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Partitioning soil respiration: quantifying the artifacts of the trenching method

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Abstract Total soil respiration (Rt) is a combination of autotrophic (Ra) and heterotrophic respiration (Rh). Root exclusion methods, such as soil trenching, are often utilized to separate these components. This method involves severing the rooting system surrounding a plot to remove the Ra component. However, soil trenching has potential limitations including (1) reduced water uptake in trenched plots that increases soil water content, which is one of the environmental controllers of Rt in many ecosystems, and (2) increased available carbon substrate for Rh caused by recently severed dead roots. We present a methodology that utilizes a bayesian modeling framework to quantify the magnitude of artifacts from a large trenching manipulation experiment. Thus methodology corrects Rh and Ra observations at daily to seasonal time scales. This study finds that the artifacts, due to recently severed roots, persist over a 2 years study period and the artifacts due to altered soil moisture had the greatest impact during drought conditions.

Keywords Autotrophic respiration · Bayesian modeling · Heterotrophic respiration · Trenching

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Abbreviations

Ra	Autotrophic respiration
Rh	Heterotrophic respiration
Rt	Total soil respiration
RSR	Recently severed roots
SMT	Soil moisture and temperature

Introduction

Terrestrial carbon (C) stocks contain two to three times the amount of C as is present in the atmosphere (Ciais et al. 2013) and in northeastern US temperate forests, soils are commonly in excess of 50% of total ecosystem C content (Fahey et al. 2005). In temperate forests, total soil respiration (Rt) is a major flux of C to

the atmosphere (Lavigne et al. 2003; Bond-Lamberty and Thomson 2010). Often Rt is measured as one flux, but comprised of two components, autotrophic respiration, (root-rhizosphere and associated microbial bacteria, Ra) and heterotrophic respiration ("free living" microbial bacteria, Rh).

Ra and Rh are large, poorly constrained components of the terrestrial carbon budget (Schulze et al. 2010). Estimates of the relative contribution of Ra to Rt vary spatially and seasonally from 10 to 90% (Hanson et al. 2000) with many studies showing roughly 30–60% using various partitioning techniques (Gaudinski et al. 2001; Gaumont-Guay et al. 2008; Comstedt et al. 2011; Savage et al. 2013), and meta-analysis (Subke et al. 2006).

Several methods are used to partition Ra and Rh, including isotopic analysis of respired C, (Cisneros-Dozal et al. 2006), tree girdling (Högberg et al. 2001), and trenching, (Drake et al. 2012; Savage et al. 2013). The trenching method is frequently used to separate the components of Rt (Ra and Rh) to address the influence of environmental controls on each component (Comstedt et al. 2011; Savage et al. 2013). Trenching involves severing the rooting system surrounding a treeless plot to remove photosynthetic carbon allocation belowground. Any measured respiration from the trenched plot is considered Rh. Ra is estimated by subtracting Rh from Rt measured in an adjacent un-trenched control plot.

While widely used, the trenching method disturbs soils and raises concerns over methodological artifacts. In upland soils, reductions in water uptake owing to the loss of plant roots in trenched plots increases moisture content, a primary control on Rt in many ecosystems (Savage and Davidson 2001; Jassal et al. 2008). It has been observed that soil moisture is greater in trenched plots compared to control plots, a result of eliminating transpiration through roots (Savage et al. 2013). Since soil moisture is an important driver of Rh, this artifact may have important implications for partitioning when using the trenching method. In a modeling effort using partial least squares projection, Comstedt et al. (2011) found that increased soil moisture in trenched plots accounted for a 29% overestimation of Rh of a spruce forest in Norway. This artifact may be most pronounced in dry seasons and well-drained soils. In a recent study in grasslands Balogh et al. (2016) found that Rh was the dominant component of Rt during dry and drought-induced conditions. The relative contribution of Ra to Rt shows a seasonal trend (Savage et al. 2013), and artifacts from trenching may influence measured responses differently depending on seasonal moisture patterns.

Recently severed roots (RSR) temporarily increase available carbon substrate for Rh (Epron et al. 1999; Subke et al. 2006). A study (Scott-Denton et al. 2006), found that RSR continue to respire, utilizing stored starches, for several months after trenching but before decomposing, suggesting that RSR remains an important source of CO_2 production for months to years (Matamala et al. 2003; Diaz-Pines et al. 2010). Estimates of the artifact due to RSR range from 16% (Comstedt et al. 2011) to 25% (Epron et al. 1999) overestimate of Rh. Carbon loss due to RSR is dependent on root biomass and soil conditions and may need to be a site-specific estimate (Subke et al. 2006) for proper quantification.

The response of Ra and Rh to biotic and abiotic drivers differs, where Ra is linked closely to above-ground processes (Tang et al. 2005; Savage et al. 2013) and Rh is linked closely to factors such as soil temperature, moisture and substrate supply (Davidson et al. 2006, 2012). There is a further complicating link between Ra and Rh through rhizosphere processes. Rh can be stimulated by root exudates and by the process of "soil priming" (Cheng 2009; Finzi et al. 2015), wherein plant C allocation to roots and rhizosphere microbes, stimulates soil organic matter decomposition. Conversely, eliminating root inputs could reduce Rh due to priming (Cheng 2009; Finzi et al. 2015). Therefore, the presence or absence of a rooting system and associated rhizosphere can have an effect on Rh. In a simulation study, Finzi et al. (2015) estimated that 10–30% of the total soil mass may be influenced by rhizosphere processes and that 15% of Rh could be attributed to priming in a hypothetical temperate forest. This suggest the loss of roots from the trenching method may have offsetting effects by reducing Rh due to loss of soil priming, while simultaneously increasing Rh due to the addition of carbon from RSR.

To properly account for this important C flux in global carbon budget, these components of Rt must be quantified and well constrained. Our objective is to determine daily, seasonal and annual contributions of Ra and Rh relative to Rt using the trenching method. We use experimental and simulation approaches to estimate the influence of the artifacts of the trenching

method on estimates of *Rh* and *Ra* carbon loss from soils in a temperate deciduous forest. A Bayesian modeling framework is used to quantify and estimate uncertainty derived from two artifacts: (1) fresh C input due to RSR and, (2) changes in soil moisture and temperature (SMT). Pairing this approach with high temporal frequency measurements of soil CO_2 efflux and environmental conditions provides a novel opportunity to assess the impacts of artifacts at inter-annual, annual, seasonal and daily time steps.

Method

Site description

This study was conducted at the Harvard Forest near Petersham, Massachusetts USA (42832N, 72811W). The site is a 75 year old well-drained mixed hardwood forest. The dominant tree species is beech (*Fagus grandifolia*) and red oak (*Quercus rubra*). Soils are classified as Canton fine sandy loam, Typic Distrochrepts. Due to agricultural use in the 19th century, the upper mineral soil is partially disturbed. The mean annual temperature is $+ 8.5$ °C and the mean annual precipitation is 1050 mm. See (Compton and Boone 2000) for further descriptions.

Trenching method

Pre-treatment soil respiration, moisture and temperature were collected during the spring through early fall of 2012. In the late fall of 2012, a trench was dug (50 cm depth) around a treeless 5×5 m area, severing all roots leading into the treatment plot. Water impermeable plastic tarp was placed along the walls of the trenched plot to prevent new root in-growth and backfilled. Understory vegetation within the trenched plot was clipped in early spring 2013 and 2014.

Four automated chambers were placed within the trenched plot and four in an adjacent control plot. Given the constrained space and the disturbance due to the trenching method, we used one large trenched plot with four replicate chambers within it as opposed to four separate trenched plots. We recognize this setup would be considered "pseudo-replication" with respect to treatment effects, however in our experience spatial heterogeneity in soil respiration within 5 m can be as great as between 25 m (Borken et al. 2006;

Giasson et al. 2013). The coefficient of variation (CV) in the trenched plot averaged 27% which is higher than the 22% CV measured in a fully replicated experiment at a similar location at the Harvard Forest (Borken et al. 2006).

Soil respiration measurements

Automated chambers were utilized in conjunction with the trenching method to partition *Rt* into its components, *Rh* and *Ra*. Respiration was measured for each chamber at 90 min intervals from early spring through late fall of 2012 (pre trenching), 2013 and 2014. For details on the automated system and flux calculations see Savage et al. (2014).

Respiration was not measured after trenching until early spring of 2013 (5 months). This allowed time for freshly severed roots to partially decompose and thereby reduce the potential CO_2 "pulse" from trenching. Soil temperature and moisture (10 cm depth, Campbell Scientific CS650 water content and temperature probes), were measured at 90 min intervals, concurrent with respiration. All data were collected and stored using a Campbell Scientific CR1000 datalogger. Measurements from the non-trenched plot (control) represent *Rt*, the combined *Rh* and *Ra*. Fluxes from the trenched plot represent *Rh* and the difference ($Rt - Rh$) represents *Ra*.

Root decomposition measurements

To account for carbon loss derived from decomposing roots, we conducted a 2-year root decomposition study. Roots were collected from the study area, classified by three size categories and placed in mesh bags. Forty root decomposition bags, 20 fine roots (< 1 mm diameter), 10 medium roots (1–5 mm diameter) and 10 coarse roots (> 5 mm diameter) were placed in the organic soil horizon of the trenched plot on Jan. 10, 2013 and root mass was measured over the course of 2013 and 2014 (5 measurement points over a total of 678 days). Four fine, two medium, and two coarse root decomposition bags were collected during each of the five sample periods. Upon collection, roots were dried (60 °C for 48 h) and weighed. In late 2014 only, new fine roots were observed to be growing through decomposition bags sampled from the trenched plot. These roots were not included in the decomposition measurement but may indicate a new

artifact of this method that arises after a prolonged treatment period.

Recently severed root (RSR) artifacts

A simple decay model Eq. (1) was used in a Bayesian modeling framework Eq. (2) to determine a site specific decay parameter (k) for root decomposition;

$$Mt = Mo * e^{-kt} \tag{1}$$

where Mt is the final root mass (g C), Mo is the initial root mass (g C), t is time (d) and k is the decay constant (d^{-1}). The posterior and joint probability distribution of the model are shown in Eq. (2).

$$
\begin{aligned}
&\left[Mo, k, \sigma_p^2 | Mt \right] \\
&\propto \prod_{i=1}^{n} gamma\left(Mt_i \Big| \frac{(Mo * \exp^{-kti})^2}{\sigma_p^2}, \frac{Mo * \exp^{-kti}}{\sigma_p^2} \right) \\
&\times\ beta(k|\alpha, \beta)\ \times\ gamma(Mo|\alpha, \beta) \\
&\times\ gamma(\sigma_p|\alpha, \beta)
\end{aligned}
\tag{2}
$$

Mo and Mt and process model uncertainty (σ_p) were given uninformed gamma prior distributions and the decay constant (k) was given an uninformed beta prior distribution. For this model we used MCMC methods implemented within the Rjags package (R 3.0.2). The model was run for 20,000 iterations with three chains, discarding the first 5000 and using the remaining iterations to calculate posterior estimates. The Gelman–Rubin convergence diagnostic was used to assess convergence and Bayesian p value to determine model suitability. Model R code and prior information are available in Supplementary Material S1_RSR. To scale from the decomposition bags to the plot, decay constant (k) was then utilized in Epron et al. (1999) C_{loss} model—Eq. (3);

$$C_{loss} = (1 - a) * c * M * \left(1 - e^{-kt}\right) \tag{3}$$

where, M is the site root biomass (g C m^{-2}; Abramoff and Finzi 2016), c is the initial carbon concentration in roots (44%; from Epron et al. 1999), and a is the fraction of carbon incorporated into soil organic matter (0.22; from Epron et al. 1999). Root biomass was categorized into fine (< 2 mm diameter) and coarse (> 2 mm diameter). Due to the difference in root size categories for estimates of root biomass

(Abramoff and Finzi 2016), and our decomposition bags, we grouped root decomposition bag categories of fine and medium into "fine +medium root" and equated that with root biomass estimate category of "fine". Coarse root decomposition bag category was equated with "coarse" root biomass. A decay constant (k) was determined for each of fine + medium and coarse roots Eq. (2). C_{loss} was determined for fine + medium roots and for coarse roots (Eq. 3) on a per day time step. This C_{loss} estimate from decaying roots was subtracted from observed Rh in the trenched plot (Rh_{RSR}).

Soil moisture and temperature (SMT) artifacts

We utilized a previously developed soil temperature and moisture model (Savage et al. 2009) in a Bayesian modeling framework Eq. (5) to determine the artifact of SMT, Eq. (4);

$$R = R_{ref} * Q^{\left(\left(\frac{T-10}{10}\right)\right)} * B^{(WCopt - WC)^2} \tag{4}$$

where R (mg C m^{-2} h^{-1}) represents respiration (R_h or R_t): T is soil temperature (°C), R_{ref} is R at 10 °C (mg C m^{-2} h^{-1}), Q is a unitless expression of the increase in R for each increase in 10 °C. WC is soil moisture content (cm^3 H$_2$O cm^{-3} soil), and WC$_{opt}$ is the optimal moisture content (cm^3 H$_2$O cm^{-3} soil), B modifies the shape of the quadratic fit.

The posterior and joint probability distribution of the model are shown in Eq. (5).

$$
\begin{aligned}
&\left[Rref, Q, B, \sigma_p^2, | R \right] \\
&\propto \prod_{i=1}^{n} norm\left((\log(R) | \log(R), Rref, Q, B, \sigma_{P,} \right) \\
&\times\ gamma(Rref|\alpha, \beta)\ \times\ gamma(Q|\alpha, \beta) \\
&\times\ beta(B|\alpha, \beta)\ \times\ gamma(\sigma_p|\alpha, \beta)
\end{aligned}
\tag{5}
$$

R_{ref}, Q and B were given informed priors based on previous modeling work at Harvard Forest (Savage et al. 2013). The process model uncertainty (σ_p) was given an uninformed gamma prior. Bayesian modeling was implemented in the same manner as for the RSR model. Prior information and R model code available in Supplementary Material S2_SMT. It should be noted that the SMT model utilized for this research is site-specific, and that researchers utilizing this method

for correction at alternative sites may need to use a different site specific temperature and moisture model. A brief explanation of how to alter the Bayesian modeling R code for alternative models is presented in Supplementary Material S2_SMT.

Equation (5) was used to model the artifact of SMT in the trenched plot with soil temperature and moisture from the control plot. Parameters from this model were used with soil temperature and moisture measured in the control plot, which are assumed the "true unaltered" temperature and moisture conditions. The percent difference between modeled results were used to "correct" observed *Rh*. This correction (*Rh*$_{SMT}$) is assumed to be *Rh* that would have been measured in the trenched plot had SMT not been altered by trenching. The percent change was applied to observed *Rh*; either increasing or decreasing observed *Rh* dependent on moisture and temperature (Fig. 1).
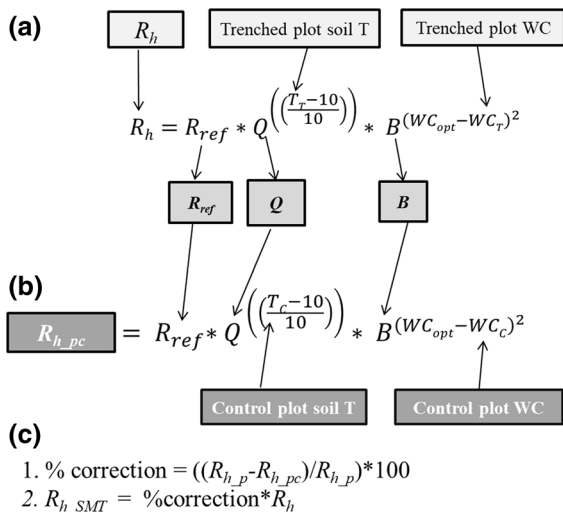
Fig. 1 Flow diagram of SMT correction due to trenching artifacts related to altered soil temperature and moisture. (*a*) Using the temperature and moisture model, defined in Eq. (4), observed heterotrophic respiration (*R*$_h$), soil temperature (T$_T$) and moisture (WC$_T$) from the trenched plot were used to parameterize R$_{ref}$ and Q and to generate a predicted estimate of heterotrophic respiration (R$_{h\_p}$). (*b*) Derived parameters R$_{ref}$ and Q, from (a), where then used in Eq. (4), along with soil temperature (T$_C$) and moisture (WC$_C$) from the control plot to predict heterotrophic respiration under natural soil temperature and moisture conditions (R$_{h\_pc}$). (*c.1*) The % correction in predicted *R*$_{h\_p}$ and R$_{h\_pc}$, (*c.2*) % correction was then used to correct observed *R*$_h$ resulting in R$_{h\_SMT}$, heterotrophic respiration under natural soil temperature and moisture conditions

Bayesian statistics

An inverse Bayes factor (BayesFactor package R3.3.1) was used to determine strength of support for the difference between seasonal and mean daily *Rh* and *Rt*. The null hypothesis was defined as no difference between the control and trenched observations. Inverse Bayes factors were computed for each day of year and strength of support for the null hypothesis categorized (Jarosz and Wiley 2014). An inverse Bayes factor > 3 indicates strong positive support.

Results
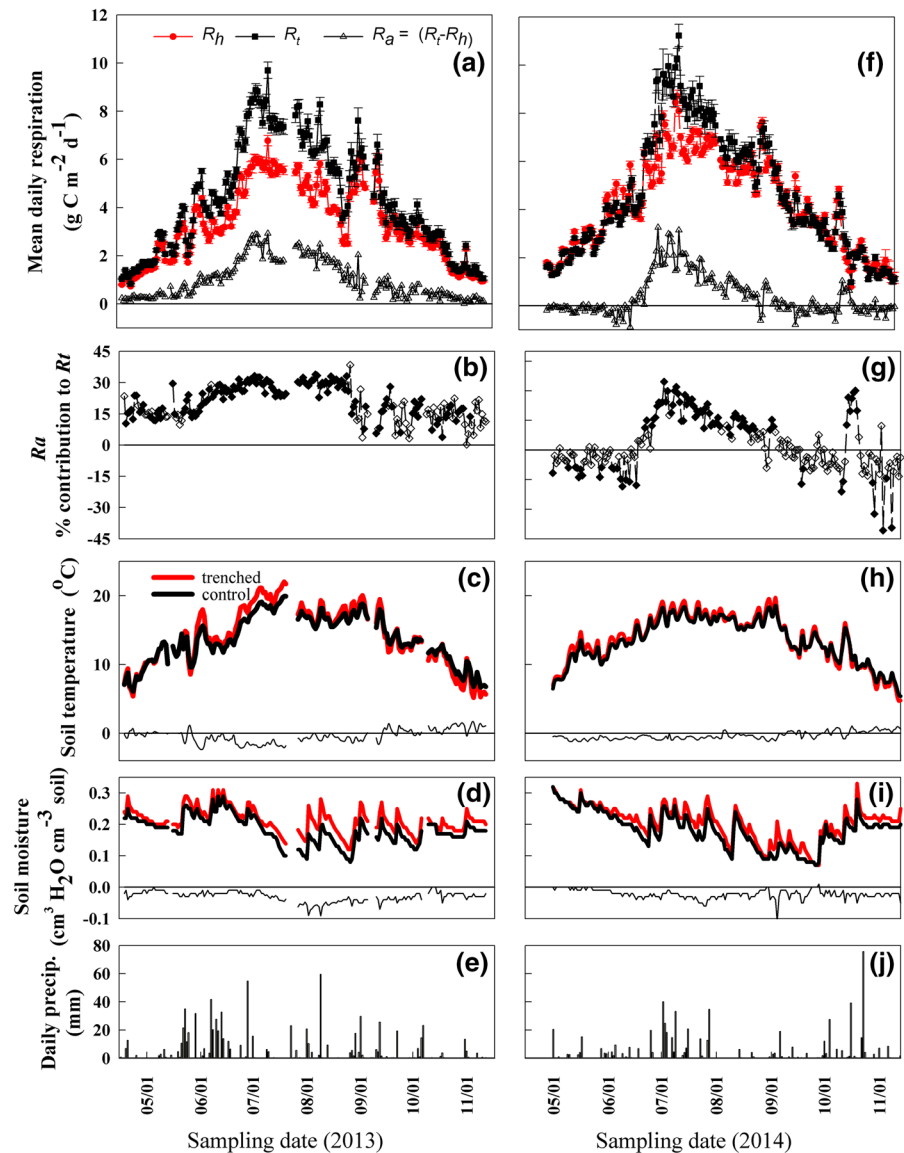
Seasonal respiration estimates

Prior to trenching in 2012, *Rt* measured from control and pre-trenched plots were not different (Bayes factor < 1), and the pre-trenched plot showed lower soil moisture compared to the control plot (Supplementary Material S3). Post trenching, mean daily *Rt*, *Rh* and *Ra* showed a seasonal pattern in both 2013 and 2014 (Fig. 2a, f). On a daily time-step, the percent contribution of *Ra* to *Rt* varied considerably (Fig. 2b, g) and peaked in mid-summer months when temperatures were warmest and trees most active. *Ra* made a smaller contribution to *Rt* in spring and fall. Maximum percent contribution of *Ra* to *Rt* in 2013 was 38 and 34% in 2014.

In 2013, mean soil temperature in the trenched plot was 0.8 °C warmer than the control plot and 0.4 °C warmer in 2014 (Fig. 2c, h). In 2013, the mean soil moisture in the trenched plot was 2% wetter than the control plot and 3% wetter in 2014 (Fig. 2d, i). In 2013, the differences between trenched and control plot soil moisture were greatest in the mid-summer (8%), but in 2014 they were largest in the early spring (5%). In 2013, and for a longer period in 2014, soil moisture was below previously observed thresholds for "drought stress" of 0.12 cm^{-3} H$_2$O cm^{-3} soil as defined for this site in Savage and Davidson (2001).

During spring and fall 2014, *Rh* was greater than *Rt* (Fig. 2f, inverse Bayes factor > 3). In early spring of 2014, when roots were less active and soils were relatively dry, fluxes in the trenched plot (*Rh*) exceeded the control (*Rt*) by up to 18% (Fig. 2f). In the fall, *Rh* exceeded *Rt,* by up to 40%. During the

Fig. 2 2013 and 2014 mean daily summary of respiration (**a** and **f**), and % *Ra* contribution to *Rt* (**b** and **g**), filled diamonds indicate strong level of support for differences based on inverse Bayes factors (> 3). Also mean daily soil temperature at 10 cm, (**c** and **h**; the thin black line is soil temperature control—soil temperature trenched), mean daily soil moisture at 10 cm, (**d** and **i**; the thin black line is volumetric soil moisture (VSM) control-VSM trenched) and daily precipitation (**e** and **j**). % *Ra* contribution to *Rt* is $\%Ra = ((Rt - Rh)/Rt)*100$

summer months, when roots were most active, *Rt* exceeded *Rh*.

Seasonal estimates of *Rt*, *Rh* and *Ra* for 2013 and 2014 were determined by summing respiration rates over each sampling season (Table 1). Missing data were linearly interpolated between sample dates. On a daily time-step, the percent contribution of *Ra* to *Rt* varied considerably (Fig. 2b, g), with greatest contribution from *Ra* occurring during the mid-summer months. Growing season estimates of *Rt* were similar in both 2013 and 2014 (Table 1). However, the seasonal average percent contribution of *Ra* to *Rt*

was higher in 2013 (23%) than in 2014 (9%). These seasonal estimates are not corrected for any artifact of the trenching method.

Artifacts of RSR

After 678 days of decomposition in the field, roots had lost 40–60% of their initial mass. Root decay constant (k) for fine + medium roots and for coarse roots were determined (Table 2). Mean decay values were similar to those found by McClaugherty et al. (1984) and Epron et al. (1999). Decay constants and estimates of

Table 1 Total seasonal fluxes (2013 was 209 days and 2014 was 201 days) with corrected estimates of Rh, corrected for both RSR (Rh_{RSR}) and root plus SMT ($Rh_{RSR + SMT}$)

Year	Rt	Rh	Ra (% Ra/Rt) uncorrected	Carbon loss from RSR	Rh_{RSR}	$Rh_{RSR + SMT}$	$Ra_{RSR + SMT}$	% Ra/Rt corrected
2013	916	707	209 (*23*)	45	662 (*628–687*)	603 (*553–656*)	313 (*260–363*)	34 (*28–37*)
2014	935	852	83 (*9*)	32	820 (*798–837*)	735 (*700–770*)	200 (*165–235*)	21 (*17–25*)

All units are g C m^{-2} season^{-1}. Gelman–Rubin test passed. 95% confidence intervals for parameter estimates from Eqs. (2) and (5) were used to derive uncertainty ranges (underlined italic). In 2013, % C loss from roots was 6.5 and 3.7% in 2014

Table 2 Decay constant k (scaled from daily to a yearly value) from Eq. (1) and upper and lower 95% confidence interval in brackets

Root class	Mean biomass M (g C m^{-2})	Mean decay constant, k (yr^{-1})
Fine + medium	714 (433–995)	0.26 (0.24–0.32)
Coarse	291 (23–559)	0.27 (0.18–0.35)

M is the mean biomass estimate and 95% confidence interval. Model Bayesian p value = 0.47. Gelman–Rubin test passed

root biomass (Table 2) were used to estimate carbon loss, Eq. (3), due to RSR for 2013 and 2014 (Table 1). This carbon loss was subtracted from observed Rh to "correct" values for artifacts due to RSR (Rh_{RSR}; Table 2). The annual effects of RSR on observed Rh was greater in 2013 (6.5%) compared to 2014 (3.7%; Table 1).

Artifacts of SMT

Rh was modeled using Eq. (4) and soil temperature and moisture measured within the trenched plot (Table 3). The trenched plot showed higher soil temperature and moisture compared to the control plot, (Fig. 2). We applied the modeled parameter estimates from Eqs. (5) to (4) and predicted respiration from the trenched plot using the soil moisture and temperature from the control plot. Using the percent difference between these modeled values, we subtracted this estimate from observed Rh to correct Rh for the artifacts of SMT (Fig. 2).

The modeled soil temperature and moisture content function had an overall effect of reducing measured estimates of Rh by 8% in 2013 and by 10% in 2014 (Table 3). Uncertainty in parameter estimates are defined by the 95% probability intervals of R_{ref}, Q and

Table 3 Model parameters and (95% confidence intervals)

	2013 (DOY 108–317)	2014 (DOY 121–321)
Trenched (Rh)	Rref = 67.9 (*67.2–69.5*)	Rref = 91.4 (*90.8–92.1*)
	Q10 = 3.9 (*3.8–4.0*)	Q10 = 4.1 (*4.0–4.2*)
	B = 0.86 (0.84–0.88)	*B = 0.88 (0.87–0.89)*
	soil T mean = 14.4	soil T mean = 13.5
	WC mean = 0.22	WC mean = 0.21
% correction Rh due to moisture and temperature artifacts	8.2	10.0
	(4–10)	(8–11)

Gelman–Rubin passed for 2013 and 2014. 2013 Bayesian p = 0.48, 2014 Bayesian p = 0.67. Soil T is soil temperature (°C) and WC is soil moisture content (cm^3 H$_2$O cm^{-3} soil). % ranges for corrections are based on using max and min ranges given for model parameters

B (Table 3) and these intervals were used to bracket the range of estimated corrections due to SMT.

Total corrected respiration

Figure 3 shows the daily time-step of Rt, Rh and Rh corrected for RSR (Rh_{RSR}) and for the combination of RSR and SMT ($Rh_{RSR+SMT}$). Greatest rates of correction occurred during the mid-summer, particularly related to SMT differences. Due to observed Rh exceeding Rt in the spring and fall of 2014, negative Ra were estimated (Fig. 2g). After corrections due to artifacts (Fig. 3b), in particular due to moisture and temperature, corrected Ra ($Ra_{RSR+SMT}$), is now positive in the spring 2014, and fall of 2014, however the magnitude of the correction in the spring was much greater than observed in the fall of 2014 (Fig. 3b).

Total flux with corrected estimates, showed a greater overall magnitude of correction in 2014 compared to 2013, primarily driven by the artifacts of SMT (Tables 1 and 3). The overall new seasonal estimate of Ra contribution to Rt, after corrections were applied was 34% in 2013 and 21% in 2014 (Table 1).

Discussion

Prior to corrections for the artifacts of trenching, Ra contributed 23% to Rt over the 2013 growing season, similar to other uncorrected estimates at the Harvard Forest using the trenching method (23% Ra; Savage et al. 2013), but lower than the Ra contribution estimated from a hemlock stand girdling study (35% Ra; Orwig et al. 2013) and a ^{14}C isotopic partitioning study, (41% Ra; Gaudinski et al. 2001). In the second year following trenching, the uncorrected contribution of Ra was 9%, much lower than that observed by other

Fig. 3 Daily C flux for observed fluxes in the control (black open squared) and trenched (black open circle) plots. **a** Thick black line is corrected for RSR and thick grey line corrected for RSR and SMT. **b** Black is observed Ra, light grey corrected Ra for RSR and dark grey corrected Ra for RSR and SMT
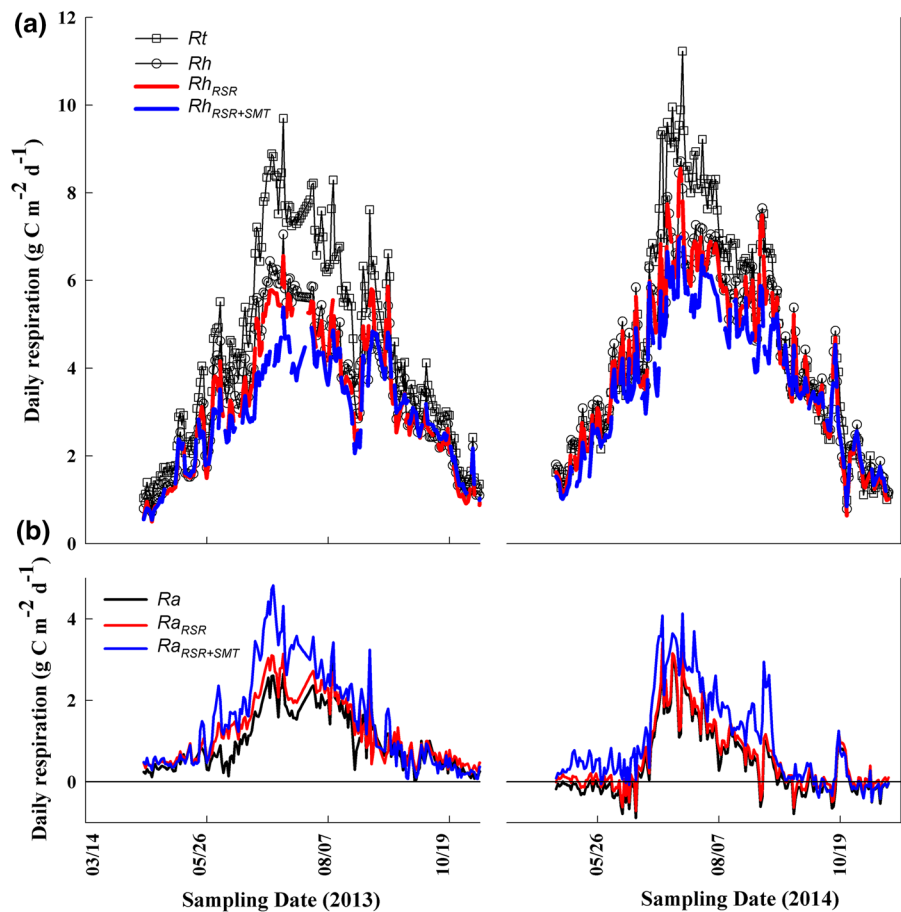
studies and methodologies. After corrections due to RSR and SMT artifacts, the percent contribution of Ra to Rt was 34% in 2013 and 21% in 2014. Clear trends emerged in the daily, seasonal and annual impacts of severed roots, moisture and temperature artifacts.

On a daily time step, RSR had a small impact on Rh (Fig. 3; Table 1). On an annual time scale, however, RSR decomposition had a significant impact on the estimate of Rh in the trenched plot, however it was smaller than the calculated soil moisture artifact. This was true in both years of the study during which time 40–60% of root mass was lost, and the effect of RSR is likely to have persisted into the future had the study continued. Based on the decay constant (k, Table 2) and initial site root biomass estimates for fine roots (Mo) for this study site (Abramoff and Finzi 2016), it would take 8 years for fine roots to lose 90% of their original mass. Overall, RSR reduced observed Rh 6.5% in 2013 and 3.5% in 2014. In a meta-analysis of experiments, Subke et al. (2006) highlighted that the uncertainty associated with additional decay of roots due to trenching was site specific and depended on root biomass and soil environmental characteristics. Comstedt et al. (2011) found a similarly low influence of root artifacts in a spruce forest, however, this is in contrast to other studies that have seen greater influence (Epron et al. 2001).

New fine roots were observed growing through root decomposition bags during the final collection period in 2014. This may be the result of either herbaceous root growth regenerating from understory vegetation or roots "invading" from outside the trenched plot, either through the plastic tarps in the trenched walls or from below the tarps upwards. We suspect Ra from these roots in the trenched plots, where the uncorrected method assumes that Ra is zero, may have contributed to the larger apparent rate of Rh in 2014, and the correspondingly low rate (9%) of uncorrected Ra. This leads to some uncertainty in the applicability of trenching to estimate Ra and Rh beyond a 2 years period.

Eliminating root inputs during trenching may reduce the contribution of Rh to Rt as a result of the loss of priming of SOM decomposition (Scott-Denton et al. 2006; Finzi et al. 2015). In temperate forests, it is estimated that priming contributes 10–25% of total Rh. Thus the addition of a priming effect would result in an even lower estimate of Ra than reported here. On the other hand, presence of RSRs may have provided sufficient carbon that there was little or no lack of C for priming additional decomposition. It should be noted that measurements of the magnitude of priming effects vary widely across and within plant species (Phillips et al. 2008, 2011; Abramoff and Finzi 2016).

Studies have shown that Rh is the dominant component of Rt during dry and drought induced treatments in a temperate forest (Borken et al. 2006). Dry conditions were observed during much of 2014 at Harvard Forest, but were less evident in 2013. In the dry spring of 2014, the uncorrected Ra contribution to Rt was often < 0, as opposed to spring 2013 when Ra was consistently positive. During the dry summer months of 2014, the proportion of Ra to Rt was much lower and declined faster than observed in 2013. This indicates that the difference in moisture and temperature is a significant artifact that needs to be corrected, particularly during drought conditions and that the magnitude of the correction can differ daily and seasonally.

Ra and Rh showed seasonal trends in 2013 with Ra peaking mid-summer when trees were most active. There was a slight increase in the relative proportion of Rh to Rt in the fall (Fig. 2b, % Ra declines therefore %Rh increases). The declining trend in Ra from mid-summer to fall suggests a reduction of above ground carbohydrate transport to support belowground processes, resulting in a measured total soil respiration during the fall dominated by Rh. This result is consistent with observations throughout the Harvard Forest in stands dominated by hardwood trees where the late summer and fall decline in ecosystem respiration is dominated by belowground fluxes (Giasson et al. 2013).

Conclusions

The trenching method is a useful technique for partitioning Ra and Rh, but recognized artifacts may need to be considered on a site-specific basis. RSR affected annual and interannual estimates at this study site, and artifacts due to moisture and temperature had large impacts at daily, seasonal and annual time steps. Use of high frequency measurements allowed us to identify these artifacts, particularly during drought conditions of the 2014 spring, something that might have been missed with a longer less frequent sampling interval.

The Bayesian modeling framework allows for the generation of credible uncertainty intervals that can assist in bracketing estimates of the components of Rt, including the magnitude of the adjustments for potential artifacts. This work outlines a protocol for quantifying and correcting the artifacts of recently severed roots and altered moisture and temperature that can be easily implemented for site-specific corrections.

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