total error in NEE was calculated from adding gap-filling and random errors. Total error in partitioned R_{eco} and GPP from the ANN was based on variation across the 20 ANN predictions, expressed as 90% confidence intervals.

Error from Reichstein-partitioned R_{eco} and GPP was determined from uncertainty in model parameters. To fit the model and estimate uncertainty in model predictions we used a model-data fusion approach with a weighted least-squares optimization (Richardson *et al.*, 2008). Data-model agreement was estimated using the following cost function:

$$J = \sum_{t=1}^N \left(\frac{y(t) - p(t)}{\sigma(t)} \right)^2 \quad (21)$$

where J is the data-model mismatch, y is the observed nighttime R_{eco} , p is the modeled R_{eco} , and σ is the uncertainty in the observed flux due to random error. To estimate parameters we used an Markov chain Monte Carlo (MCMC) approach with a delayed rejection adaptive Metropolis-Hastings algorithm (DRAM) (Haario *et al.*, 2006). This technique allows for global adaptation and efficient sampling. DRAM was selected as it is less sensitive to non-Gaussian distributions and gives good mixing even under poorly informed initial parameter values.

Multiple chains ($n=3$) were run with different starting points to evaluate convergence. For each chain, we first ran 50,000 iterations without adaptation to explore the parameter space. We then initialized the next optimization using the best parameter set from the burn-in period,

running 150,000 iterations with an adaptive algorithm. Posteriors were generated using the end of this chain, where convergence had been achieved. Convergence within each chain was evaluated using 3 criteria. First, Geweke tests were used to estimate convergence for each parameter (Geweke, 1992). Second, chains were identified as converged if long-term acceptance rates were near 50% (Braswell *et al.*, 2005). Finally, the means and standard deviations of all parameters were analyzed for stability towards the end of the chain. From this final inspection, we selected 10,000 parameter sets towards the end of the chain that were used to assess uncertainty in parameter values and model predictions. As we are not evaluating the potential of the model to predict future R_{eco} at the site, we did not validate the model using independent data. 90% confidence intervals were calculated from the spread in modeled values across the 10,000 predictions.

Error in partitioned GPP and R_{eco} using isotopes was based on uncertainty in data inputs and fractionation factors. Uncertainty in NEE and *isoflux* was based on estimates of random error using the daily differencing approach described above. Uncertainty in $\delta^{13}C_a$ was based on the standard deviation of measurements during the campaign and was normally distributed. Uncertainty in $\delta^{13}C_r$ was determined by the 95% confidence interval of the intercept in the Keeling plot (Figure 1). This is a conservative approach for estimating error in $\delta^{13}C_a$ and $\delta^{13}C_r$ as variation in these terms may not only be due to sampling or random error but also changes in the fractionation of C sources over time. Fractionation due to conductance for CO₂ in the mesophyll had uncertainty based on the range of previously reported G_m across a wide variety of species under diverse environmental conditions (0.15-0.55 mol CO₂ m⁻² s⁻¹) (Flexas *et al.*, 2012), assuming a normal distribution around the mean. Previously measured variance in fractionation during photorespiration is small and therefore assumed to be negligible (Tcherkez, 2006). The

photocompensation point was assumed to have a normally distributed error of ± 10 ppm due to error from using air temperature as a proxy for leaf temperature. Error in fractionation due to Rubisco carboxylation was based on previous measurements in spinach ($26.4 \pm 0.6\text{‰}$ - $30.3 \pm 0.8\text{‰}$) (Guy *et al.*, 1993, Roeske & O'Leary, 1984), tobacco ($27.4 \pm 0.9\text{‰}$) (McNevin *et al.*, 2007), cotton ($27.1 \pm 3.5\text{‰}$) (Wong *et al.*, 1979) and soybean ($28.3 \pm 1.5\text{‰}$) (Christeller *et al.*, 1976), choosing a mean of 27.5‰ with a normally distributed error of $\pm 3\text{‰}$. It is important to note that the error associated with fractionation due to Rubisco carboxylation is highly uncertain as only a limited number of plant species have been measured with possible flaws in experimental design (see review by Wehr & Saleska, 2015). The boundary layer conductance was assumed to have a normally distributed error of $\pm 50\%$ due to uncertainty reported in McNaughton & Van den Hurk (1995). Error due to diffusion from the leaf to the substomatal cavity was assumed to be negligible.

Uncertainty in Penman-Monteith derived G_c was based on previous investigations of differences in G_c from measured G_s . G_s is known to be similar to G_c when LAI is greater than 3 (Kelliher *et al.*, 1995) and the fraction of evapotranspiration from the soil surface is small relative to that from plant surfaces. During the campaign, soil moisture was low and no rain events occurred. However, the alfalfa field LAI was 1.65. According to theoretical relationships between G_c and G_s , at an LAI of 1.65, $G_c / G_s = 1.7$ (Kelliher *et al.*, 1995). Similarly, other studies have found that errors associated with Penman-Monteith derived G_c were $\pm 25\text{-}50\%$ (Baldocchi & Meyers, 1998, Baldocchi *et al.*, 1987, Callander & Woodhead, 1981). Therefore, uncertainty in G_c was conservatively estimated at $\pm 50\%$ assuming a normal distribution of error.

While it would be ideal to assess uncertainty in CANVEG parameters G_s and C_i using measured G_s and C_i in the alfalfa system, no such data were available. Instead, we employed

measurements and CANVEG modeling results from a blue oak system to assess uncertainty in CANVEG modeled G_s and C_i (Xu & Baldocchi, 2003). Relative to leaf-level measurements throughout the blue oak canopy ($n=3$ leaves), CANVEG modeled G_s underestimated measured G_s by ~40% and overestimated C_i by ~20%. We therefore estimate potential error in modeled G_s and C_i at ± 40 and 20%, respectively, assuming a normal distribution of error.

Using Monte Carlo sampling, NEE was partitioned using each of the three isotopic partitioning methods by randomly sampling across the uncertainty in data streams to get a range of GPP and R_{eco} at each time point ($n=1000$). 90% confidence intervals were calculated from the spread across those GPP and R_{eco} values.

3.7. Light use efficiency

Daily light use efficiency (LUE) was calculated for each partitioning method using an estimate of absorbed PAR (APAR), which is a function of LAI, PAR and a light extinction coefficient ($k=0.5$) (Ruimy *et al.*, 1999), where $LUE = GPP/APAR$. Relationships between LUE and APAR were evaluated for each partitioning method using ordinary least squares regression.

4. Results

We were able to test flux partitioning during the most active period of the growing season when gas exchange was vigorous, the physiological capacity of the alfalfa field was near peak, and the weather was steady. NEE and LE were consistent throughout the campaign (Figure 3a, b) due to stable meteorological conditions (on average 21.7°C (SD=5.2), 1.2 kPa (SD=0.91) and

175.0 W m⁻² (SD=274.5) for air temperature, VPD and RNET, respectively; Figure 3c-e) with no irrigation, precipitation, or harvest events observed. Canopy height was on average 55.2 cm (SD=8.2) with a mean LAI of 1.65 (SD=0.42). $\delta^{13}C_a$ and *isoflux* were also stable throughout the campaign (Figure 3f, g), with an average $\delta^{13}C_a$ of -8.79‰ (SD=1.31). On average, $\delta^{13}C_r$ was -30.7‰ ± 2.3 (Figure 1) and the daytime isotope disequilibrium was on average -2.1‰ (Figure A5).

Figure 3. Diel plots for flux and environmental variables (a) net ecosystem exchange of CO₂ (NEE), (b) latent heat exchange (LE), (c) air temperature, (d) vapor pressure deficit (VPD), (e)

net radiation (RNET), (f) the isotopic composition of atmospheric CO₂ ($\delta^{13}\text{C}_a$) and (g) *Isoflux*. SD bars are shown representing variability across the 2-week campaign.

Overall, CANVEG performance was strong with good agreement between simulated and observed NEE (cumulative CANVEG NEE overestimated observed NEE by 5%), 30% overestimation of cumulative H, and 9% overestimation of cumulative LE (Figure A4a-c). Penman-Monteith G_c tended to be lower than CANVEG G_s , especially at midday (Figure 4a; mean $G_c = 0.41 \text{ mol m}^{-2} \text{ s}^{-1}$ (SD=0.11); mean $G_s = 0.63 \text{ mol m}^{-2} \text{ s}^{-1}$ (SD=0.23)). Employing CANVEG-modeled C_i in partitioning (CANVEG- G_s - C_i), however, led to similar midday C_i/C_a values (mean = 0.71 at noon) and Δ values (mean = 21.6‰ at noon) compared with the other two isotope partitioning approaches (C_i/C_a mean = 0.80 and 0.82 at noon for PM- G_c and CANVEG- G_s , respectively; Δ mean = 23.7 and 23.9‰ at noon for PM- G_c and CANVEG- G_s , respectively; Figure 4b, c).

Figure 4. (a) canopy or surface conductance (G_c or G_s), (b) C_i/C_a ratio (ppm ppm^{-1}), and (c) isotopic discrimination by photosynthesis during the day (Δ , ‰) for the three partitioning approaches PM- G_s , CANVEG- G_s and CANVEG- G_s-C_i . As CANVEG- G_s and CANVEG- G_s-C_i use the same G_s , only 1 is shown in panel a. SD bars are shown representing variability across the 2-week campaign. SD are not shown for C_i/C_a ratio.

Diel patterns differed between the partitioning methods (Figure 5). 90% confidence intervals based on data and model uncertainties for each partitioning method (CANVEG- G_s-C_i , CANVEG- G_s , and PM- G_c , ANN, and Reichstein) and R_{soil} measurements allowed identification of periods when the methods significantly differed. At night, CANVEG- G_s-C_i , CANVEG- G_s ,

PM- G_c -, ANN-, and Reichstein-partitioned R_{eco} overlapped and were on average higher than R_{soil} measurements (Figure 5a). During the day, CANVEG- G_s - C_i -, CANVEG- G_s -, and PM- G_c -partitioned R_{eco} at times exceeded R_{soil} while tending to fall below R_{soil} in the afternoon while ANN- and Reichstein-partitioned R_{eco} was always higher than R_{soil} (Figure 5a). ANN and Reichstein partitioned R_{eco} increased throughout the day, peaking at 15:00 while R_{soil} peaked near 17:00. In general, isotope-partitioned R_{eco} had more diel variability than ANN- and Reichstein-partitioned R_{eco} mostly due to variation in modeled G_c and G_s (Figure 4a). Daytime CANVEG- G_s - C_i -, CANVEG- G_s -, and PM- G_c -partitioned GPP were lower in magnitude compared to ANN and Reichstein partitioned GPP (Figure 5b). We calculated 90% confidence intervals based on data and model uncertainties for each partitioning method (CANVEG- G_s - C_i -, CANVEG- G_s -, and PM- G_c -, ANN, and Reichstein) and R_{soil} measurements (Figure 5).

Figure 5. (a) Ecosystem respiration (R_{eco}) and soil respiration (R_{soil}) and (b) gross primary productivity (GPP) are plotted for ANN-partitioned (ANN), Reichstein-partitioned (Reichstein), and isotope-partitioned fluxes ($PM-G_c$, $CANVEG-G_s$, $CANVEG-G_s-C_i$). Continuous ($R_{soil cont}$) and manual ($R_{soil manual}$) soil respiration measurements are also plotted. Average diel 90% confidence intervals are shown based on the uncertainty analysis for all fluxes except $R_{soil manual}$ which are shown with SD bars. ANN and Reichstein results often overlap and are therefore difficult to distinguish.

The influence of partitioning on the relationship between LUE and APAR was evaluated using ordinary least squares regression (Figure 6). All isotope partitioning methods exhibited a

significant negative relationship between LUE and APAR (slope = -1.14, -0.80, -1.07 for CANVEG- G_s - C_i , CANVEG- G_s , and PM- G_c , respectively), while standard partitioning methods exhibited little to no relationship between LUE and APAR (slope = 0.02 Reichstein, slope = ns ANN).

Figure 6. Daily LUE is plotted against daily APAR for ANN-partitioned (ANN), Reichstein-partitioned (Reichstein), and isotope-partitioned fluxes (PM- G_c , CANVEG- G_s , CANVEG- G_s - C_i). Linear ordinary least-squares fits are shown for all partitioning methods.

The significance of above-ground plant respiration (R_{plant}) during the day and night was investigated by subtracting R_{soil} from R_{eco} for each partitioning method (daytime was defined as $\text{PAR} > 5 \mu\text{mol m}^{-2} \text{s}^{-1}$). On average, standard partitioning methods ANN and Reichstein estimated

R_{plant} during the night to be $0.93 \pm 0.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ similar to isotope partitioning methods ($0.80 \pm 0.3 \mu\text{mol m}^{-2} \text{s}^{-1}$ on average across the three methods). This is not surprising as nighttime partitioning, when $\text{GPP}=0$, is identical among methods. However nighttime, as defined here, includes short time periods at dusk and dawn when GPP may be slightly below 0. During the day, standard partitioning methods estimated R_{plant} to be $2.1 \pm 0.3 \mu\text{mol m}^{-2} \text{s}^{-1}$, on average higher than isotope estimates of R_{plant} at $0.2 \pm 0.5 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Cumulative sums were computed to assess how differences between partitioning methods translated to cumulative differences over the two-week campaign (Table 1). Differences between the isotope partitioning approaches were not significant; all isotope-partitioned cumulative estimates of GPP and R_{eco} overlapped with one another. Isotope-partitioned cumulative budgets were lower in magnitude than ANN- and Reichstein-partitioned budgets for both GPP and R_{eco} . R_{soil} and isotope-partitioned R_{eco} cumulative budgets overlapped. Cumulative isotope-partitioned GPP was on average $20.6 \text{ g CO}_2\text{-C m}^{-2}$ lower (or $\sim 10\%$ lower) than the average cumulative ANN and Reichstein-partitioned GPP . Cumulative isotope-partitioned R_{eco} was on average $18.0 \text{ g CO}_2\text{-C m}^{-2}$ lower (or $\sim 13\%$ lower) than the average cumulative ANN and Reichstein-partitioned R_{eco} . If we assume the true estimates of GPP and R_{eco} lie within the range of all partitioned estimates, cumulative GPP would range between $176.8\text{-}218.4 \text{ g CO}_2\text{-C m}^{-2}$ (or $199.2 \text{ g CO}_2\text{-C m}^{-2} \pm 11\%$) and cumulative R_{eco} would range between $101.3\text{-}140.6 \text{ g CO}_2\text{-C m}^{-2}$ (or $122.6 \text{ g CO}_2\text{-C m}^{-2} \pm 17\%$).

Method	GPP (g CO ₂ -C m ⁻²)	R _{eco} (g CO ₂ -C m ⁻²)
Isotope PM-Gc	-184.3 ± 7.5	108.8 ± 7.5
Isotope CANVEG-Gs	-192.5 ± 7.0	117.0 ± 7.0
Isotope CANVEG-Gs-Ci	-196.0 ± 8.6	120.5 ± 8.6
ANN	-210.0 ± 2.6	131.5 ± 2.6
Reichstein	-213.1 ± 5.3	135.3 ± 5.3
R _{soil}	N/A	108.5 ± 5.3*

Table 1. Cumulative fluxes during the 2-week campaign for partitioned variables with 90% confidence intervals. In order to derive cumulative fluxes, gap-filled NEE was used in partitioning approaches (<10% of data were gap-filled). *R_{soil} does not account for above-ground respiration.

Analysis of long-term data (outside the isotope measurement campaign) revealed that estimates of R_{eco} and R_{soil} diverged during harvest events. One harvesting event is shown as an example of when ANN- and Reichstein-partitioned R_{eco} and R_{soil} diverge significantly (Figure 7). Both the ANN and Reichstein method predicted relatively high R_{eco} following harvest on DOY 245 compared to R_{soil}. R_{soil} measurements showed a decrease in soil respiration in response to harvesting, which neither of the modeling approaches detected. Cumulative annual fluxes were higher for ANN- and Reichstein-partitioned R_{eco} compared to R_{soil} (1809.0 ± 367.7, 2105.1 ± 85.9, 2234.0 ± 74.6 g CO₂-C m⁻² yr⁻¹ for R_{soil}, ANN R_{eco}, and Reichstein R_{eco}, respectively).

Figure 7. Example period from 2015 following a harvest event (indicated by a downward facing arrow) where estimates of $R_{\text{eco ANN}}$, $R_{\text{eco Reichstein}}$, and NEE diverge from R_{soil} . 90% confidence intervals are shown based on the uncertainty analysis for all fluxes.

5. Discussion

Partitioning NEE into GPP and R_{eco} is critical for understanding the processes regulating CO_2 exchange and for the development of predictive models. All techniques for partitioning NEE, however, have their strengths and limitations. We used an ensemble of partitioning approaches simultaneously to give insight into uncertainty in partitioning NEE under ideal meteorological and ecosystem conditions. Over the course of a two-week campaign in an actively growing agricultural ecosystem, we found that isotope-partitioned GPP and R_{eco} were on average 10-13% lower than ANN- and Reichstein-partitioned GPP and R_{eco} . This difference may be attributed to the Kok effect. Several studies have shown that nighttime leaf respiration is higher than leaf respiration during the day leading to large errors in partitioned GPP, up to 17% (Wohlfahrt *et al.*, 2005). A recent isotope partitioning study found that nighttime R_{eco} is twice as large as daytime R_{eco} during the first half of the growing season in a temperate forest and that this difference was not detectable later in the growing season (Wehr *et al.*, 2016). Our results are

congruent with these previous studies and suggest that even in the case of an alfalfa field on peat soils, where R_{soil} is a large part of the respiration budget, the Kok effect may be significant. Further, our results may reflect a time period when the Kok effect is expected to be small as the campaign began 25 days after harvest when the canopy was fully established. Whether or not the differences in partitioning are due to the Kok effect, our ensemble of partitioning techniques suggest that error in partitioned GPP and R_{eco} fluxes may be large. This amount of error has implications for constraining global carbon models and our ability to predict terrestrial carbon balance in the future. This is especially relevant for the usage of partitioned eddy covariance data publically available in the FLUXNET Database (<http://fluxnet.ornl.gov/>), a large database used to constrain global carbon models. These data typically employ standard partitioning methods such as the ANN and Reichstein approaches which rely on the assumption that nighttime R_{eco} can be used to estimate R_{eco} during the day. Moving forward, it is not clear in what systems, if any, the Kok effect can be conservatively ignored. For windy sites with high potential fluxes, steady conditions and flat topography, it may be possible to quantify the Kok effect at the canopy level using a light response curve, also known as the Kok method (Kok, 1948), such as the one shown in Figure 8. However, there is considerable uncertainty when applying eddy covariance data to the Kok method due to uncertainty in intercept values and changing light response curves over time (Bruhn *et al.*, 2011). As the implementation of new isotope analyzers such as the one employed here become more accessible, the significance of the Kok effect across diverse ecosystems can be more easily and accurately assessed and our ability to constrain uncertainty in partitioned fluxes will be greatly improved.

Figure 8. Example of how a light response curve (net ecosystem exchange of CO₂ (NEE) vs. light (PAR)) can be used to estimate the Kok effect for days 190-192. Two least squares regressions are shown: one for data above the break in the linear light response curve near the light compensation point at $\sim 200 \mu\text{mol m}^{-2} \text{s}^{-1}$ (open circles and solid line) and one for data below the break ($< 200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR; closed circles and dashed line). The y-intercept for the data above the break is an estimate of R_{eco} in the light and the y-intercept for the data below the break is an estimate of R_{eco} in the dark. The difference between the y-intercepts is an estimate of the Kok effect at the canopy scale, and is $3.6 \mu\text{mol m}^{-2} \text{s}^{-1}$ in this example.

The implementation of the CCIA-48 isotope analyzer enabled robust measurements of *isoflux* and $\delta^{13}C_r$ despite low sampling frequency and single inlet installation. Low sampling frequency (1 Hz) is known to lead to poor sampling of certain eddy sizes (Wesely & Hart, 1985). Spectral analysis of the CCIA-48 data indeed revealed spectral loss at higher frequencies. However, under-sampling can be robustly corrected for using the high frequency LI-7500 data (Detto *et al.*, 2011). The CCIA-48 isotope analyzer also enabled robust estimates of $\delta^{13}C_r$. Historically, Keeling plots used to derive $\delta^{13}C_r$ have been informed using profile measurements

throughout the canopy, where measurements at different heights can help inform sources of CO₂ (Bowling *et al.*, 2001, Wehr & Saleska, 2015). This technique is limited by spatial specificity and temporal averaging. Our approach uses turbulence theory and conditional sampling to achieve the same measurement using a single inlet above the canopy. By restricting measurements to nighttime updrafts when the range of CO₂ concentrations is high, we were able to better constrain an estimate of $\delta^{13}C_r$ that is not restricted to spatial regions near the base of the tower. Similar techniques have been used for resolving transport of air enriched in CO₂ from soil respiration to the atmosphere (Scanlon & Albertson, 2001). This approach is valuable as single inlet closed-path analyzers are simple to install and maintain in the field.

Our isotope partitioning approach advances partitioning theory by applying a canopy-scale biophysical model to estimate canopy conductance and C_i and by applying a rigorous error analysis of modeled parameters and data. However, there are multiple modeled parameters which require future research in order to further refine isotope partitioning theory. For example, we ignore fractionation during dark respiration due to previous work that did not detect significant fractionation of carbon during dark respiration in alfalfa (Klumpp *et al.*, 2005). While this fractionation may not be significant in alfalfa, it may be an important parameter in other ecosystems (Wehr & Saleska, 2015). In addition, the isotope partitioning theory applied here does not take into account the different components of R_{eco} (i.e. above- and below-ground plant respiration and soil respiration), however previous research has accounted for these components in flux partitioning theory (Wehr *et al.*, 2016, Wehr & Saleska, 2015). Future work is encouraged to measure the fluxes and isotopic compositions of the different components of R_{eco} in order to better support and advance isotope partitioning theory. Another issue concerns uncertainty associated with modeled G_s and G_c . In this study, we found little differences in

partitioned GPP and R_{eco} using Penman-Monteith derived G_s and CANVEG derived G_c . Future studies are encouraged to use measurements of G_c to gain better estimates of uncertainty in modeled G_c . For example, eddy covariance measurements of carbonyl sulfide exchange may provide rigorous estimates of G_c that can be used to test models (Commane *et al.*, 2015). In addition, a more direct approach for estimating G_c than the Penman-Monteith approach is to use water vapor exchange and restrict analyses to periods when surface evaporation is likely to be small (Wehr & Saleska, 2015). Despite all model assumptions, the isotope partitioning method provided estimates of R_{eco} and GPP with similar degrees of uncertainty compared to standard partitioning methods. Ecosystems with low nighttime turbulence and/or noisy nighttime fluxes are difficult to assess using standard partitioning methods (Reichstein *et al.*, 2005). In addition, heterogeneous landscapes such as peatlands are difficult to sample using R_{soil} measurements alone (Schrier-Uijl *et al.*, 2010). As isotope sensors continue to improve and become more accessible, these measurements will provide valuable insight into ecosystems where the application of standard partitioning methods and R_{soil} measurements are less effective.

We detected different relationships between light use efficiency and APAR when using standard- vs isotope-partitioned GPP. The light use efficiency of GPP is expected to decrease as APAR increases due to many factors including the switch from light-limited photosynthesis to CO_2 -limited photosynthesis as well as the meteorological conditions that are associated with high light (i.e. high VPD) which are expected to decrease GPP. Similarly to results presented in Wehr *et al.* (2016), we found little to no relationship between standard-partitioned LUE and APAR while significant negative relationships were detected with isotope-partitioned LUE and APAR. This further supports the need to adjust standard partitioning algorithms to account for the Kok

effect in order to not only quantify GPP but understand functional relationships between photosynthesis and light at the canopy scale.

Continuous R_{soil} data provided a valuable and reliable means of benchmarking partitioned fluxes. For example, R_{soil} measurements provided a means of estimating above-ground plant respiration ($R_{\text{plant}} = R_{\text{eco}} - R_{\text{soil}}$). Standard partitioning methods estimated R_{plant} to increase during the day compared to night while isotope partitioning methods predicted a decrease in R_{plant} during the day (~75% decrease). Strong inhibition of leaf respiration (>80%) during the day has been observed across diverse plant species (reviewed by Heskell *et al.*, 2013, Wohlfahrt *et al.*, 2005). Again, this suggests that the isotope partitioning methods detect inhibition of leaf respiration during the day. It is also important to note that at times, particularly in the late afternoon, isotope-partitioned R_{eco} dropped below R_{soil} which is unexpected. Above-ground autotrophic respiration in agricultural systems typically ranges between 37-62% of total R_{eco} (Suleau *et al.*, 2011) and was previously measured to be 35% of total R_{eco} in alfalfa (Da Costa *et al.*, 1986). Therefore, we would expect the ratio of $R_{\text{soil}}:R_{\text{eco}}$ to vary across ecosystems with a general trend that R_{soil} underestimates R_{eco} (Janssens *et al.*, 2001). However, R_{soil} measurements with low spatial replication have been known to overestimate fluxes when scaled up to the ecosystem (Goulden *et al.*, 1996, Lavigne *et al.*, 1997). Therefore assessing the spatial variability of R_{soil} across the landscape is a valuable pursuit as it can provide estimates of scaling error associated with R_{soil} measurements (Lavoie *et al.*, 2015). These results highlight the importance of constraining errors as error in R_{soil} measurements influence our interpretation of partitioned fluxes. Constraining errors will also enable better assessment of whether or not the differences among partitioned fluxes are due to errors in isotope partitioning methods or errors in standard partitioning methods.

Analysis of long-term data indicated that R_{soil} measurements detected fast changes in below-ground respiration during the day that were not identified by ANN- and Reichstein-partitioned R_{eco} or nighttime NEE. Fast changes (<24 hr) in R_{soil} are expected in response to severe defoliation due to significantly decreased labile C availability for roots and microbes (Fu & Cheng, 2004). Future efforts will be made to combine manual chamber measurements with continuous R_{soil} measurements during disturbance in order to further validate these findings. Overall, R_{soil} measurements provided a valuable benchmark and gave insight into short-term respiratory responses to disturbance, however these measurements do not account for aboveground respiration and have poor spatial replication and are therefore not a surrogate for but a compliment to partitioning approaches.

6. Conclusions

We employed an ensemble of partitioning methods to better constrain uncertainty in partitioned NEE fluxes GPP and R_{eco} under ideal conditions. The newly-developed CCIA-48 CO_2 isotope analyzer provided robust measurements of ecosystem *isoflux* and estimates of $\delta^{13}\text{C}_r$ using an above-canopy inlet. These measurements were employed in a series of isotope-partitioning approaches each using different estimates of canopy conductance and C_i . Despite uncertainty in modeled parameters and differences among the isotope-partitioning approaches, all isotope-partitioned estimates of GPP and R_{eco} overlapped with one another with low uncertainty. Overall, isotope-partitioned GPP and R_{eco} were smaller than ANN- and Reichstein-partitioned GPP and R_{eco} . Our results agree with previous studies suggesting that standard partitioning approaches (ANN and Reichstein) that extrapolate nighttime R_{eco} relationships to the day may overestimate daytime R_{eco} because they do not model a daytime reduction in plant

respiration (the Kok effect). This has implications for the interpretation of partitioned eddy covariance data across the world, a critical data source for constraining terrestrial carbon cycling. These results also have implications for modeling where poorly understood uncertainty in partitioned GPP and R_{eco} can lead to unknown amounts of uncertainty in model predictions. The application of similar isotope measurements in other more complex ecosystems will provide valuable insight into the error associated with NEE partitioning, especially if conducted in conjunction with a biophysical model and compared with standard partitioning (i.e. Reichstein and ANN) and R_{soil} measurements.

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Appendix

Figure A1. Allan Deviation plot for CO_2 , $\delta^{13}\text{C}$, $\delta^{17}\text{O}$, and $\delta^{18}\text{O}$ measured by the CCIA-48 instrument in the chamber with the Multiport Inlet Unit (MIU) and the reference gas. Precision specifications of the CCIA-48 are also given for each species. Note that precision specifications for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ overlap and are therefore indistinguishable in the figure.

Figure A2. Sample raw transmission (Volts) spectrum of the CO₂ isotopologue peaks (¹²CO₂, ¹³CO₂, ¹⁸OCO, ¹⁷OCO) measured during the campaign by the CCIA-48 instrument. Data point refers to the order of data collected on the detector. The red dotted line is the baseline used for fitting the spectrum.

Figure A3. Comparison between soil surface CO₂ flux manual chamber measurements and continuous measurements including the forced diffusion (FD) method and the profile method employing an empirical model and the Moldrup model for estimation of D_s . Both diffusion models yielded higher fluxes compared with manual measurements during the campaign, however the empirical model gave closer estimates to manual measurements. A one-to-one line is shown as reference (black dotted line).

Figure A4. CANVEG results compared to (a) observed NEE, (b) H, and (c) LE. SD bars are shown representing variability across the 2-week campaign.

Figure A5. Diel variation in isotope disequilibrium ($D = \delta^{13}C_r - (\delta^{13}C_a - \Delta/1+\Delta)$) is shown for each isotope partitioning method (PM- G_c , CANVEG- G_s , CANVEG- G_s - C_i); SD bars represent variability across the 2-week campaign. On average daytime $D = -3.1, -2.6, -0.6$ ‰ for PM- G_c , CANVEG- G_s , CANVEG- G_s - C_i , respectively.