

# Diel patterns of autotrophic and heterotrophic respiration among phenological stages

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## Abstract

Improved understanding of the links between aboveground production and allocation of photosynthate to belowground processes and the temporal variation in those links is needed to interpret observations of belowground carbon cycling processes. Here, we show that combining a trenching manipulation with high-frequency soil respiration measurements in a temperate hardwood forest permitted identification of the temporally variable influence of roots on diel and seasonal patterns of soil respiration. The presence of roots in an untrenched plot caused larger daily amplitude and a 2–3 h delay in peak soil CO<sub>2</sub> efflux relative to a root-free trenched plot. These effects cannot be explained by differences in soil temperature, and they were significant only when a canopy was present during the growing season. This experiment demonstrated that canopy processes affect soil CO<sub>2</sub> efflux rates and patterns at hourly and seasonal time scales, and it provides evidence that root and microbial processes respond differently to environmental factors.

**Keywords:** autotrophic, diel patterns, heterotrophic, phenology, soil respiration, temperate

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## Introduction

Efflux of CO<sub>2</sub> from the soil surface, hereafter referred to as total soil respiration ( $R_t$ ), generally exhibits a diel pattern that is related to temperature. While it is logical that variation in temperature should affect biological enzymatic processes of soil microorganisms, plant roots and their mycorrhizal symbionts, the temperature response of  $R_t$  is not always simple, because (1) heterotrophic respiration ( $R_h$ ) and autotrophic respiration ( $R_a$ ) may have variable responses (Gu *et al.*, 2008); and (2) supply of carbon substrate to roots could also follow temporal patterns related to aboveground processes (Davidson & Holbrook, 2009; Kuzyakov & Gavrichkova, 2010). Teasing out these sources of variation in  $R_t$  is important, so that estimates of the temperature response of  $R_h$  and decomposition of soil organic matter are not obscured or inflated by other covarying processes (Davidson *et al.*, 2006a,b). Improved understanding of the links between aboveground production and allocation of photosynthate to belowground processes will also improve our understanding of controls on belowground carbon cycling processes (Trumbore, 2006).

Significant lags between peak  $R_t$  and peak soil temperature on diel time scales and inferred lags of hours to days with aboveground environmental cues, such as light and vapor pressure deficits, provide evidence for

partial decoupling of  $R_t$  from strict control by only the belowground environment (Tang *et al.*, 2005; Liu *et al.*, 2006; Savage *et al.*, 2009). These lags could be related either to the time required for recent photosynthate transport through the phloem to the roots or from a pressure wave function related to phloem loading to affect root activity (Davidson & Holbrook, 2009; Kuzyakov & Gavrichkova, 2010). If recent photosynthate is the primary source of carbohydrates for  $R_a$  and possibly also for  $R_h$  through root exudates, then temporal variation in  $R_t$  may be linked to plant phenological patterns at hourly to seasonal time scales. In ecosystems that experience strong seasonality, such as temperate hardwood forests, the link between aboveground and belowground processes is likely to vary with season, depending upon the presence and activity of the canopy.

Several methods have been developed and evaluated to tease out the components of  $R_t$ , such as isotopic analyses, and removing  $R_a$  input through tree girdling and root exclusion experiments (Hanson *et al.*, 2001; Kuzyakov, 2006; Subke *et al.*, 2006). Trenching involves severing the rooting system leading into a plot to remove the  $R_a$  component within the plot. This method has some potential limitations. Reduced water uptake in trenched plots could change soil water content, which is one of the environmental controllers of  $R_t$  in many ecosystems. Eliminating root inputs could reduce heterotrophic decomposition of SOM via lack of priming (Cheng, 2009). On the other hand, the severed dead roots may temporarily increase available carbon substrate for  $R_h$ .

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Although quantitative estimates of  $R_a$  and  $R_h$  may be uncertain due to possible artifacts, the trenching method offers new possibilities for revealing temporal patterns when combined with high temporal frequency soil respiration measurements. Automated soil respiration measurements permit analysis of diel patterns across seasons. The objective of this study was to reveal whether and when the contribution of  $R_a$  alters diel patterns of  $R_t$  relative to  $R_h$ . We hypothesize that  $R_a$  is of sufficient magnitude when the forest canopy is present that the diel pattern of  $R_t$  is shifted relative to the diel pattern of  $R_h$ . We also hypothesize that estimates of the temperature sensitivity of  $R_t$ , as expressed by a simple  $Q_{10}$  function, will overestimate the temperature sensitivity of heterotrophic decomposition of soil carbon ( $R_h$ ), because of seasonally varying contributions of  $R_a$  that are confounded with temperature.

## Materials and methods

Soil respiration was measured at the Harvard Forest near Petersham, Massachusetts USA (42°32'N, 72°11'W). This site is at 340 m elevation and is characterized by a mixed hardwood forest (dominate species *Q. rubra* and *A. rubrum*), approximately 60-years-old and 16–20 m height. Soils are well drained and classified as Canton fine sandy loam, Typic Distrochrepts. The mean annual temperature is +7.9 °C and the mean annual precipitation is 1100 mm.

On Nov 6, 2008, a trench was excavated to 1 m depth around a 5 m × 5 m area, severing all roots leading into the trenched plot. Plastic tarp was placed along the walls of the trench and then backfilled. Three automated soil respiration chambers were placed in each of the trenched and un-trenched plots. This site has little understory vegetation, but what was present in the treatment plot was clipped on May 29, 2009. Automated measurements of soil respiration were made once per half hour for each chamber. Pretrenching flux measurements for all six chambers were made from Aug 22nd to Nov 5th, 2008, and posttrenching measurements were made from Apr 22th through Oct 31st, 2009. For a description of the automated system and data quality procedures, see Savage & Davidson (2003), Savage *et al.* (2008).

The measured fluxes from the trenched plot represent the heterotrophic component ( $R_h$ ) of soil respiration, whereas total soil respiration ( $R_t = R_a + R_h$ ) was measured in the control plot. The difference between measured fluxes represents auto-trophic respiration ( $R_a = R_t - R_h = \text{control plot flux} - \text{trenched plot flux}$ ). However, it should also be remembered that  $R_a$  also includes mycorrhizal respiration and potentially some microbial respiration of fresh root exudates. We did not attempt to estimate the effects of increased labile carbon (from severed roots) within the trenched plots, due to large uncertainties in estimation of fine root decay rates. To minimize the impact of severed roots, the measurements in the trenched plot were started 6 months after the trenching.

Within the control and trenched plots, volumetric soil moisture was measured at 8–16 cm depth every 15 min using

Campbell Scientific Water Content Reflectometer (CS616) probes. Data were aggregated to half hourly intervals to correspond to respiration measurement rates. Soil temperature was measured (type T-thermocouple) at 10 cm depth at half hourly intervals. Air temperature, precipitation, and photosynthetically active radiation (PAR) were measured every 15 min at the Harvard Forest meteorological station, located approximately 1.6 km from the study site. Data were aggregated to half hourly sampling intervals.

Occasional malfunction of the data logging system resulted in some missing soil moisture data (14%) in the control and trenched plots. These data gaps were filled by using soil moisture data from a soil pit approximately 1 mile away (Savage & Davidson, 2001) that was instrumented with the same water content probes at similar depths. When soil moisture probes from both sites were functioning, their correlations were strong:  $R^2 = 0.80$  ( $P < 0.0001$ ).

Statistical models used for the seasonal relationship of measured respiration rates and environmental variables temperature and moisture follow Savage *et al.* (2009):

$$R = R_{\text{ref}} \times Q^{\frac{(T_s - 10)}{10}} \quad (1)$$

$$R = R_{\text{ref}} \times D^{(M_{\text{opt}} - M)} \times Q^{\frac{(T_s - 10)}{10}} \quad (2)$$

where,  $R$  represents respiration, either  $R_t$ ,  $R_h$ , or  $R_a$ ,  $R_{\text{ref}}$  is the reference respiration ( $\text{mg C m}^{-2} \text{ hr}^{-1}$ ) at 10 °C,  $Q$  is the  $Q_{10}$  function which is the change in respiration with a 10 °C change in temperature,  $T_s$  is half hourly soil temperature 10 cm depth ( °C),  $D$  is a unit less parameter,  $M$  is soil moisture ( $\text{cm}^3 \text{ H}_2\text{O cm}^{-3}$  soil) from 8–16 cm,  $M_{\text{opt}}$  is the optimum soil moisture which, based on our previous work at the Harvard Forest (Savage *et al.*, 2009), is set at the maximum% soil moisture content for the control and trenched plot.

Diel patterns of respiration for  $R_t$ ,  $R_h$ , and  $R_a$  were modeled using a sine wave function:

$$R = yo + A \times \sin\left(\left(\frac{2 \times \pi \times \text{TOD}}{2400}\right) + C\right) \quad (3)$$

where  $yo$  represents the mean respiration rate over the time period modeled ( $\text{mg C m}^{-2} \text{ hr}^{-1}$ ),  $A$  is diel amplitude ( $\text{mg C m}^{-2} \text{ hr}^{-1}$ ),  $C$  corresponds to the shift of minimum and maximum diel peaks (radians), and  $\text{TOD}$  is time of day in hundreds.

Weighted absolute deviations (WAD) were used to fit all models:

$$\Omega = \sum_{i=1}^n \frac{|y_i - y_{\text{pred}}|}{\sigma(\delta i)} \quad (4)$$

where  $y_i$  is the observed respiration ( $\text{mg C m}^{-2} \text{ hr}^{-1}$ ),  $y_{\text{pred}}$  is the predicted respiration ( $\text{mg C m}^{-2} \text{ hr}^{-1}$ ), and  $\sigma(\delta i)$  is a weighting function. Following a procedure proposed by Hollinger & Richardson (2005) using a paired observation approach, the random error was characterized by an estimate of its standard deviation, and is used as the weighting function  $\sigma(\delta i)$  in Eqn (4). Model parameters were then optimized by minimizing Eqn (4) using the Gauss-Newton method in R2.7.1. As the WAD method does not produce an  $R^2$  value, the method developed by Richardson *et al.* (2006a,b) to derive a

Figure-of-Merit ( $FMR^2$ ) value was used. The  $FMR^2$  value from the WAD model fitting procedure is similar to the multiple correlation coefficient  $R^2$  from the OLS fitting procedure.

The trenching experiment was not replicated, due to the prohibitive costs of building and maintaining large numbers of automated soil respiration chambers distributed across a large area. Distribution of the six chambers was constrained by the need to connect each one to a single, centralized system for gas analysis, valve operation, and data logging. There is also a tradeoff between having more small plots with a greater edge effect in each due to higher perimeter-to-area ratio, or fewer large plots with less edge effect. In this study, the coefficient of variation (CV) of soil respiration was 27% within the trenched plot and 21% within the control plot, which is similar to the 22% CV measured during a pretreatment period among six fully replicated plots of a previous throughfall exclusion experiment at the Harvard Forest (Borken *et al.*, 2006). We conclude that there is as much spatial variation within these 5 m × 5 m plots as among them.

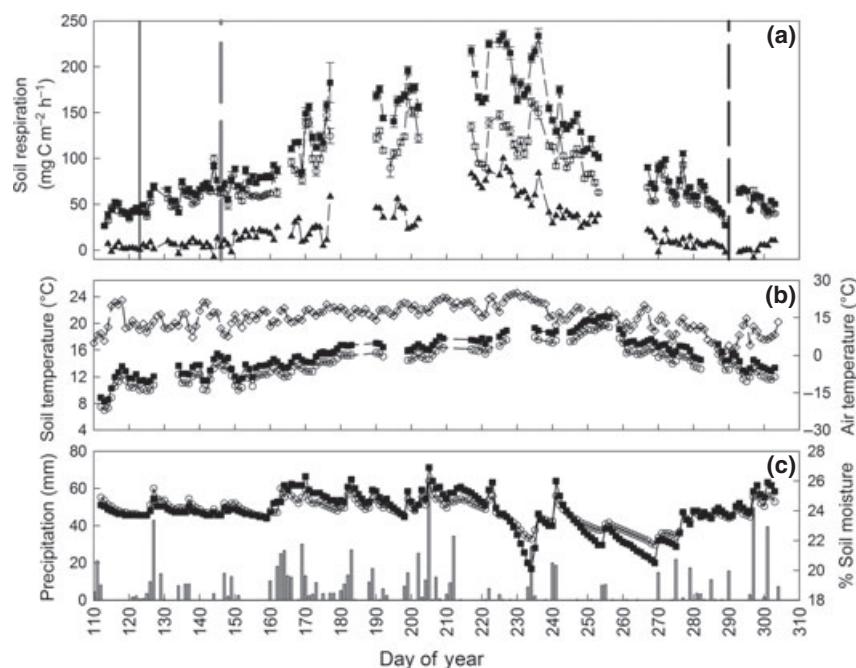
Nevertheless, true replication would be necessary if our objective were to make quantitative estimates of mean  $R_t$ ,  $R_h$ , and  $R_a$  that are meant to be representative of the entire forest. Instead, our objective was to demonstrate temporal patterns, which tend to be similar among plots within the forest despite differences in plot means for any single time point (Davidson *et al.*, 1998; Savage & Davidson, 2001). The two plots were compared during a pretreatment calibration period, and the posttreatment divergence between plots can then be attributed to the treatment. For this purpose, within-plot variation among the triplicate chambers was used for

analysis of differences between plots. These analyses are not meant to imply that the plots are quantitatively representative of spatial variation within the forest, but rather to demonstrate differences in temporal variation between these two specific plots. Bootstrapping was used to estimate parameter space and 95% confidence intervals for Eqns. (1), (2), and (3). Monte Carlo (MC) simulation was used to determine significant differences between model parameters and model outcome. R2.7.1 and Microsoft Excel were used for all statistical analysis.

To determine daily sums of  $R_a$ , any missing hourly  $R_a$  data were filled by linearly interpolating between missing flux values. Gap filled data were only used in the determination of daily  $R_a$  sums and only missing time periods <2 h were interpolated. Daily sums of gross ecosystem exchange (GEE) are from the Ecological Monitoring Site (EMS: <http://harvardforest.fas.harvard.edu/data-archives>, see Urbanski *et al.*, 2007 for further site details) eddy covariance tower at Harvard Forest which is <1 mile from the location where the trenching experiment was conducted. Daily sum of GEE is the difference between net ecosystem exchange (NEE) and ecosystem respiration (Reco) summed per day.

## Results

Mean soil respiration in the trenched plot ( $R_h$ ) and control plot ( $R_t$ ) were nearly identical prior to spring bud break in 2009 and began diverging thereafter (Fig. 1a). The difference between plots ( $R_a$ ) peaked



**Fig. 1** (a) Mean daily values  $R_t$  (solid square),  $R_h$  (open circle), and  $R_a$  (closed triangle) which is the difference between control-trenched and StErr bars, (b) soil temperature at 10 cm- closed squares control, open circles trenched, open diamonds-air temperature, (c) moisture at 8–16 cm closed squares control, open circles trenched and bars precipitation. Vertical black line is bud break, vertical dashed light gray line is 75% leaf out and vertical dashed dark gray line is leaf fall.

during midsummer and then declined to near zero by autumn leaf fall. Combining all 2009 data, a repeated measures ANOVA confirmed that  $R_t$  was significantly greater than  $R_h$ . In contrast, during the pretrenching measurement period, DOY 240-256 of 2008, the plot that was to be trenched later that year had higher mean soil respiration compared with the control plot, although the difference was driven by high fluxes recorded in one chamber (Table 1). Later in the autumn of 2008 (DOY 286-289), but still before the trenching, there were no differences between plots in mean respiration.

Soil temperatures were slightly different between treatments, with the control being generally about 1 °C warmer than the trenched plot (Fig. 1b). This difference may be the result of a slight difference in the depth of the placement of the sensors rather than a difference created by treatment effects. There were no differences between plots in diel amplitude of soil temperature or the time of day of peak soil temperature.

Contrary to expectations, the control plot was generally wetter than the trenched plot (Fig. 1c). However, during the few dry periods of 2009, there was a more rapid decrease in soil moisture in the control plot compared with the trenched plot, indicating that root uptake in the control plot may have been greater during these periods. In no case, however, did soil moisture fall below  $0.12 \text{ cm}^3 \text{ cm}^{-3}$ , which is the threshold previously identified, below which water limitation to soil respiration is clear (Savage & Davidson, 2001).

Through visual examination of the 2009 seasonal trends, data were partitioned into apparent phenological stages: early spring (DOY 112-145, pre-full-leaf out), spring-summer (DOY 146-210), summer-fall (DOY 211-

260), and fall (DOY 261-304, after leaf fall), with Eqn. (3) applied to each subset of data. Binning the data by time of day for each phenological period, the parameters of the sine wave function differed for  $R_t$  and  $R_h$  (Table 1, Fig. 2). As expected, peak  $R_h$  occurred at about 17:30–18:00 hours during the summer, roughly coincident with peak 10 cm soil temperature at 17:00 hours. In contrast, peak  $R_t$  occurred at 20:00–21:00 hours, and peak  $R_a$  ( $R_t - R_h$ ) occurred at about 22:00 hours (not shown in table).

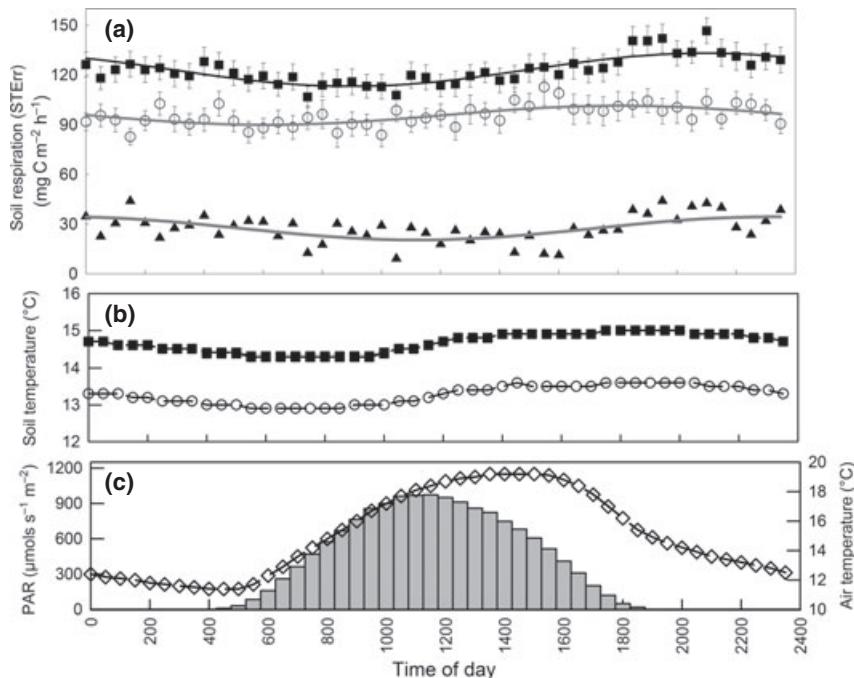
Hysteresis was evident in the diel respiration and soil temperature data for both  $R_t$  and  $R_h$  (Fig. 3).  $R_h$  showed a clockwise hysteresis pattern with a narrow minor radius whereas  $R_t$  showed a counterclockwise pattern with a rounder minor radius, indicating a lag between fluxes and soil temperature at 10 cm depth. The best correlations with soil temperature across the measurement periods were with a 0.5-, 2.5-, and 5-h lag for  $R_h$ ,  $R_t$ , and  $R_a$ , respectively (Fig. 4). The difference was statistically significant for the offset term (c) in the diel sine wave model [Eqn. (3)] only for the summer period (Table 1). The daily amplitude term (A) in Eqn. (3) was also greater for  $R_t$  than  $R_h$ ; the difference was statistically significantly for all time periods except the early spring and was different in the summer (Table 1). The mean flux term ( $y_0$ ) was significantly higher for  $R_t$  for all time periods (Table 1), although the difference was small in the early spring. In contrast, there were no differences between the same two plots in amplitude or time of day for peak respiration prior to trenching in 2008.

Across the entire measurement period, the  $Q_{10}$  [Eqn. (1)] for  $R_h$ ,  $R_t$ , and  $R_a$  were 2.9, 4.1, and 9.7, respectively, with nonoverlapping confidence intervals

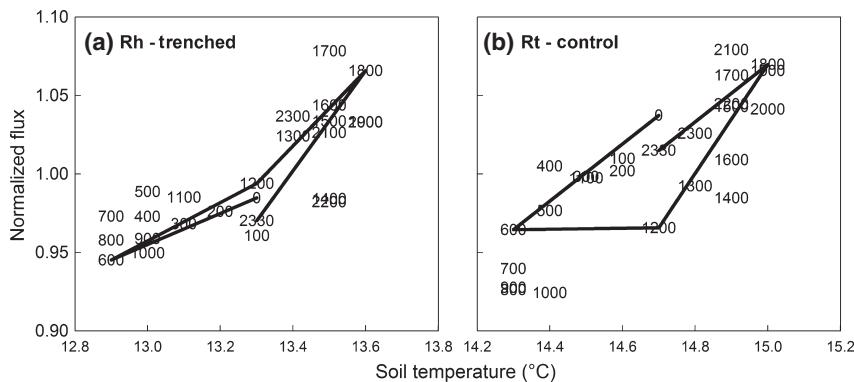
**Table 1** Diel sine wave models for  $R_h$  and  $R_t$  at phenological stages—Eqn. (3)

DOY	Plot	$y_0$	A	c	FMR <sup>2</sup>
2009a 112-145 early spring	$R_h$	52.3 <sup>a</sup> (51.1-53.6)	4.78(3.10-6.30)	3.41 (1700 hrs)(3.07-3.78)	0.42
	$R_t$	55.7 <sup>b</sup> (54.7-56.9)	4.06(2.52-5.60)	3.69 (1630 hrs)(3.23-4.12)	0.38
2009b 146-210 spring-summer	$R_h$	95.6 <sup>c</sup> (93.5-97.7)	6.00 <sup>k</sup> (3.10-9.04)	3.12 <sup>s</sup> (1800 hrs)(2.59-2.63)	0.37
	$R_t$	124.4 <sup>d</sup> (122.1-126.5)	10.36 <sup>l</sup> (7.36-13.36)	2.38 <sup>t</sup> (2100 hrs)(2.07-2.68)	0.67
2009c 211-260 summer-early fall	$R_h$	110.8 <sup>e</sup> (109.1-112.7)	3.76 <sup>m</sup> (1.46-6.09)	3.26 <sup>u</sup> (1730 hrs)(2.40-4.45)	0.12
	$R_t$	166.4 <sup>f</sup> (163.9-168.8)	10.30 <sup>n</sup> (7.09-13.60)	2.66 <sup>v</sup> (2000 hrs)(2.30-3.04)	0.58
2009d 261-304 fall	$R_h$	58.0 <sup>g</sup> (57.0-59.0)	4.52 <sup>o</sup> (3.09-5.93)	2.83(1900 hrs)(2.48-3.18)	0.67
	$R_t$	64.9 <sup>h</sup> (63.8-66.0)	6.29 <sup>p</sup> (4.73-7.82)	2.59 (2000 hrs)(2.36-2.85)	0.76
2009e 112-304 seasonal	$R_h$	82.4 <sup>i</sup> (81.2-83.4)	3.95 <sup>q</sup> (2.50-5.60)	3.26 (1730 hrs)(2.84-3.66)	0.59
	$R_t$	106.9 <sup>j</sup> (105.5-108.4)	6.40 <sup>r</sup> (4.31-8.44)	2.57 (1930 hrs)(2.22-2.92)	0.81
2008f 240-256	$R_h$	219.5 <sup>w</sup> (213.5-225.6)	13.54(5.35-22.01)	2.33 (2000 hrs)(1.66-3.11)	0.03
	$R_t$	139.7 <sup>x</sup> (136.6-142.9)	10.64(6.17-15.23)	2.90 (1900 hrs)(2.47-3.32)	0.03

$R_h$ =heterotrophic respiration-trenched plot,  $R_t$ =total respiration-control plot,  $y_0$ =mean diel respiration for that period ( $\text{mg C m}^{-2} \text{ hr}^{-1}$ ), A=dial amplitude ( $\text{mg C m}^{-2} \text{ hr}^{-1}$ ), c=phase shift indicating peak timing of respiration, FMR<sup>2</sup>=Figure of merit. Parameter plus 95% confidence range in italic brackets, Significant difference in parameter between  $R_h$  and  $R_t$  indicated by different superscript letters for each phenological period and within each parameter column.



**Fig. 2** (a) mean half hourly diel pattern for the control  $R_t$  (solid square), trenched  $R_h$  (open circle) plots, and  $R_a$  (closed triangle) for the spring/early summer phenological period (DOY 146–210).  $R_a$  values are  $R_t - R_h$ . (b) soil temperature, (c) air temperature (open diamonds), and PAR (bars).

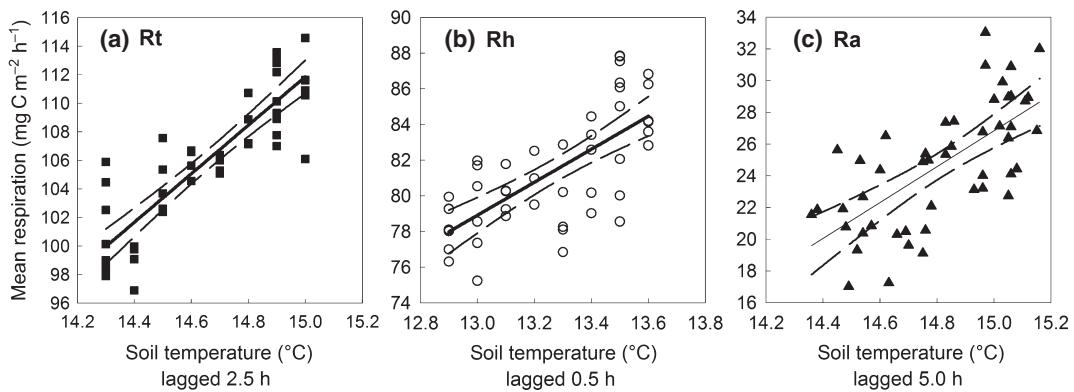


**Fig. 3** Normalized mean hourly flux (DOY 112–305) for: (a) trenched plot  $R_m$  vs. soil temperature at 10 cm, and (b) control plot  $R_t$  vs. soil temperature. Flux values normalized by taking each mean hourly flux/daily mean flux. Black lines join points at times 0, 600, 1200, 1800 and 2300 h to help define the elliptical pattern.

(Table 2). The addition of soil moisture [Eqn. (2)] improved model fits for both  $R_t$  and  $R_h$ , but the estimated  $Q_{10}$  remained higher for  $R_t$  compared with  $R_h$  (Table 2), indicating that differences in soil moisture due to trenching did not affect the relative differences in apparent temperature sensitivity.

Daily sum of  $R_a$  was compared with daily sum of gross ecosystem exchange (GEE), Fig 5a). There was a significant, nonlinear correlation between daily  $R_a$  and daily GEE across the sampling season. Examination of

the relationship between GEE and  $R_a$  with lagged time steps of greater than a day showed no improvement in the correlation between GEE and  $R_a$ . At a daily time step, there was no correlation, nor lagged correlation between daily sum of PAR and daily sum of  $R_a$  (Fig. 5b). During the early spring period when leaf out was < 75%, and the late fall during senescence, there was little change in daily sum of  $R_a$  at any PAR value (Fig 5b, open symbols). When leaves were present on the trees, there is a strong link between daily sum of

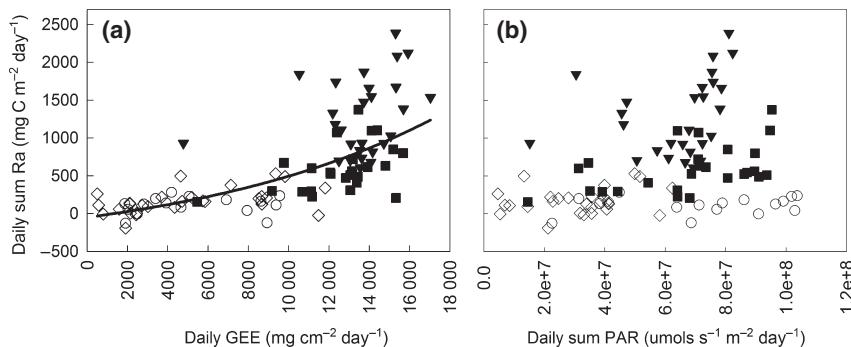


**Fig. 4** Mean daily flux for: (a)  $R_t$  ( $R_t = 17.0 \times \text{soilT-143}$ ,  $R^2 = 0.76$ ), (b)  $R_h$  ( $R_h = 9.2 \times \text{soilT-41}$ ,  $R^2 = 0.50$ ), and (c)  $R_a$  ( $R_a = 11.3 \times \text{soilT-143}$ ,  $R^2 = 0.46$ ) lagged with soil temperature. Dashed lines are 95% confidence intervals.

**Table 2** Seasonal Models of heterotrophic ( $R_h$ ), autotrophic ( $R_a$ ), and total ( $R_t$ ) soil respiration.

	Equation 1	Equation 2
$R_h$ -Trenched	$R_{\text{ref}} = 54.76$ (53.94–55.61) $Q_{10} = 2.9$ (2.8–3.0) $\text{FMR}^2 = 0.37$	$R_{\text{ref}} = 62.03$ (60.57–63.62) $Q_{10} = 3.3$ (3.2–3.4) $D = 0.80$ (0.77–0.82) $\text{FMR}^2 = 0.40$
$R_t$ -Control	$R_{\text{ref}} = 49.75$ (48.91–50.65) $Q_{10} = 4.1$ (4.0–4.2) $\text{FMR}^2 = 0.54$	$R_{\text{ref}} = 56.20$ (55.09–57.38) $Q_{10} = 5.0$ (4.8–5.1) $D = 0.47$ (0.45–0.49) $\text{FMR}^2 = 0.59$
$R_a - (R_t - R_h)$	$R_{\text{ref}} = 7.71$ (6.96–8.11) $Q_{10} = 9.7$ (9.2–11.3) $\text{FMR}^2 = 0.18$	$R_{\text{ref}} = 14.00$ (10.63–13.57) $Q_{10} = 11.2$ (9.7–12.0) $D = 0.86$ (0.88–0.92) $\text{FMR}^2 = 0.22$

Italic brackets are 95% confidence intervals, FMR<sup>2</sup> figure of merit



**Fig. 5** (a) Daily sum of  $R_a$  and Gross Ecosystem Exchange (GEE), (b) daily sum  $R_a$  and daily sum of photosynthetically active radiation (PAR). Solid symbols denote time periods when leaves were present on trees, open symbols when there was < 75% leaf out. Circles DOY 112–145, squares DOY 146–210, triangles DOY 211–260, and diamonds DOY 261–304. Model line Daily sum  $R_a = \exp(6.10948 + 7.982 \times 10^{-4} \times \text{GEE}) - 500$ ,  $R^2 = 0.58$ ,  $P < 0.0001$

PAR and  $R_a$ , with  $R_a$  increasing with increased daily sum of PAR (Fig 5b, closed symbols). Similar results were found when comparing maximum daily vapor

pressure deficit (VPD) with  $R_a$  (data not shown) with no apparent correlation on a daily time step nor over lagged time intervals >1 day.

## Discussion

These data provide evidence that the contribution of root respiration ( $R_a$ ) to total soil respiration ( $R_t$ ) varies on both diel and seasonal time scales. The effect was clearest when the canopy was present in this deciduous temperature forest, indicating that aboveground phenology affects the magnitude and timing of  $R_a$  and its relative contribution to  $R_t$ . Prior to bud break, daily amplitude and peak time of soil respiration were not different between  $R_t$  and  $R_h$ , and the daily mean was only slightly higher for  $R_t$ , indicating little effect of root respiration. After bud break, however,  $R_t$  showed higher mean daily respiration, larger daily amplitude of soil respiration, and a later time of day for the peak flux during the spring and summer. Once trees began to senesce in autumn, the  $R_a$  contribution to  $R_t$  declined, and, although the mean diel respiration and amplitude remained significantly greater for  $R_t$  than  $R_h$ , there was no longer a significant difference in peak timing of respiration. This indicates that during autumn,  $R_t$  was predominately heterotrophic with some continuing modest contribution from  $R_a$ , but not enough to significantly alter the diel pattern of  $R_t$ .

The higher mean daily flux when roots were present and active in the control plot in the late spring and summer is not surprising, because it has been well understood that  $R_a$  contributes a significant fraction of  $R_t$  (Hanson *et al.*, 2001). It follows that if  $R_a$  has a diel cycle roughly corresponding to that of  $R_h$ , then the amplitude of the  $R_t$  diel cycle would also be larger than the amplitude of diel variation in  $R_h$  alone.

Different time lags between photosynthetic activity radiation (PAR) and vapor pressure deficit (VPD) with soil carbon efflux varying from 1 day to several days have been reported across different forest ecosystems (Ekblad *et al.*, 2005; Tang *et al.*, 2005; Vargas *et al.*, 2011). Where photosynthesis is limited by sun, the daily sum of PAR could be correlated with substrate production available for both aboveground and belowground respiration. High values of maximum daily VPD may be linked to stomata closure and to reduce moisture loss via transpiration, in turn reducing daily production of photosynthate. However, for this study, we were unable to find any time lag between  $R_a$  and any of these variables: GEE, PAR, or VPD. This may be a unique result in this study site or for this particular sampling year because no periods of moisture stress were experienced during this study period. There was a clear correlation on a daily time step between GEE and  $R_a$ , where high rates of GEE corresponding directly to days of high  $R_a$  efflux. The lack of a lag between above- and belowground activity at daily time steps at this site may be indicative of a fast response to climate variables.

With no apparent lag between above- and belowground activity at timescales  $>1$  day, we then considered diel timescales. Variation in soil respiration at diel timescales could be attributed to either the biological processes, such as linkages between aboveground and belowground processes and plant and microbe responses to climate, or the soil physical effects on gas transport. Modeling efforts have shown that lags between surface soil carbon effluxes and soil temperature measured at a particular depth can, in part, be explained by heat and CO<sub>2</sub> transport processes induced by different soil heat profiles between the morning and the afternoon (Phillips *et al.*, 2011). Through modeling of belowground processes, plots of surface flux vs. soil temperature show distinct elliptical patterns (Phillips *et al.*, 2011) and the direction and 'roundness' of the minor axis of the ellipse indicate the amount of lag between peak surface flux and peak soil temperature. However, Martin *et al.* (2012) found that diel soil flux patterns could not completely be explained by changes in diffusion rates or depth of production in a ponderosa pine stand; the biological effect plays the major role in controlling the diel patterns.

In this study, although diel hysteresis for both  $R_h$  and  $R_t$  with soil temperature was observed, the relationship was more linear and the hysteresis effect less pronounced for  $R_h$  than  $R_t$  (Fig. 3). This result in the trenched plot indicates that the soil physical processes of gas transport caused very little hysteresis. However, for the control plot,  $R_t$ , where roots were present, there was a distinct, rounder elliptical pattern, indicating that it was the presence of roots that contributed to changing the timing between peak surface carbon efflux and peak soil temperature. If the lag between surface flux and temperature at any given depth was controlled solely by physical processes through the soil, the time of peak soil respiration for both  $R_t$  and  $R_h$  would be identical and only the magnitude of the flux would be different. This study is consistent with previous reports of lags of several hours between peak soil temperature and peak soil respiration (Tang *et al.*, 2005; Liu *et al.*, 2006; Savage *et al.*, 2009), but here we show through root exclusion that the delay in the hour of peak soil respiration rate was due to the presence of roots.

The time required for photosynthate, to travel many meters from the canopy of a tree to its roots is probably on the order of many hours to a few days (Kuzyakov & Gavrichkova, 2010; Mencuccini & Hölttä, 2010; Vargas *et al.*, 2010). However, more rapid transmission of pressure/concentration waves through the phloem, which could provide roots with information on shoot processes, is theoretically possible and could explain increases in root respiration within hours of increases in shoot photosynthesis (Thompson & Holbrook, 2004;

Davidson & Holbrook, 2009). In this study, peak  $R_a$  occurred at about 22:00 hours, which was about 5 h after peak 10 cm soil temperature, 8 h after peak air temperature, and 10 h after peak photosynthetically active radiation. The correlation between daily summed GEE and daily summed  $R_a$ , with no lags at daily time steps, suggest that daily allocation of C to roots may be related to the same day's photosynthesis. Furthermore, the hourly data support the hypothesis that a link between aboveground activity and belowground response ( $R_a$ ) also occurs at the sub-daily level, with a lag on the order of several hours.

Lack of true replication and possible artifacts of the trenching approach preclude confidence in quantitative estimates of  $R_a$  relative to  $R_h$ . For this pairwise comparison of plots,  $R_a$  contributed 46% of  $R_t$  at the mid-summer peak of  $R_a$ . Integrated over the entire measurement period,  $R_a$  contributed 22% of  $R_t$ . While this is within reported ranges (Hanson *et al.*, 2001), we suspect that these are underestimates of  $R_a$ , because decomposition of dead roots during 2009 resulting from the previous autumn's trenching could elevate the estimate of  $R_h$ , causing  $R_a$  to be underestimated. Estimates of this effect in the literature range from 16 to 45%, with the effect most pronounced within the first 4–5 months after trenching and declining to minimal levels after 4 months to 2 years (Lee *et al.*, 2003; Comstedt *et al.*, 2010). As trenching was conducted six months prior to the flux measurements in the present experiment, we expect that the effects of severed roots due to trenching was a small, but unknown component of  $R_t$ .

Differences in soil moisture between control and trenched plots is another potential artifact of the trenching experiment. In a trenching experiment in a pine forest in Sweden, Comstedt *et al.* (2010) found that differences in soil moisture contributed to an estimated 29% of  $R_h$ . However, it should be noted that in this study, differences in soil moisture between control and trenched plots were generally less than 2%, and soil moisture remained well above previously observed thresholds where drought has been shown to affect soil respiration.

While these uncertainties could have an important effect on the quantitative estimate of  $R_a$ , analysis of the timing of  $R_a$  on diel and seasonal time scales are less likely to be affected.

The seasonally varying input of  $R_a$  to  $R_t$  affected the apparent  $Q_{10}$  of  $R_t$ . Using soil respiration spanning seasons to calculate a  $Q_{10}$  yields a higher value when roots are present, because it includes the increasing influence of growing root biomass from spring to summer, which is confounded with increasing temperature (Boone *et al.*, 1998; Epron *et al.*, 1999; Gaumont-Guay *et al.*, 2008). Similarly, the high  $Q_{10}$  calculated for  $R_a$  reflects the com-

bined effect of both the temperature sensitivity of root respiration per gram of live root and also presumably an increase in root biomass from spring to summer.

Improved understanding of belowground carbon cycling processes will require identification and quantification of the linkages with aboveground processes. We have shown that root inputs to soil CO<sub>2</sub> production vary seasonally and are of sufficient magnitude to alter seasonal and diel patterns of soil respiration. For soil respiration measurements to be used as a constraint in ecosystem carbon cycling models, this variation in the seasonal and daily contribution of roots must be represented and better quantified.

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## References

- Boone RD, Nadelhoffer KJ, Canary JD, Kaye JP (1998) Roots exert a strong influence on the temperature sensitivity of soil respiration. *Nature*, **396**, 570–572.
- Borken WB, Savage K, Davidson EA, Trumbore SE (2006) Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil. *Global Change Biology*, **12**, 177–193.
- Cheng W (2009) Rhizosphere priming effect: its functional relationships with microbial turnover, evapotranspiration, and C–N budgets. *Soil Biology and Biochemistry*, **41**, 1795–1801.
- Comstedt D, Boström B, Ekblad A (2010) Autotrophic and heterotrophic soil respiration in a Norway spruce forest: estimating the root decomposition and soil moisture effects in a trenching experiment. *Biogeochemistry*, **104**, 121–132. doi:10.1007/s10533-010-9491-9.
- Davidson EA, Holbrook NM (2009) Is temporal variation of soil respiration linked to the phenology of photosynthesis? In: *Phenology of ecosystem processes: applications in global change research* (ed Noormets A), Springer, New York.
- Davidson E, Belk E, Boone R (1998) Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. *Global Change Biology*, **4**, 217–227.
- Davidson E, Janssens IJ, Luo Y (2006a) On the variability of respiration in terrestrial ecosystems: moving beyond  $Q_{10}$ . *Global Change Biology*, **12**, 154–164.
- Davidson EA, Savage KE, Trumbore SE, Borken W (2006b) Vertical partitioning of CO<sub>2</sub> production within a temperate forest. *Global Change Biology*, **12**, 944–956.
- Ekblad A, Boström B, Holm A, Comstedt D (2005) Forest soil respiration rate and  $d^{13}C$  is regulated by recent above ground weather conditions. *Oecologia*, **143**, 136–142.
- Epron D, Farque L, Lucot E, Badot P-M (1999) Soil CO<sub>2</sub> efflux in a beech forest: dependence on soil temperature and soil water content. *Annals of Forest Science*, **56**, 221–226.
- Gaumont-Guay D, Black TA, Barr AG, Jassal RS, Nesic Z (2008) Biophysical controls on rhizospheric and heterotrophic components of soil respiration in a boreal black spruce stand. *Tree Physiology*, **28**, 161–171.
- Gu L, Hanson PJ, Post WM, Liu Q (2008) A novel approach for identifying the true temperature sensitivity from soil respiration. *Global Biogeochemical Cycles*, **22**, GB4009. doi:10.1029/2007GB003164.
- Hanson PJ, Edwards NT, Garten CT, Andrews JA (2001) Separating root and soil microbial contributions to soil respiration: a review of methods and observations. *Biogeochemistry*, **48**, 115–146.

- Hollinger D, Richardson A (2005) Uncertainty in eddy covariance measurements and its application to physiological models. *Tree Physiology*, **25**, 873–885.
- Kuzakov Y (2006) Sources of CO<sub>2</sub> efflux from soil and review of partitioning method. *Soil Biology & Biochemistry*, **38**, 425–448.
- Kuzakov Y, Gavrichkova O (2010) Time lag between photosynthesis and carbon dioxide efflux from soil: a review of mechanisms and controls. *Global Change Biology*, **16**, 3386–3406.
- Lee M, Nakane K, Nakatsubo T, Koizumi H (2003) Seasonal changes in the contribution of root respiration to total soil respiration in a cool-temperate deciduous forest. *Plant and Soil*, **255**, 311–318.
- Liu Q, Edwards N, Post W, Gu L, Ledford J, Lenhart S (2006) Temperature-independent diel variation in soil respiration observed from a temperate deciduous forest. *Global Change Biology*, **12**, 2136–2145.
- Martin JG, Phillips CL, Schmidt A, Irvine J, Law BE (2012) High-frequency analysis of the complex linkage between soil CO<sub>2</sub> fluxes, photosynthesis and environmental variables. *Tree Physiology*, **32**, 49–64.
- Mencuccini M, Hölttä T (2010) The significance of phloem transport for the speed with which canopy photosynthesis and belowground respiration are linked. *New Phytologist*, **185**, 189–203.
- Phillips CL, Nickerson N, Risk D, Bond BJ (2011) Interpreting diel hysteresis between soil respiration and temperature. *Global Change Biology*, **17**, 515–527.
- Richardson AD, Braswell B, Hollinger D *et al.* (2006a) Comparing simple respiration models for eddy flux and dynamic chamber data. *Agriculture and Forest Meteorology*, **141**, 219–234.
- Richardson AD, Hollinger D, Gurba G *et al.* (2006b) A multi-site analysis of random error in tower-based measurements of carbon and energy fluxes. *Agriculture and Forest Meteorology*, **136**, 1–18.
- Savage K, Davidson E (2001) Interannual variation of soil respiration in two New England forests. *Global Biogeochemical Cycles*, **15**, 337–350.
- Savage K, Davidson E (2003) A comparison of manual and automated systems for soil CO<sub>2</sub> flux measurements: trade-offs between spatial and temporal resolution. *Journal of Experimental Botany*, **54**, 891–899.
- Savage K, Davidson EA, Richardson AD (2008) A conceptual and practical approach to data quality and analysis procedures for high-frequency soil respiration measurements. *Functional Ecology*, **22**, 1000–1007, doi:10.1111/j.1365-2435.2008.01414.x.
- Savage K, Davidson EA, Richardson AD, Hollinger DY (2009) Three scales of temporal resolution from automated soil respiration measurements. *Agricultural and Forest Meteorology*, **149**, 2012–2021.
- Subke J-A, Inglima I, Cotrufo FM (2006) Trends and methodological impacts in soil CO<sub>2</sub> efflux partitioning: a metaanalytical review. *Global Change Biology*, **12**, 921–943.
- Tang J, Baldocchi D, Xu L (2005) Tree photosynthesis modulates soil respiration on a diurnal time scale. *Global Change Biology*, **11**, 1298–1304.
- Thompson MV, Holbrook NM (2004) Scaling phloem transport: information transmission. *Plant Cell and Environment*, **27**, 509–519.
- Trumbore SE (2006) Carbon respired by terrestrial ecosystems-recent progress and challenges. *Global Change Biology*, **12**, 141–153.
- Urbanski S, Barford C, Wofsy S *et al.* (2007) Factors controlling CO<sub>2</sub> exchange on timescales from hourly to decadal at Harvard Forest. *Journal of Geophysical Research*, **112**, G02020, doi:10.1029/2006JG000293.
- Vargas R, Baldocchi DD, Allen MF *et al.* (2010) Looking deeper into the soil: biophysical controls and seasonal lags of soil CO<sub>2</sub> production and efflux. *Ecological Applications*, **20**, 1569–1582.
- Vargas R, Baldocchi DD, Bahn M *et al.* (2011) On the multi-temporal correlation between photosynthesis and soil CO<sub>2</sub> efflux: reconciling lags and observations. *New Phytologist*, **191**, 1006–1017.