

# Endogenous circadian regulation of carbon dioxide exchange in terrestrial ecosystems

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## Abstract

It is often assumed that daytime patterns of ecosystem carbon assimilation are mostly driven by direct physiological responses to exogenous environmental cues. Under limited environmental variability, little variation in carbon assimilation should thus be expected unless endogenous plant controls on carbon assimilation, which regulate photosynthesis in time, are active. We evaluated this assumption with eddy flux data, and we selected periods when net ecosystem exchange (NEE) was decoupled from environmental variability in seven sites from highly contrasting biomes across a 74° latitudinal gradient over a total of 36 site-years. Under relatively constant conditions of light, temperature, and other environmental factors, significant diurnal NEE oscillations were observed at six sites, where daily NEE variation was between 20% and 90% of that under variable environmental conditions. These results are consistent with fluctuations driven by the circadian clock and other endogenous processes. Our results open a promising avenue of research for a more complete understanding of ecosystem fluxes that integrates from cellular to ecosystem processes.

**Keywords:** biosphere-atmosphere interactions, canopy conductance, circadian clock, ecosystem exchange, gas exchange, global change, photosynthesis, source/sink regulation

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## Introduction

One of the major assumptions underlying studies of land-atmosphere exchange is that daily fluctuations in the net ecosystem exchange of CO<sub>2</sub> (NEE) are driven almost exclusively by direct physiological responses to changes in irradiance, temperature, humidity, and other environmental factors (Hollinger *et al.*, 1994; Sellers *et al.*, 1997; Chapin *et al.*, 2002; Hanson *et al.*, 2004). This

assumption arose largely because leaf photosynthesis, which represents the major input of carbon to an ecosystem, responds strongly and rapidly to changes in the meteorological environment. However, it is increasingly recognized that diurnal changes in NEE could also result from a variety of endogenous processes (Hennessey & Field, 1991; Wingler *et al.*, 2000; Franks, 2004; Doughty *et al.*, 2006; Resco *et al.*, 2009a; Brodersen *et al.*, 2010), or indirect physiological responses to the environment.

NEE is the difference between gross ecosystem exchange and respiration. Thus, variation in both carbon assimilation and respiration determine fluctuations in

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daily NEE. We will focus here on the physiological drivers of carbon assimilation that affect NEE, as discussions on how direct and indirect responses of respiration to environmental variation may affect NEE have been addressed elsewhere (Davidson *et al.*, 2006; Höglberg & Read, 2006; Trumbore, 2006; Bahn *et al.*, 2010).

Under the standard physiological convention, where increasing carbon assimilation is represented as more positive NEE, the diurnal pattern of NEE during the growing season often follows a common trend whereby NEE increases during the morning, reaches a maximum around noon, and declines thereafter. A variety of endogenous factors affecting carbon assimilation have been reported to accelerate the afternoon decline in NEE. For instance, reduced carbon assimilation resulting from afternoon stomatal closure is often observed, reflecting the loss of hydraulic conductivity (Franks, 2004; Brodersen *et al.*, 2010). Moreover, stomatal limitations tend to intensify under high light, causing photorespiration to act as a major energy sink in C3 plants, which further contributes to the afternoon photosynthetic reduction (Wingler *et al.*, 2000). The reduction in NEE over the course of an afternoon might also result from the feedback inhibition of photosynthesis due to starch accumulation in the chloroplast (Paul & Foyer, 2001).

The circadian clock has also been hypothesized to influence the daily pattern of NEE (Doughty *et al.*, 2006; Resco *et al.*, 2009a). Under constant light and temperature, leaf carbon assimilation in a variety of species has been reported to follow a diurnal pattern that increases in the morning and decreases after noon (Hennessey & Field, 1991; Webb, 2003; Dodd *et al.*, 2004). This is because the circadian clock acts as a central 'orchestrator' of plant activity (*sensu* Harmer *et al.*, 2000; Mcclung, 2009) and directly controls (amongst other processes) stomatal opening and the transcription of genes underlying Rubisco activity as a function of time of day (Hennessey & Field, 1991; Liu *et al.*, 1996; Dodd *et al.*, 2004; Doughty *et al.*, 2006). The core mechanism of the circadian clock is a 'central oscillator' responsible for generating cycles of ca. 24 h period under constant levels of light and temperature over a broad range of temperatures. This oscillator is a molecular mechanism involving a series of highly conserved transcriptional-translational positive and negative feedback loops (Mcclung, 2009). Unlike the previously cited endogenous mechanisms, which only contribute to the afternoon decline in NEE, the circadian clock at the leaf level mirrors the typical daily trend in carbon assimilation observed in the field (Hennessey & Field, 1991; Dodd *et al.*, 2004; Doughty *et al.*, 2006).

The vast majority of experiments on circadian regulation of leaf gas exchange have been conducted with model species, under low levels of light (photosynthe-

tically active radiation, PAR, <200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), and in lab settings. Direct evidence that this molecular clock mechanism is an important driver of leaf photosynthesis in the field is scarce (Williams & Gorton, 1998), and even more scarce are tests on whether its effect may scale up to affect a whole ecosystem. However, preliminary studies suggest that this is likely. Doughty *et al.* (2006) observed that the uptake of  $\text{CO}_2$  in a tropical forest in Pará, Brazil increased almost fivefold (from 3.6 to 16.1  $\mu\text{mol m}^{-2} \text{s}^{-1}$  between 0645 h and 0945 h), whereas light, air temperature and soil respiration rates remained relatively constant. Doughty *et al.* (2006) also measured leaf photosynthesis under constant light and temperature and concluded that the increase in NEE generally coincided with the measured increase in leaf photosynthesis under a constant environment.

Here, we test the assumption that daily NEE fluctuations are driven almost exclusively by direct physiological responses to environmental cues, and that NEE would remain constant over a day given constant environmental conditions. Instead of the logistically challenging approach of enclosing entire ecosystems within a chamber at constant temperature and light levels, we employed data filtering and statistical modeling techniques to remove the potential effects of variation in the external environment on NEE. We applied these techniques to NEE that was measured with the eddy covariance technique together with concurrently measured environmental data from sites in each of seven biomes across a 74° latitudinal gradient: tropical forest (Tapajos), chaparral (Sky Oaks), savanna (Santa Rita Mesquite), hardwood temperate forest (Bartlett), conifer temperate forest (Howland), subalpine conifer forest (Niwot Ridge), and tundra (Barrow; see Table 1). The dataset included half-hourly observations over a total of 36 site-years (Tables 2 and 3).

Our filtering approach extracted a subset of observations during the growing season for which environmental variables (e.g. temperature, humidity, soil water, light) were nearly constant. This filtering defined our data set of observations made under 'constant environmental conditions'. Because carbon assimilation is co-regulated by leaf biochemistry and stomatal conductance, we also calculated canopy conductance on the filtered dataset by inverting a widely used evapotranspiration model (Monteith & Unsworth, 2008; see Appendix S1 in Supporting Information).

## Methods

### Eddy covariance data

We selected seven sites from the AmeriFlux network, representative of contrasting climate regions and vegetation types

**Table 1** Sites description, location, references and how to access the data file (ftp address, date accessed, file name, and size)

Site	Vegetation type	Location	References	Data file
Barrow	Tundra	Alaska. 71.32° (N); 156.62° (W)	Harazono <i>et al.</i> (2003)	ftp://cdiac.ornl.gov/pub/ameriflux/data/Level2/Sites_ByName/Barrow/with_gaps/ (17/08/2009)uakbarr_1999_L2.csv (5.0 MB); uakbarr_2000_L2.csv (5.0 MB); uakbarr_ 2001_L2.csv (5.0 MB); uakbarr_2002_L2.csv (5.0 MB)
Bartlett	Hardwood temperate forest	New Hampshire. 44.06° (N); 71.28° (W)	Jenkins <i>et al.</i> (2007)	ftp://cdiac.ornl.gov/pub/ameriflux/data/Level2/Sites_ByName/Bartlett_ Experimental_Forest/with_gaps/ (09/09/2009)AMF_USBar_2004_L2_WG_ V002.csv (4.2 MB); AMF_USBar_2005_L2_WG_V002.csv (4.2 MB); AMF_USBar_ 2006_L2_WG_V002.csv (4.0 MB); AMF_USBar_2007_L2_WG_V002.csv (4.2 MB)
Howland	Coniferous temperate forest	Maine. 45.20° (N); 68.74° (W)	Hollinger <i>et al.</i> (2004); Savage <i>et al.</i> (2009)	ftp://cdiac.ornl.gov/pub/ameriflux/data/Level2/Sites_ByName/Howland_Forest_ Main/with_gaps/ (1/10/2009)AMF_USHo1_2000_WG_V001.csv (4.5 MB); AMF_USHo1_2001_WG_V001.csv (4.5 MB); AMF_USHo1_2002_WG_V001.csv (4.5 MB); AMF_USHo1_2003_WG_V001.csv (4.5 MB); AMF_USHo1_2004_WG_ V001.csv (4.5 MB)
Niwot Ridge	Taiga	Colorado. 40.03° (N); 105.54° (W)	Hu <i>et al.</i> (2010)	ftp://cdiac.ornl.gov/pub/ameriflux/data/Level2/Sites_ByName/Niwot_ Ridge/with_gaps/ (17/08/2009)AMF_USNR1_2002_L2_WG_V003.csv (4.3 MB); AMF_USNR1_2003_L2_WG_V003.csv (4.4 MB); AMF_USNR1_2004_L2_WG_ V003.csv (4.3 MB); AMF_USNR1_2005_L2_WG_V003.csv (4.4 MB)
Sky Oaks	Mediterranean chaparral	California. 33.37° (N); 116.62°(W)	Luo <i>et al.</i> (2007)	ftp://cdiac.ornl.gov/pub/ameriflux/data/Level2/Sites_ByName/Sky_Oaks_Old/ with_gaps/ (19/08/2009)uscaskyo_1997_L2.csv (4.2 MB); uscaskyo_ 1998_L2.csv (5.1 MB); uscaskyo_1999_L2.csv (5.1 MB); uscaskyo_2000_L2.csv (5.0 MB); uscaskyo_2001_L2.csv (5.1 MB); uscaskyo_2002_L2.csv (5.0 MB); uscaskyo_ 2003_L2.csv (5.1 MB); uscaskyo_2004_L2.csv (5.0 MB); uscaskyo_2005_L2.csv (5.0 MB)
SRM	Savanna	Arizona. 31.90°(N); 110.83° (W)	Scott <i>et al.</i> (2009)	ftp://cdiac.ornl.gov/pub/ameriflux/data/Level2/Sites_ByName/Santa_Rita_ Mesquite_Savanna/with_gaps/ (06/07/2009)AMF_USSRM_2004_L2_ WG_V003.csv (4.5 MB); AMF_USSRM_2005_L2_WG_V003.csv (4.5 MB); AMF_ USSRM_2006_L2_WG_V003.csv (4.5 MB); AMF_USSRM_2007_L2_WG_ V003.csv (4.5 MB)
Tapajos	Tropical forest	Pará. -3.01° (S); 54.97° (W)	Goulden <i>et al.</i> (2004)	http://www.ess.uci.edu/%7EElba/ (23/11/2009)JLBA-ECO_CD04_TNF_KM83_ METEOROLOGY_AND_FLUXES_MEASURED.zip (8 MB)

**Table 2** Environmental filters applied to daytime data, such that NEE variation may be considered independent of the environment

Site	Months	Years (sample size)	PAR ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$R_{\text{net}}$ ( $\text{Wm}^{-2}$ )	SWC (%)	$T_a$ ( $^{\circ}\text{C}$ )	$T_s$ ( $^{\circ}\text{C}$ )	RH (%)	VPD (kPa)	$T_l$ ( $^{\circ}\text{C}$ )	$u$ ( $\text{ms}^{-1}$ )	WD ( $^{\circ}$ )	$u^*$ ( $\text{ms}^{-1}$ )	H ( $\text{Wm}^{-2}$ )	LE ( $\text{Wm}^{-2}$ )
Barrow	7-8	1999-2002 (1977)	>500 (<0.001)	NF (<0.010)	NA	NF (<0.010)	NF (<0.040)	70-90 (<0.040)	NF (<0.040)	NF (<0.010)	NF (<0.040)	NF (<0.040)	.25-.47 (<0.040)	NF (<0.010)	NF (<0.010)
Bartlett	6-7	2004-2007 (352)	>1000 (<0.001)	NF (<0.040)	NF (<0.010)	NF (<0.010)	NF (<0.010)	40-70 (<0.040)	1.5-1.8 (<0.001)	NF (<0.040)	NF (<0.040)	NF (<0.040)	>0.275 (<0.010)	NF (<0.010)	NF (<0.040)
Howland	5-9	2000-2004 (740)	1000-1600 (<0.010)	>0.36 (<0.040)	NF (<0.010)	NF (<0.010)	NF (<0.010)	40-80 (<0.040)	0.5-1.5 (<0.040)	NF (<0.010)	NF (<0.010)	NF (<0.010)	>0.25 (<0.001)	125-250 (<0.040)	100-200 (<0.040)
Niwot Ridge	6-8	2002-2005 (275)	1000-1600 (<0.010)	NF (<0.010)	<20 (<0.010)	NF (<0.010)	0-13 (<0.010)	NF (<0.001)	0.5-1 (<0.010)	NF (<0.010)	NF (<0.010)	NF (<0.040)	>0.1 (<0.040)	<300 (<0.040)	NF (<0.010)
Sky Oaks	5-6	1997-2006 (596)	>1000 (<0.001)	NF (<0.040)	300-700 (<0.001)	20-26 (<0.010)	NF (<0.010)	NF (<0.010)	NF (<0.010)	NF (<0.010)	NF (<0.010)	NF (<0.040)	>0.275 (<0.001)	NF (<0.040)	>140 (<0.010)
Santa Rita	8-9	2004-2007 (160)	>1000 (<0.010)	NF (<0.001)	NF (<0.010)	25-32 (<0.040)	26-32 (<0.040)	40-60 (<0.001)	NF (<0.010)	NF (<0.040)	NF (<0.010)	NF (<0.010)	>0.15 (<0.040)	NF (<0.010)	100-400 (<0.040)
Messquite															
Tapajos	1-12	2000-2004 (2114)	>700 (<0.001)	NF (<0.040)	300-600 (<0.001)	22-28 (<0.010)	NF (<0.010)	60-80 (<0.010)	NF (<0.010)	NF (<0.010)	NF (<0.010)	NF (<0.010)	0.2-0.8 (<0.010)	NF (<0.010)	NF (<0.040)

NF indicates that no filter was necessary to apply for the associated variable because NEE and the variable already appeared decoupled ( $R^2 < 0.04$ ). Numbers in parentheses below each filter range indicate the  $R^2$  of a simple regression model between filtered NEE and each variable, except under the column 'years', where parentheses indicate the sample size. PAR is photosynthetically active radiation,  $\text{PAR}_{\text{dir}}$  is the fraction of direct to total PAR (and has relative units),  $R_{\text{net}}$  is net radiation, SWC is soil water content,  $T_a$  is air temperature,  $T_s$  is soil temperature, RH is relative humidity, VPD is vapor pressure deficit,  $T_l$  is leaf temperature,  $u$  is wind speed, WD is wind direction,  $u^*$  is friction velocity, H is sensible heat, and LE is latent heat. No SWC data were available from Barrow or Sky Oaks.

**Table 3** Environmental filters applied to nighttime data, such that NEE variation may be considered independent of the environment

Site	Months	Years (sample size)	PAR ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$R_{\text{net}}$ ( $\text{Wm}^{-2}$ )	SWC (%)	$T_a$ ( $^{\circ}\text{C}$ )	$T_s$ ( $^{\circ}\text{C}$ )	RH (%)	VPD (kPa)	$T_f$ ( $^{\circ}\text{C}$ )	$u$ ( $\text{ms}^{-1}$ )	WD ( $^{\circ}$ )	$u^*$ ( $\text{ms}^{-1}$ )	H ( $\text{Wm}^{-2}$ )	LE ( $\text{Wm}^{-2}$ )
Bartlett	6–7	2004–2007 (136)	<5 (<0.010)	NF (0.001)	NF (<0.010)	NF (<0.010)	<18 (<0.010)	50–95 (<0.040)	NF (<0.010)	NF (<0.010)	NF (<0.010)	>100 (<0.010)	>0.275 (<0.040)	NF (<0.040)	NF (<0.010)
Howland	5–9	2000–2004 (422)	<5 (<0.001)	NF (<0.010)	NF (<0.001)	10–15 (<0.001)	15–18 (<0.001)	NF (<0.001)	0.01–0.05 (<0.010)	NF (<0.010)	NF (<0.010)	NF (<0.010)	0.25–0.6 (<0.010)	NF (<0.040)	NF (<0.010)
Niwot Ridge	6–8	2002–2005 (1243)	<5 (<0.001)	NF (<0.010)	NF (<0.040)	15–10 (<0.040)	NF (<0.010)	NF (<0.010)	NF (<0.010)	NF (<0.010)	NF (<0.010)	NF (<0.010)	>0.1 (<0.040)	NF (<0.010)	<30 (<0.040)
Sky Oaks	5–6	1997–2006 (365)	<5 (<0.010)	NF (<0.040)	NA	NF (<0.010)	0–15 (<0.010)	NF (<0.010)	NF (<0.010)	NF (<0.040)	NF (<0.010)	360–200 (<0.010)	>0.275 (<0.010)	NF (<0.010)	NF (<0.040)
Santa Rita Mesquite	8–9	2000–2004 (156)	<5 (<0.010)	NF (<0.001)	7–14 (<0.040)	NF (<0.040)	NF (<0.040)	NF (<0.040)	NF (<0.040)	NF (<0.010)	NF (<0.010)	NF (<0.040)	>0.15 (<0.040)	NF (<0.040)	15–40 (<0.040)
Tapajos	1–12	2000–2004 (4598)	<5 (<0.010)	NF (<0.001)	NF (<0.040)	22–28 (<0.010)	NF (<0.010)	60–80 (<0.001)	NF (<0.001)	NF (0.001)	NF (<0.040)	NF (<0.001)	0.2–0.8 (<0.001)	NF (<0.010)	NF (<0.001)

NF indicates that no filter was necessary to apply for the associated variable because NEE and the variable already appeared decoupled ( $R^2 < 0.04$ ). Numbers in parentheses below each filter range indicate the  $R^2$  of a simple regression model between filtered NEE and each variable, except under the column 'years', where parentheses indicate the sample size. PAR is photosynthetically active radiation,  $R_{\text{net}}$  is net radiation, SWC is soil water content,  $T_a$  is air temperature,  $T_s$  is soil temperature, RH is relative humidity, VPD is vapor pressure deficit,  $T_f$  is leaf temperature,  $u$  is wind speed, WD is wind direction,  $u^*$  is friction velocity, H is sensible heat, and LE is latent heat. No SWC data were available from Sky Oaks and no nighttime data were available from Barrow due to the short duration of summer nights at high latitude.

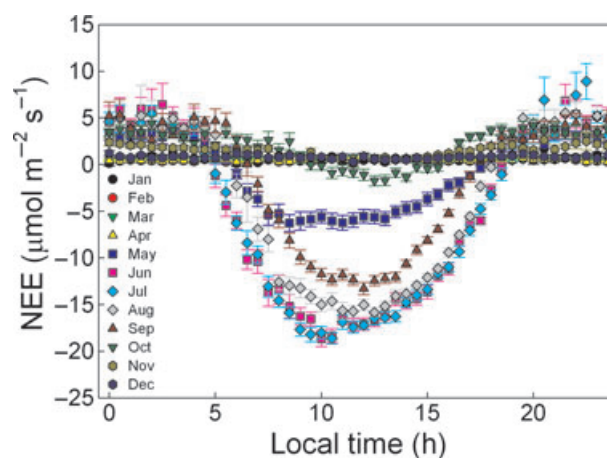
(Table 1). Because of the data filtering described below, we were particularly interested in stations with relatively long records (Barrow, Niwot Ridge, and Sky Oaks), or with additional soil respiration measurements (Bartlett, Howland, SRM, Tapajos), allowing comparison between the net CO<sub>2</sub> ecosystem exchange (NEE) and soil respiration (SR) patterns. Methods for collecting and processing the NEE and SR data have been described elsewhere (Table 1) and are typical for the AmeriFlux network. The NEE observations at sites with tall canopies were determined as the sum of the eddy covariance flux above the vegetation and the change in air column storage within the canopy space when data were available.

The flux data used in this study were processed and screened to ensure only high-quality data were used in this analysis. For all sites, this includes: (1) screening out data when rain/snow/fog interfered with the instruments, (2) despiking of high frequency data before computation of covariances, (3) elimination of data collected under unfavorable wind directions, (4) applying a site-specific  $u^*$  filter to eliminate periods when turbulence was inadequate for representative measurements, and (5) eliminating periods that did not meet stationarity criteria. For sites with closed-path gas analyzers (Barlett, Howland, Niwot Ridge and Tapajos), the concentration time series were shifted to account for timing offsets, and frequency response corrections were applied to account for high frequency filtering induced by the tubing. The effect of sensible and latent heat fluxes on density fluctuations of CO<sub>2</sub> fluxes was corrected (Webb *et al.*, 1980) at sites with open-path analyzers (Barrow, Sky Oaks and Santa Rita Mesquite).

### Data filtering

We partitioned the effect of the environment on NEE from that of endogenous plant rhythms by extracting a subset of the data when the environmental effect on NEE was negligible. We selected a subset of the data when: (a) there were no seasonal differences in the daily NEE patterns (we selected only the months with highest NEE within the growing season; Fig. 1); (b) the ranges of observed environmental variables — PAR, net radiation, the ratio of direct to total photosynthetically active radiation (Appendix S2), soil water content, air and soil temperature, relative humidity, vapor pressure deficit, wind speed and direction, friction velocity, and latent and sensible heat fluxes—were narrow enough (Tables 2 and 3) that they did not significantly affect NEE ( $r^2$  between NEE and each variable was lower than 0.01–0.04), and (c) there was little day-to-day variation in environmental variables and in antecedent conditions such that the environmental conditions over the 2–3 days prior to each NEE observation were similar to the day of measurement. These requirements ensured that photochemical energy supply and environmental factors directly affecting carbon assimilation were effectively constant.

For clarity, we explain the procedure followed in data filtering using the example of the Bartlett site, although the same procedure was followed at each of the seven sites. First, we discarded all daytime and nighttime observations during peri-



**Fig. 1** Daily Net Ecosystem Exchange pattern for each month at Bartlett. We selected only data from June and July, as half-hourly differences between these months were, on average,  $<1.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Error bars indicate standard errors ( $n = 70,128$ ).

ods when the friction velocity ( $u^*$ ) was below a minimum threshold. This threshold was based on previous publications for the site (Table 2;  $0.375 \text{ m s}^{-1}$  in Bartlett), and was determined to eliminate any statistical relationship between NEE and  $u^*$  ( $P > 0.1$ ). Second, we avoided seasonal effects by selecting only those growing season months with a comparable daily pattern of NEE (June and July; differences across each half-hour bin are smaller than  $1.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ , Fig. 1). Only these 2 months were used in subsequent analyses. Third, these data were divided into night ( $\text{PAR} < 5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and day. For daytime analyses, we selected NEE values that occurred on the asymptotic (saturating) part of the NEE vs. PAR curve. Variation in PAR within this asymptotic region explained less than 1% of the variation in NEE data ( $\text{PAR} > 1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  in Bartlett). PAR cut-off values for the other sites are given in Table 2. Fourth, we used a simple regression to quantify the correlation between NEE and the diffuse PAR fraction, air temperature ( $T_a$ ), soil temperature ( $T_s$ ), net radiation ( $R_{\text{net}}$ ), soil water content (SWC), relative humidity (RH), vapor pressure deficit (VPD), wind speed ( $u$ ), wind direction (WD), friction velocity ( $u^*$ ), sensible (H) and latent (LE) heat flux. We selected the environmental variable that had the largest effect on NEE (VPD for Bartlett,  $r^2 = 0.21$ ,  $p < 0.01$ ), and defined values within a narrow range of VPD, such that VPD variation accounted for less than 1% of NEE variation (for Bartlett,  $\text{VPD} = 1.5\text{--}1.8 \text{ kPa}$ ,  $r^2 < 0.01$ ;  $P = 0.97$ ). Fifth, we analyzed the correlation of this subset of NEE data with the abovementioned environmental variables after applying the VPD filter, and observed that RH was then the most important variable affecting NEE. Hence, we selected NEE values with RH between 40% and 70%, and NEE variation then became independent of RH ( $r^2 = 0.01$ ;  $P = 0.039$ ). We followed this procedure until we obtained a subset of the NEE data where all of the environmental variables had a negligible ( $r^2 < 0.04$ ) effect on NEE.

The filter also included  $u$ , WD, and  $u^*$  to avoid the potential influence of changing meteorological conditions, such as daily

changes in local wind patterns affecting the footprint of the eddy covariance measurement, or micro-to-mesoscale meteorological effects such as convection. The effect of early-morning releases of nighttime-stored CO<sub>2</sub> in tall forest canopies was assumed to be negligible because the filter selected data at least 2.5–3 h after dawn (defined as PAR >5 μmol m<sup>-2</sup> s<sup>-1</sup>; Table 4).

We also assessed whether: (a) daily NEE was within one standard deviation of the mean NEE for the study period (determined for the aforementioned subset) to ensure that filtered NEE data were representative of the larger NEE dataset; and (b) daily NEE up to 2 days before a particular NEE observation was within one standard deviation of the mean NEE. This assessment was necessary to minimize day-to-day variation and ensure comparable antecedent conditions; our goal was to exclude NEE data from days for which the near-past environmental conditions could have been quite different from the day in question (Resco *et al.*, 2009b; Savage *et al.*, 2009). However, the magnitude of daily variation in NEE under a constant environment after and before applying this filter for antecedent conditions was not significantly different (*t*-test; *P* = 0.8).

Finally, we discarded individual half-hour bins with a sample size less than 5, to avoid the effects of a low sample size. Final sample sizes varied across stations, ranging from 160 to 2114 data points per site for daytime and from 136 to 4598 for nighttime data (Tables 2 and 3). On average, there were 104 data points for any given time period and site. Normality of the filtered dataset was tested with a quantile-quantile plot.

Because leaf and air temperature can be temporarily decoupled (Smith, 1978; Helliker & Richter, 2008), we also calculated leaf temperature (*T<sub>l</sub>*; Blanken & Black, 2004):

$$T_l = T_a + \left( \frac{1}{\rho C_p} \right) \frac{H}{G_a} \quad (\text{Eqn 1})$$

where *T<sub>a</sub>* is air temperature, *ρ* and *C<sub>p</sub>* the density and specific heat of air, respectively, *H* the sensible heat flux, and *G<sub>a</sub>* the aerodynamic conductance (calculation in Appendix S1). We

observed that, after filter application, the effect of leaf temperature was also negligible (*r*<sup>2</sup> < 0.01, Tables 2 and 3).

### Statistical analyses

If regulation of NEE by endogenous processes is present, variation in the filtered dataset should vary significantly with time. However, incorporating the effects of changes in the environment should not significantly increase the goodness-of-fit of a model based solely on time. This was tested by fitting a linear model that only included time (model 1):

$$NEE_f = a + b \cos(2\pi t/24) + c \sin(2\pi t/24) + \varepsilon \quad (\text{Eqn 2})$$

where *NEE<sub>f</sub>* indicates filtered NEE, *t* indicates time (in hours) and *a*, *b*, and *c* indicate fitting parameters. We compared the performance of model 1 to that of a mixed model (*sensu* Crawley, 2007) that extended Eqn 2 to also include PAR, *T<sub>a</sub>*, and VPD as continuous random terms (model 2). The models were fit using maximum likelihood by assuming that the errors (*ε*) are normally distributed; ten-thousand Monte Carlo iterations were run to obtain maximum likelihood estimates (MLEs) and approximately 95% confidence intervals. Appendix S3 presents the R code for the analyses. Only VPD, *T<sub>a</sub>*, and PAR were selected in the mixed model, as these are considered the main drivers of daytime ecosystem assimilation (Lasslop *et al.*, 2010).

To avoid the potentially problematic assumptions of normally distributed errors and of the functional forms assumed in the linear models above, we also parameterized the Self-Organizing Linear Output map (SOLO; Hsu *et al.*, 2002), an Artificial Neural Network that has been proposed for use as a benchmark to evaluate the performance of land surface models (Abramowitz, 2005). The relationship between inputs and outputs in SOLO is established in three stages. First, the Self Organized Feature Map (SOFM; Kohonen, 1989) is used to classify input data into different nodes. A linear regression is then performed between input and output data within each node. This results in a piecewise linear regression of the training data. Following the same logic as in the previous analysis, we

**Table 4** Temporal changes in NEE and canopy conductance (*G<sub>c</sub>*), the effect of PAR on filtered NEE and time periods selected by the filter

Site	Diurnal ΔNEE (μmol m <sup>-2</sup> s <sup>-1</sup> )	Filtered ΔNEE (μmol m <sup>-2</sup> s <sup>-1</sup> )	Filtered ΔNEE normalized (%)	Filtered Δ <i>G<sub>c</sub></i> normalized (%)	Sensitivity to changes in PAR (±95% CI)	Dawn-dusk (h)	Daytime filtering (h)
Barrow	2.16	1.76	89.57	48.06	0.05 (±0.17)	00.00–23.30	07.00–20.30
Bartlett	6.86	3.56	20.18	25.34	−0.39 (±1.13)	04.00–19.30	09.00–15.30
Howland	7.41	4.77	32.88	38.41	0.53 (±0.61)	03.30–19.30	07.00–15.30
Niwot Ridge	4.84	4.51	40.69	50.08	0.29 (±0.59)	04.30–19.00	07.30–16.00
Sky Oaks	1.55	1.62	24.96	21.80	−0.04 (±0.58)	04.00–20.00	08.00–16.00
SRM	3.29	3.25	44.77	44.77	0.32 (±1.19)	06.30–19.30	09.30–16.00
Tapajos	15.66	17.24	84.76	61.64	−0.77 (±0.66)	06.00–18.30	08.30–16.30

ΔNEE (minimum NEE – maximum NEE) from Fig. 2 across the study period before (diurnal) and after (filtered) applying the filter, ratio of filtered ΔNEE and Δ*G<sub>c</sub>* to minimum NEE and *G<sub>c</sub>* after filtering (filtered ΔNEE and Δ*G<sub>c</sub>* normalized, respectively), sensitivity of endogenous NEE to the changes in PAR that occurred during the filtered dataset, time of dawn and dusk (PAR >5 μmol m<sup>-2</sup> s<sup>-1</sup>) and the part of the day that resulted after filtering in local time.

compared the performance of two different SOLO parameterizations for each site. The first one included time as the only input (model 3), and the second one included time, PAR,  $T_a$ , and VPD (model 4).

These different models were compared with the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC). Simplifying, both criteria evaluate the maximized value of the likelihood function (related to 'goodness-of-fit') and 'penalize' for increasing model parameters, with BIC putting greater emphasis on penalization. The model with the lowest AIC and BIC is deemed the better model; AIC and BIC are computed as:

$$\text{AIC} = -2L(\text{MLE}) + 2p \quad (\text{Eqn 3})$$

$$\text{BIC} = -2L(\text{MLE}) + \log(n)p \quad (\text{Eqn 4})$$

where  $L(\text{MLE})$  is the likelihood function evaluated at the maximum likelihood estimates,  $p$  is the number of parameters and  $n$  is the number of observations. Akaike Information Criterion reduction ( $\Delta\text{AIC}$ ) was slightly modified from Burnham & Anderson (2002) and calculated as  $\text{AIC}_t - \text{AIC}_{te}$ , where  $\text{AIC}_t$  indicates AIC values for models incorporating only time (models 1 and 3), and  $\text{AIC}_{te}$  is for models incorporating time and environmental variables (models 2 and 4).  $\Delta\text{BIC}$  was computed similarly to  $\Delta\text{AIC}$ . It is important to note that we only compared models 1 with 2 and 3 with 4, and no attempt was made for a full comparison of the 4 models. In addition, the root mean square error and mean absolute error were calculated for models 3 and 4. Statistical analyses were implemented in R (R Development Core Team, 2011), using the packages 'MCMCpack' for linear regression with Monte Carlo simulations (Martin *et al.*, 2011), 'lme4' for mixed models (Bates *et al.*, 2011) and 'languageR' (Baayen, 2011) to extract confidence intervals in mixed model estimates.

## Results

The filter was effective in removing the direct effects of the environment on filtered NEE; the coefficient of determination ( $r^2$ ) between filtered NEE and each environmental variable was always lower than 0.04 (Tables 2 and 3). However, a similar temporal pattern between filtered and nonfiltered NEE emerged at all sites even after decoupling NEE from environmental variation. Indeed, time of day was the only covariate significantly correlated with filtered daytime NEE, and the significance of time persisted across all sites except the chaparral site (Fig. 2a-g). A large portion of the day was left out at all sites as a result of selecting only high PAR values. For instance, data for only 6.5 h, from 09:30 to 16:00 hours, were available at the savanna site (Fig. 2f). However, even in this extreme case, there is a statistically significant and visually apparent decline in both filtered and nonfiltered NEE through time.

Following Burnham & Anderson (2002),  $\Delta\text{AIC}$  shows that model 1 was more likely than model 2 at Bartlett,

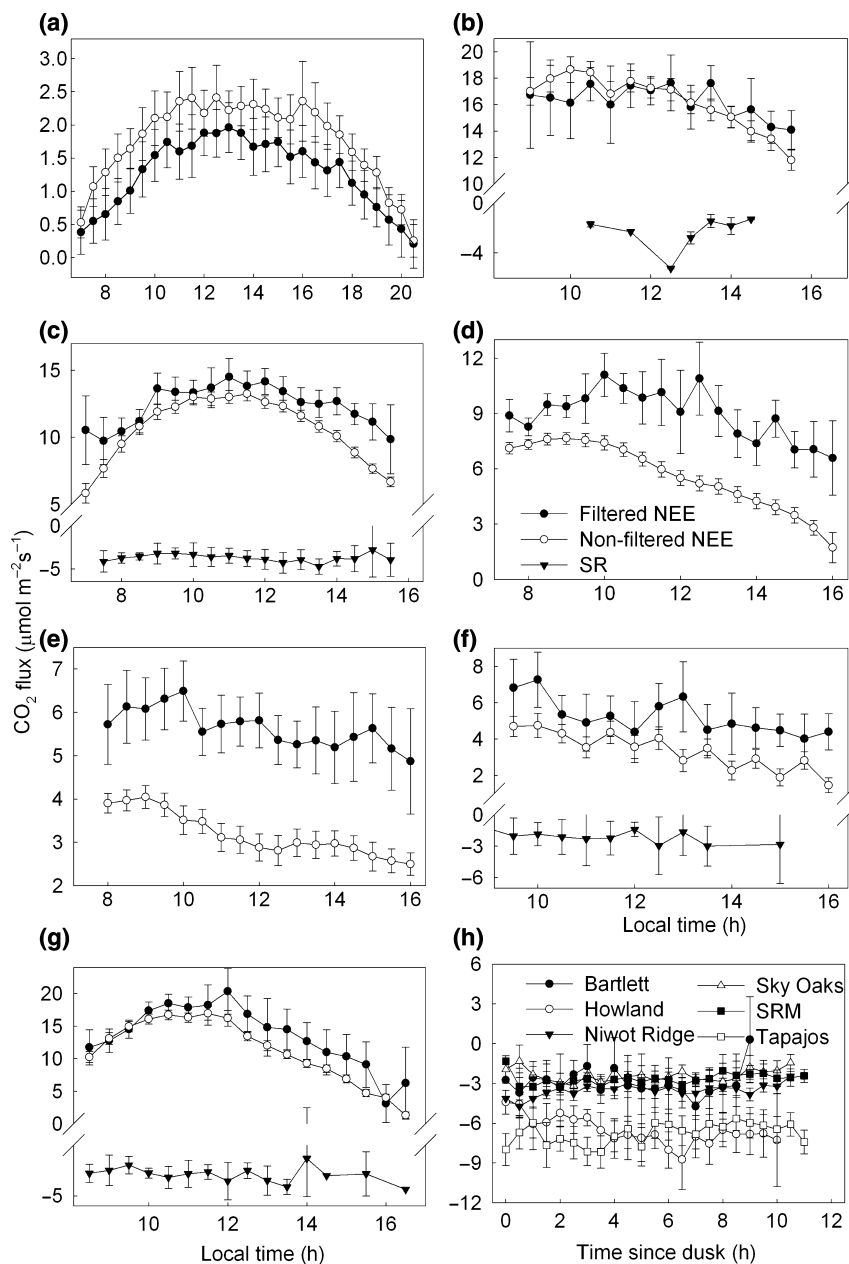
Niwot Ridge, and Santa Rita Mesquite; that models 1 and 2 were equally likely at Tapajos; and that model 2 was more likely than model 1 at Barrow, Howland, and Sky Oaks.  $\Delta\text{BIC}$  indicates that model 1 is more likely than model 2 at all sites except Barrow and Sky Oaks. Thus, we can conclude that the inclusion of PAR,  $T_a$ , VPD in model 2 did not significantly improve model performance as compared with considering only time (model 1) at all sites except at the tundra and chaparral sites. That is, the effect of time on filtered NEE supercedes the effect of these environmental variables (Fig. 2a-g, S6, Table 5).

However, it could be argued that this statistical modeling approach is arbitrary and not very flexible as, amongst other potential problems, certain assumptions about the distribution of the data and functional relationships are made. Thus, one may argue that a non-parametric approach, such as the SOLO algorithm, is more appropriate. Results from the SOLO algorithm further indicate a nonsignificant effect of environmental variables at nearly all sites. We observed a smaller AIC in the model that was solely dependent on time (model 3) than on the model with time and environmental drivers (model 4) at all sites except the chaparral site (Table 5). The root mean square error and the mean absolute error for both models are very similar, and the increase in AIC observed in model 4 was largely due to increasing the number of parameters. Examination of the 95% CI in Fig. 2 also indicate that Sky Oaks is the only site where no statistical differences across half-hour average values are observed.

A sensitivity analysis reinforced the finding that environmental variation exerted a negligible effect on filtered NEE. It could be argued that, despite the restrictive filtering, PAR could still exert some influence over NEE under constant conditions. However, the effect of changing PAR under constant conditions was rather limited. Following the Bartlett example, we observed that the estimated slope of the relationship between filtered NEE and PAR was  $-0.00062$ . Mean half-hour PAR at Bartlett varied by  $632.2 \mu\text{mol m}^{-2} \text{s}^{-1}$  (spanned from 1732.3 (at 12:30 hours) to 1100.2  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (at 16:00 hours) after filtering, a range of  $632.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The expected change in the filtered NEE to this change in PAR (that is,  $632.2 \times 0.00062$ ) was  $0.39 \mu\text{mol m}^{-2} \text{s}^{-1}$ , which could not explain the much larger variation in NEE observed under constant conditions (range  $=3.56 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; Fig. 2c; Table 4). The sensitivity of NEE to changes in PAR for all sites is given in Table 4, where the 95% CI revealed that no slope was statistically different from zero.

The filter selects for a limited set of NEE values, and this selection is based on visual inspection of the relationship between NEE and each environmental variable.





**Fig. 2** Temporal variation in Net Ecosystem Exchange (NEE) of CO<sub>2</sub> and soil respiration (SR). Daytime data for (a) Barrow (tundra), (b) Bartlett (hardwood temperate forest), (c) Howland (coniferous temperate forest), (d) Niwot Ridge (subalpine conifer forest), (e) Sky Oaks (chaparral), (f) Santa Rita Mesquite (mesquite savannah), and (g) Tapajos (tropical forest). (h) Nighttime variation in filtered NEE as a function of time since dusk (when PAR < 5 μmol m<sup>-2</sup> s<sup>-1</sup>). The X and Y axis are scaled differently because the period with constant conditions and flux magnitude, respectively, differed across sites. Error bars represent 95% CI Sample size is given in Tables S2 and S3. ‘Non-filtered NEE’ represents NEE for the same part of the year as filtered NEE.

It could thus be argued that if different thresholds had been selected for the acceptance of data, a different result would have emerged. In our preliminary analyses, we selected different combinations of environmental conditions until the final *r*<sup>2</sup> conditions were met, and the temporal pattern of filtered NEE remained unchanged (data not shown).

There were differences in the pattern of filtered NEE across sites. A morning increase in filtered NEE can be seen at the tundra (Fig. 2a), conifer temperate (Fig. 2c), subalpine coniferous (Fig. 2d), and tropical forest (Fig. 2g) sites. An afternoon decline in filtered NEE is apparent at all sites. Filtered NEE is higher than nonfiltered NEE at all sites except the tundra (Fig. 2a),

**Table 5** Parameter estimates and model comparison

Site	<i>a</i>	<i>b</i>	<i>c</i>	AIC – Model 1	AIC – Model 2	ΔAIC	BIC – Model 1	BIC – Model 2	ΔBIC
Barrow	0.75 (±0.20)	-1.11 (±0.24)	-0.44 (±0.17)	3851.50	3718.81	132.69	3871.52	3783.90	87.62
Bartlett	10.94 (±6.38)	-6.97 (±6.96)	1.08 (±1.87)	981.32	985.25	-3.93	993.70	1025.47	-31.77
Howland	4.95 (±1.54)	-9.36 (±1.78)	1.25 (±0.72)	3973.44	3935.64	37.8	3991.77	3995.20	-3.43
Niwot Ridge	5.57 (±1.25)	-3.88 (±1.64)	1.42 (±0.54)	1185.85	1193.79	-7.94	1200.08	1240.03	-39.95
Sky Oaks	6.98 (±1.08)	1.29 (±1.25)	0.70 (±0.39)	2827.70	2296.78	530.92	2845.41	2353.55	491.86
Santa Rita Mesquite	7.57 (±3.59)	2.57 (±4.03)	1.78 (±1.19)	840.87	843.98	-3.11	853.34	884.51	-31.17
Tapajos	-5.38 (±4.27)	-18.75 (±3.83)	-14.79 (±3.48)	10904.01	10902.97	1.04	10925.07	10971.41	-46.34

Site	AIC – Model 3	AIC – Model 4	ΔAIC	RMSE – Model 3 ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	RMSE – Model 4 ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	MAE – Model 3 ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	MAE – Model 4 ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )
Barrow	3860.91	3862.66	-1.75	1.37	1.36	1.04	1.05
Bartlett	1003.03	1026.81	-23.78	4.85	4.84	3.84	3.85
Howland	3987.85	3991.89	-4.04	3.76	3.71	2.85	2.78
Niwot Ridge	1225.68	1260.22	-34.54	2.45	2.50	1.93	1.91
Sky Oaks	2686.18	2628.49	57.69	2.38	2.22	1.92	1.76
Santa Rita Mesquite	739.28	779.66	-40.38	2.05	2.15	1.56	1.66
Tapajos	10914.85	10978.85	-64	10.96	11.11	8.03	8.25

Parameter estimates for model 1 (Eqn 2); comparison of models 1 and 2 and models 3 and 4. Reduction ( $\Delta$ ) Akaike and Bayesian Information Criteria (AIC and BIC) were used to compare the performance of models 1 and 2.  $\Delta$ AIC, Root Mean Square Error (RMSE) and Mean Absolute Error (MAE) were used to compare the performance of models 3 and 4.

hardwood temperate (Fig. 2b) and tropical forest sites (Fig. 2g).

It could also be argued that short-term lag effects are interfering with the response of filtered NEE. Prior conditions that do not match the filter criteria may be affecting NEE within the filtered dataset if, for instance, sudden changes from low to high PAR occur from the morning to the afternoon. We thus correlated NEE at time *t*, with PAR, VPD, and *T<sub>a</sub>* at time *t-x*, (where *x* ranges from 0.5 to 12 h). We did not observe any systematic linkage between short-term antecedent conditions and actual NEE.

**Discussion**

*NEE is not exclusively driven by direct physiological responses to environmental cues*

Based on the filtered dataset, we observed significant, rhythmic, daily variation in NEE under nearly constant environmental conditions during the daytime (Fig. 2a-g), but not during nighttime (Fig. 2h) at six of seven sites. This temporal behavior followed a generally similar pattern among sites and had a magnitude comparable to the daily NEE variation within each site (Fig. 2a-g, Table 4). The ratio of NEE variation to the maximum NEE under constant environmental conditions ranged

from 20% to 90%, with an average across sites of 48% (Table 4). Despite the large variation in the filtered dataset with time, each environmental variable under the constant environment typically explained less than 1% of NEE variation (Tables 2 and 3). This is in contrast with current conventional wisdom that daily NEE variations result almost exclusively from direct physiological responses to environmental cues.

Net ecosystem exchange is the difference between gross ecosystem-level carbon assimilation and ecosystem respiration, which is often dominated by the soil CO<sub>2</sub> efflux (Janssens *et al.*, 2001). The rhythmic behavior of daytime NEE under constant environmental conditions appears to be caused by changes in gross ecosystem-level assimilation because the filtered soil respiration data exhibited either an invariant or a different temporal pattern (Fig. 2a-g), at least at those sites with available data. Moreover, temporal changes in the rates of foliage and stem respiration would not explain the observed temporal variation in NEE because respiration is a negative contribution to NEE that tends to peak around noon (Hibbard *et al.*, 2005), which would have led to reduced NEE around that time, while we observed the opposite pattern. The presence of daytime temporal variation in NEE (i.e., a significant time effect) under constant environmental conditions, and its absence during nighttime, suggests the potential for a

strong endogenous control of photosynthesis, which in turn affects NEE.

Despite our restrictive filtering procedure, it could still be argued that other environmental or biological factors could have produced the temporal variation in observed NEE. For example, changes in the geometry of leaf angles and azimuth relative to solar elevation (Falster & Westoby, 2003) could have contributed to the observed NEE patterns. This could be particularly troublesome at the tundra site (Barrow) where monocotyledons and dicotyledons show contrasting orientations and canopy positions (Tieszen, 1978). However, leaf angles only influence carbon assimilation when radiation is direct; under diffuse radiation, the angular distribution of light is isotropic and does not change throughout the day. We repeated the filtering analysis at Barrow under high diffuse radiation (>70%) and found that the filtered NEE pattern observed at Barrow is not the result of the changes in the leaf energy balance or canopy illumination which would result from varying leaf angle (data not shown).

#### *Possible mechanisms leading to a diurnal pattern of filtered NEE*

Variation in net carbon assimilation under constant conditions could be driven by one or more endogenous mechanisms, including fluctuations in chloroplast carbohydrate concentrations, changes in stomatal conductance, photorespiration, or circadian regulation of gas exchange. Accurately quantifying the explicit importance of each of these different mechanisms would require very detailed experiments under controlled environments. Nevertheless, some hypotheses may be drawn from this study.

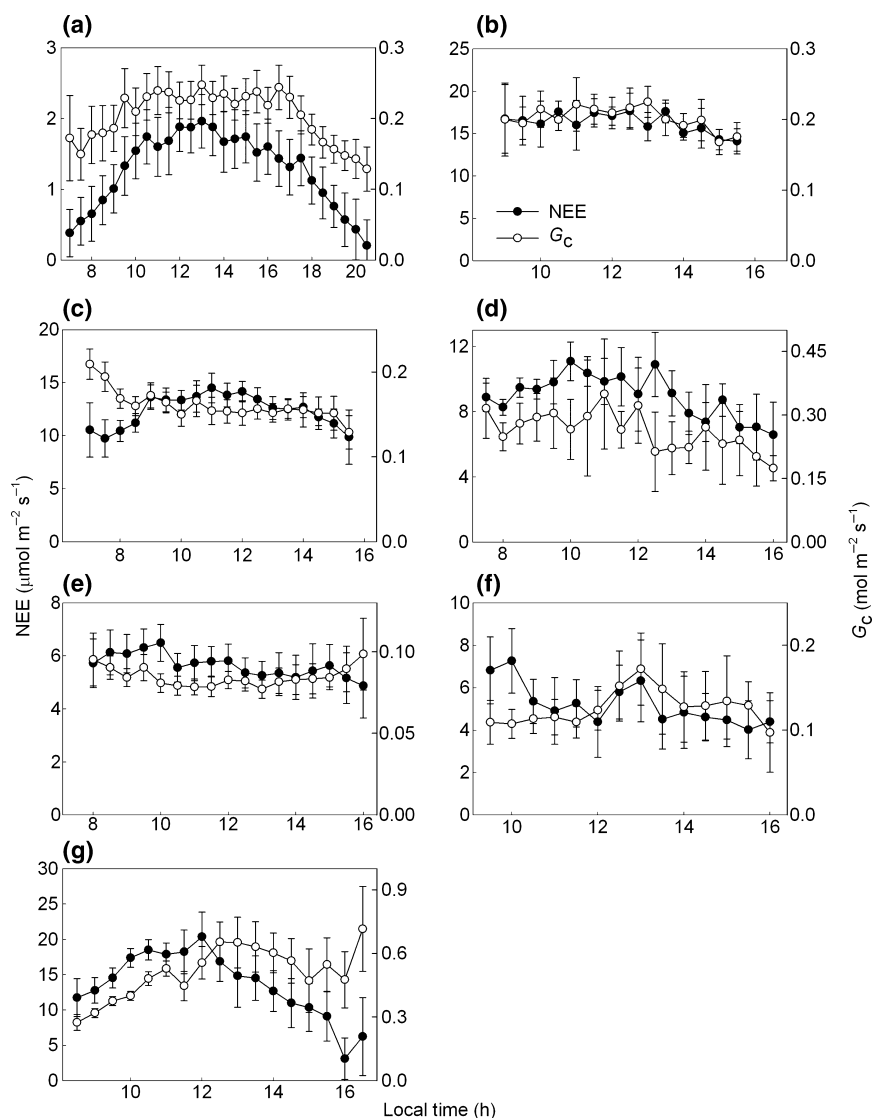
Fluctuations in carbohydrate concentrations could affect the daytime pattern of filtered NEE if source/sink dynamics drive carbon assimilation. If the sink of carbohydrates (any part of the plant to which carbohydrates are transported) saturates, a feedback inhibition on the source (leaves) carbon fixation could occur. Conversely, a large demand for carbohydrates in sink tissues could lead to greater photosynthesis. Increased carbohydrate concentrations lead to a feedback inhibition of Rubisco activity (Paul & Foyer, 2001), and this feedback could partially explain the afternoon decline in CO<sub>2</sub> uptake under constant environmental conditions observed at the temperate hardwood (Fig. 2b) and savanna (Fig. 2f) sites. However, the increasing rate of CO<sub>2</sub> uptake observed during the morning (from ~7 to 10 local time) at the tundra (Fig. 2a), conifer temperate (Fig. 2c), subalpine coniferous (Fig. 2d), and tropical forest (Fig. 2g) sites cannot be explained by carbohydrate accumulation. Moreover, as foliage

carbohydrate concentration is minimal at dawn (Smith & Stitt, 2007), we should expect maximum assimilation early in the morning if this were the main process controlling NEE under constant conditions.

Lower CO<sub>2</sub> uptake in the morning and afternoon under constant environmental conditions could be driven by reductions in either stomatal conductance or biochemical capacity (Farquhar & Sharkey, 1982). Daily changes in stomatal conductance are concurrently driven by endogenous processes (e.g., the circadian clock, increasing losses in hydraulic conductivity, morning stomatal 'sluggishness'; Dodd *et al.*, 2004; Franks, 2004) as well as by direct feedback and, potentially, feedforward loops from direct responses to environmental changes (Raschke, 1975; Buckley, 2005). Reduced stomatal conductance early and late in the day would be expected to influence photosynthetic carbon uptake leading to a draw-down of intercellular CO<sub>2</sub> concentration. A decline in intercellular CO<sub>2</sub> driven by stomatal closure could occur if the diurnal proportional change in conductance was larger than in NEE. In our filtered dataset, the changes in NEE could not be solely attributed to changes in canopy conductance. The diel change in canopy conductance was either proportionally smaller than the changes in NEE (Fig. 3a, e, f, g; Table 4) or largely decoupled from the changes in NEE (Fig. 3b, c, d). For instance, carbon assimilation at the temperate conifer forest (Fig. 3c) increased between 07:00 hours and 09:00 hours (local time) and decreased in the afternoon, whereas canopy conductance decreased between 07:00 hours and 09:00 hours and remained fairly constant afterward (Fig. 3c). This implies that the temporal patterns in NEE under constant environmental conditions are not due solely to variations in stomatal conductance. The absence of strong stomatal limitations in the afternoon does not suggest an *a priori* reason to expect diel changes in the strength of photorespiration leading to a diurnal CO<sub>2</sub> uptake pattern in the filtered dataset (Wingler *et al.*, 2000).

We therefore propose that circadian regulation of biochemical capacity is an important driver of daily NEE patterns. In particular, circadian regulation is the most plausible explanation for the morning increase in CO<sub>2</sub> uptake at the tundra (Fig. 2a), temperate conifer (Fig. 2c), subalpine coniferous (Fig. 2d), and tropical forest (Fig. 2g), and is a plausible explanation for at least part of the afternoon decline in CO<sub>2</sub> uptake under constant conditions observed at all sites.

We note that the possible endogenous controls on gas exchange are not mutually exclusive, but probably co-occur, and may shift in relative importance as the day advances. For instance, stomatal sluggishness may be more important in the morning than carbohydrate



**Fig. 3** Relationship between Net Ecosystem Exchange (NEE) and canopy conductance ( $G_c$ ) for the filtered dataset. (a) Barrow (tundra), (b) Bartlett (hardwood temperate forest), (c) Howland (coniferous temperate forest), (d) Niwot Ridge (subalpine conifer forest), (e) Sky Oaks (chaparral), (f) Santa Rita Mesquite (mesquite savanna), and (g) Tapajos (tropical forest). The X and Y axis are scaled differently because the period with constant conditions and flux magnitude, respectively, differed across sites. Error bars represent 95% CI. Sample size is given in Table S2. ‘Non-filtered NEE’ represents NEE for the same part of the year as filtered NEE.

feedbacks, which may increase in importance later in the day, whereas the circadian clock effect is likely to remain constant throughout the day.

It is important to note that endogenous regulation will not increase NEE when external conditions are limiting. Instead, endogenous regulation will yield a predictable, time varying ‘base-line’ carbon uptake capacity that will be further modified by the environmental conditions at any given moment. At the tundra site (Fig. 2a), the filter selected for constant, yet sub-optimal conditions for carbon assimilation, which resulted in lower NEE in the filtered dataset as com-

pared with the non-filtered dataset. The higher NEE values of the filtered dataset at the conifer temperate and subalpine forests, chaparral, and savanna sites indicate that the filter selected for constant and close to optimal environmental conditions.

Our hypothesis that the circadian clock affects diel patterns of NEE may sound surprising at first. It has been often argued that “nothing in biology makes sense except under the light of evolution” (Dobzhansky, 1973). Much current research on NEE lies, either implicitly or explicitly, on evolutionary assumptions about the adaptation of organisms to climatic or other

environmental factors, many of which show high interannual variability and limited predictability (such as rainfall or temperature regimes; Chapin *et al.*, 2002). However, the photoperiod is a highly predictable and constant (across years) environmental variable that exerts a central control on carbon assimilation. All photoperiod responses studied to date are regulated by the circadian clock (Yakir *et al.*, 2007), and circadian regulation of gas exchange has been explicitly linked with evolutionary success (Dodd *et al.*, 2004).

### Implications

Perhaps the most important implication in a circadian regulation of NEE is that it opens a new and promising understanding of ecological systems that truly and directly connects molecular mechanisms with land surface processes, in previously unrecognized ways. Another important conceptual implication stems from the fact that circadian rhythms are cell-autonomous systems entrained by environmental cues of light and temperature (Harmer, 2009). This implies that asynchronous circadian regulation, not only within different plant functional types (e.g., canopy trees and below-canopy shrubs), but also within a single organism (e.g., shade vs. sun leaves, photosynthesis vs. phloem loading) and, perhaps, even within a single leaf (when the outer portion is more exposed to different light and temperature regimes than the inner portion) is likely to occur as these different components will be exposed to different oscillations in the light and temperature regimes. However, this cell autonomous process seems to lead to an emergent ecosystem synchrony of gas exchange.

This emergent synchrony in endogenous regulation of NEE could perhaps pave the way to a novel generation of integrated, gene-to-ecosystem NEE models with endogenous processes as the core mechanism, and exogenous responses that would modify this core or base-line behavior. However, we first need to clarify the mechanisms underlying gas exchange regulation by the clock beyond the molecular scale.

Hewitt *et al.* (2011) incorporated circadian regulation of isoprene emissions as a high order polynomial function of time, which may be justified considering our poor mechanistic understanding of the underlying processes. For carbon assimilation models, we suspect that incorporation of empirical time-vary functions, meant to represent endogenous effects, into current mechanistic models of plant photosynthesis may often lead to only limited improvements to model fit or predictive ability. This was demonstrated by Williams & Gorton (1998), who incorporated sinusoidal temporal variation

in electron transport, carboxylation capacity, and stomatal conductance into a mechanistic model, and found only a small (but significant) improvement in the fit of the model to field data. However, we do not believe it is appropriate to represent endogenous processes within a modeling framework by adding an empirical function of time without having previously removed any environmental effects. Any increase in model fit by adding such an empirical function may just be the result of the spurious correlation of time with light, temperature or other environmental cues, or from the inaccurate mathematical representation of how these factors affect leaf biochemistry.

Close synchronization of endogenous controls with the typical daily oscillations of light, temperature, and evaporative demand usually occur in the field (Doughty *et al.*, 2006; Webb, 2003; Yerushalmi & Green, 2009; Fig. 2 a-g). Light and temperature show strong diurnal trends, and their variation is highly correlated with time. Endogenous plant rhythms also show strong diurnal trends that are similarly correlated with time. Empirical and mechanistic models of carbon assimilation already incorporate daily variations of light and temperature and are thus likely to indirectly absorb a large part of the variation in daily carbon assimilation that may be attributed to endogenous plant rhythms. In other words, the tight association between the daily pattern of light and temperature with endogenous rhythms obscures the potential importance of endogenous controls, and creates a situation where strong correlations between NEE and the physical environment may be interpreted as exclusive causation, overlooking the possibility that NEE may be controlled by a combination of endogenous and exogenous factors.

A seemingly logical conclusion would be that endogenous regulation is insignificant as its effect is already captured by our current modeling approaches, albeit indirectly. However, this assumption could reduce the predictive ability of such models, and may result in incorrect interpretations of how different environmental drivers govern NEE. That is, a danger in ignoring endogenous regulation is that current models based on the direct effects of the physical environment on leaf physiology (Hanson *et al.*, 2004) may produce a 'correct' result for a partially 'wrong' reason, potentially compromising their ability to predict CO<sub>2</sub> fluxes and carbon budgets under future no-analog, novel conditions. Thus, we argue for new research aimed at understanding the effect of different endogenous processes, and particularly the circadian clock, on NEE. In the longer term, this may lead to novel and deeper understanding of ecological systems that integrates from genes to ecosystems.

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## References

- Abramowitz G (2005) Towards a benchmark for land surface models. *Geophysical Research Letters*, **32**, L22702.
- Baayen RH (2011) languageR: Data Sets and Functions with "Analyzing Linguistic Data: A Practical Introduction to Statistics". R Package Version 1.2. Available at: <http://CRAN.R-project.org/project=languageR> (accessed 23 October 2011).
- Bahn M, Kutsch W, Heinemeyer A (2010) Synthesis: emerging issues and challenges for an integrated understanding of soil carbon dynamics. In: *Soil Carbon Dynamics. An Integrated Methodology* (eds Kutsch W, Bahn M, Heinemeyer A), pp. 257–271. Cambridge University Press, Cambridge, UK.
- Bates D, Maechler M, Bolker B (2011) lme4: Linear Mixed-Effects Models Using S4 Classes. R package version 0.999375-42. Available at: <http://CRAN.R-project.org/package=lme4> (accessed 23 October 2011).
- Blanken PD, Black TA (2004) The canopy conductance of a boreal aspen forest, Prince Albert National Park, Canada. *Hydrological Processes*, **18**, 1561–1578.
- Brodersen CR, Mcelrone AJ, Choat B, Matthews MA, Shackel KA (2010) The dynamics of embolism repair in xylem: *in vivo* visualizations using high-resolution computed tomography. *Plant Physiology*, **154**, 1088–1095.
- Buckley TN (2005) The control of stomata by water balance. *New Phytologist*, **168**, 275–292.
- Burnham KP, Anderson DR (2002) *Model Selection and Multi-Model Inference: A Practical Information-Theoretic Approach*. Springer-Verlag, New York.
- Chapin FS, Matson PA, Mooney HA (2002) *Principles of Terrestrial Ecosystem Ecology*. Springer-Verlag, New York, USA.
- Crawley MJ (2007) *The R Book*. Wiley, Chichester, UK.
- Davidson EA, Janssens IA, Luo Y (2006) On the variability of respiration in terrestrial ecosystems: moving beyond  $Q_{10}$ . *Global Change Biology*, **12**, 154–164.
- Dobzhansky T (1973) Nothing in biology makes sense except under the light of evolution. *American Biology Teacher*, **35**, 125–129.
- Dodd AN, Parkinson K, Webb A (2004) Independent circadian regulation of assimilation and stomatal conductance in the *ztl-1* mutant of *Arabidopsis*. *New Phytologist*, **162**, 63–70.
- Doughty C, Goulden ML, Miller S, Da Rocha H (2006) Circadian rhythms constrain leaf and canopy gas exchange in an Amazonian forest. *Geophysical Research Letters*, **33**, L15404.
- Falster DS, Westoby M (2003) Leaf size and angle vary widely across species: what consequences for light interception? *New Phytologist*, **158**, 509–525.
- Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. *Annual review of plant physiology and plant molecular biology*, **33**, 317–345.
- Franks PJ (2004) Stomatal control and hydraulic conductance, with special reference to tall trees. *Tree Physiology*, **24**, 865–878.
- Goulden M, Miller S, Da Rocha H, Menton M, De Freitas H, Figueira A, De Sousa C (2004) Diel and seasonal patterns of tropical forest CO<sub>2</sub> exchange. *Ecological Applications*, **14**, S42–S54.
- Hanson PJ, Amthor JS, Wullschlegel SD *et al.* (2004) Oak forest carbon and water simulations: model intercomparisons and evaluations against independent data. *Ecological Monographs*, **74**, 443–489.
- Harazono Y, Mano M, Miyata A, Zulueta RC, Oechel WC (2003) Inter-annual carbon dioxide uptake of a wet sedge tundra ecosystem in the Arctic. *Tellus*, **55B**, 215–231.
- Harmer SL (2009) The circadian system in higher plants. *Annual Review of Plant Biology*, **60**, 357–377.
- Harmer SL, Hogenesch JB, Straume M *et al.* (2000) Orchestrated transcription of key pathways in *Arabidopsis* by the circadian clock. *Science*, **290**, 2110–2113.
- Helliker BR, Richter SL (2008) Subtropical to boreal convergence of tree-leaf temperatures. *Nature*, **454**, 511–514.
- Hennessey TL, Field CB (1991) Circadian rhythms in photosynthesis: oscillations in carbon assimilation and stomatal conductance under constant conditions. *Plant Physiology*, **96**, 831–836.
- Hewitt CN, Ashworth K, Boynard A *et al.* (2011) Ground-level ozone influenced by circadian control of isoprene emissions. *Nature Geoscience*, **4**, 671–674.
- Hibbard KA, Law BE, Reichstein M, Sulzman J (2005) An analysis of soil respiration across northern hemisphere temperate ecosystems. *Biogeochemistry*, **73**, 29–70.
- Högberg P, Read DJ (2006) Towards a more plant physiological perspective on soil ecology. *Trends in Ecology & Evolution*, **21**, 548–554.
- Hollinger DY, Kelliher FM, Byers JN, Hunt JE, Mcsevevny TM, Weir PL (1994) Carbon dioxide exchange between an undisturbed old-growth temperate forest and the atmosphere. *Ecology*, **75**, 134–150.
- Hollinger DY, Aber J, Dail B *et al.* (2004) Spatial and temporal variability in forest-atmosphere CO<sub>2</sub> exchange. *Global Change Biology*, **10**, 1689–1706.
- Hsu K, Gupta HV, Gao X, Sorooshian S, Imam B (2002) Self-organizing linear output map (SOLO): an artificial neural network suitable for hydrologic modeling and analysis. *Water Resources Research*, **38**, 1302.
- Hu J, Moore DJP, Burns SP, Monson RK (2010) Longer growing seasons lead to less carbon sequestration by a subalpine forest. *Global Change Biology*, **16**, 771–783.
- Janssens IA, Lankreijer H, Matteucci G *et al.* (2001) Productivity overshadows temperature in determining soil and ecosystem respiration across European forests. *Global Change Biology*, **7**, 269–278.
- Jenkins JP, Richardson AD, Braswell BH, Ollinger SV, Hollinger DY, Smith ML (2007) Refining light-use efficiency calculations for a deciduous forest canopy using simultaneous tower-based carbon flux and radiometric measurements. *Agricultural Forest Meteorology*, **143**, 64–79.
- Kohonen T (1989) *Self-Organization and Associative Memory*. Springer, New York.
- Lasslop G, Reichstein M, Papale D *et al.* (2010) Separation of net ecosystem exchange into assimilation and respiration using a light response curve approach: critical issues and global evaluation. *Global Change Biology*, **16**, 187–208.
- Liu Z, Taub CC, Mcclung CR (1996) Identification of an *Arabidopsis thaliana* ribulose-1,5-bisphosphate carboxylase/oxygenase activase (RCA) minimal promoter regulated by light and the circadian clock. *Plant Physiology*, **112**, 43–51.
- Luo H, Oechel WC, Hastings SJ, Zulueta R, Qian Y, Kwon H (2007) Mature semiarid chaparral ecosystems can be a significant sink for atmospheric carbon dioxide. *Global Change Biology*, **13**, 386–396.
- Martin AD, Quinn KM, Park JH (2011) MCMCpack: Markov chain Monte Carlo in R. *Journal of Statistical Software* **42**(9): 1–21. Available at: <http://www.jstatsoft.org/v42/i09/> (accessed 23 October 2011).
- Mcclung CR (2009) Linking the loops. *Science*, **323**, 1440–1441.
- Monteith J, Unsworth M (2008) *Principles of Environmental Physics*. Academic Press, San Diego, CA.
- Paul MJ, Foyer CH (2001) Sink regulation of photosynthesis. *Journal of Experimental Botany*, **52**, 1383–1400.
- R Development Core Team, 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, Available at: <http://www.R-project.org/> (accessed 23 October 2011).
- Raschke K (1975) Stomatal action. *Annual review of plant physiology and plant molecular biology*, **26**, 309–340.
- Resco V, Hartwell J, Hall A (2009a) Ecological implications of plants' ability to tell the time. *Ecology Letters*, **12**, 583–592.
- Resco V, Ewers BE, Sun W, Huxman TE, Weltzin JF, Williams DG (2009b) Drought-induced hydraulic limitations constrain leaf gas exchange recovery from drought after precipitation pulses in the C<sub>3</sub> woody legume, *Prosopis velutina*. *New Phytologist*, **181**, 672–682.
- Savage K, Davidson E, Richardson AD, Hollinger DY (2009) Three scales of temporal resolution from automated soil respiration measurements. *Agricultural and Forest Meteorology*, **149**, 2012–2021.
- Scott R, Jenerette G, Potts D, Huxman TE (2009) Effects of seasonal drought on net carbon dioxide exchange from a woody-plant-encroached semiarid grassland. *Journal of Geophysical Research*, **114**, G04004.
- Sellers PJ, Dickinson RE, Randall DA *et al.* (1997) Modeling the exchanges of energy, water, and carbon between continents and the atmosphere. *Science*, **275**, 502–509.
- Smith WK (1978) Temperatures of desert plants: another perspective on the adaptability of leaf size. *Science*, **201**, 614–616.
- Smith AM, Stitt M (2007) Coordination of carbon supply and plant growth. *Plant, Cell & Environment*, **30**, 1126–1149.
- Tieszen LL (ed) (1978) *Vegetation and Production Ecology of an Alaskan Arctic Tundra*. Springer-Verlag, New York.
- Trumbore SE (2006) Carbon respired by terrestrial ecosystems – recent progress and challenges. *Global Change Biology*, **12**, 141–153.

Webb AR (2003) The physiology of circadian rhythms in plants. *New Phytologist*, **160**, 281–303.

Webb E, Pearman G, Leuning R (1980) Correction of flux measurements for density effects due to heat and water vapour transfer. *Quarterly Journal of the Royal Meteorological Society*, **106**, 85–100.

Williams WE, Gorton HL (1998) Circadian rhythms have insignificant effects on plant gas exchange under field conditions. *Physiologia Plantarum*, **103**, 247–256.

Wingler A, Lea PJ, Quick WP, Leegood RC (2000) Photorespiration: metabolic pathways and their role in stress protection. *Philosophical Transactions: Biological Sciences*, **355**, 1517–1529.

Yakir E, Hilman D, Harir Y, Green RM (2007) Regulation of output from the plant circadian clock. *FEBS Journal*, **274**, 335–345.

Yerushalmi S, Green RM (2009) Evidence for the adaptive significance of circadian rhythms. *Ecology Letters*, **12**, 970–981.

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

- Appendix S1.** Estimation of canopy conductance.
- Appendix S2.** Estimation of diffuse and direct radiation.
- Appendix S3.** R code for models 1 and 2.
- Appendix S4.** Supplementary references.

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