FINAL TECHNICAL REPORT
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Project Title: Partitioning CO_2 fluxes with isotopologue measurements and modeling to understand mechanisms of forest carbon sequestration

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1. Project Summary and Objectives

This project combines automated *in situ* observations of the isotopologues of CO_2 with root observations, novel experimental manipulations of belowground processes, and isotope-enabled ecosystem modeling to investigate mechanisms of below- vs. aboveground carbon sequestration at the Harvard Forest Environmental Measurements Site (EMS). The proposed objectives, which have now been largely accomplished, include:

A. Partitioning of net ecosystem CO2 exchange (NEE) into photosynthesis and respiration using long-term continuous observations of the isotopic composition of NEE, and analysis of their dynamics ;
B. Investigation of the influence of vegetation phenology on the timing and magnitude of carbon allocated belowground using measurements of root growth and indices of belowground autotrophic vs. heterotrophic respiration (via trenched plots and isotope measurements);
C. Testing whether plant allocation of carbon belowground stimulates the microbial decomposition of soil organic matter, using *in situ* rhizosphere simulation experiments wherein realistic quantities of artificial isotopically-labeled exudates are released into the soil; and
D. Synthesis and interpretation of the above data using the Ecosystem Demography Model 2 (ED2).

2. Highlights

Accomplishments:

- Our isotopic eddy flux record has completed its 5th full year and has been used to independently estimate ecosystem-scale respiration and photosynthesis.
- Soil surface chamber isotopic flux measurements were carried out during three growing seasons, in conjunction with a trenching manipulation.

Key findings to date (listed by objective):

A. *Partitioning of Net Ecosystem Exchange:*
    1. Ecosystem respiration is lower during the day than at night—the first robust evidence of the inhibition of leaf respiration by light (the "Kok effect") at the ecosystem scale.
    2. Because it neglects the Kok effect, the standard NEE partitioning approach overestimates ecosystem photosynthesis (by ~25%) and daytime respiration (by ~100%) in the first half of the growing season at our site, and portrays ecosystem photosynthetic light-use efficiency as declining when in fact it is stable until autumnal senescence.

B. *Vegetation Phenology and belowground allocation:*

    Findings:

    1. Autotrophic respiration (*Ra*) showed a seasonal pattern, peaking in mid-summer when trees were most active.
    2. The effective age of the substrate for belowground respiration is less than 2 weeks.
    3. Above and belowground phenology are more synchronous in deciduous hardwood stands than evergreen hemlock stands.
    4. The decline in root respiration rates in the fall is related to temperature rather than acclimation of root respiration or substrate limitations.

    Methodological Issues:

    5. The isotopic signatures of autotrophic and heterotrophic respiration are too similar for isotopic partitioning of belowground respiration into these two components at our site—in keeping with the recent findings of Bowling et al. (2015) in a subalpine conifer forest.
    6. Artifacts of the trenching method, such as changes in soil moisture and increased carbon substrate from the newly severed roots, are significant and need to be quantified when determining daily to annual estimates of autotrophic and heterotrophic respiration.

C. Effects of simulated exudates on priming of microbial decomposition:

    The stoichiometry of root exudates influences both the amount and the mechanism by which priming occurs. At low C:N, SOC loss is caused by an increase in microbial efficiency. At high C:N, SOC loss is caused by an increase in microbial biomass.

D. Modeling with the Ecosystem Demography Model (ED2):
    1. Incorporation of ^{13}C tracking to create an isotopically-enabled Ecosystem Demography v2 model (ED2)
    2. State-of-the-art parameter optimization methodology developed for improving ED2 model predictions and parameters.
    3. Significantly improved model predictions of growth- and maintenance-related carbon fluxes and ^{13}C fluxes

3. Detailed Progress and Findings

The major contributing institutions for each aspect of the following work are identified as follows: UA = University of Arizona (Saleska, Wehr), BU = Boston University (Finzi), WH = Woods Hole Research Center (Davidson, Savage), HU = Harvard University (Moorcroft).

3.1. Ecosystem-Atmosphere CO_2 Exchange

We (UA) completed and published (Wehr and Saleksa, 2015) substantially improved theory for using isotopic eddy flux measurements to partition the net ecosystem-atmosphere CO_2 exchange (i.e. NEE) into ecosystem photosynthesis (i.e. gross ecosystem production, GEP) and daytime ecosystem respiration (DER). Our (UA) findings from isotopic partitioning of 3 years of NEE measurements (2011-2013) are described below and in a manuscript that is about to enter its 2nd round of review at *Nature* (Wehr et al. 2015). We (UA) also continued to measure the ecosystem-atmosphere exchange of $^{12}CO_2$, $^{13}CO_2$, and $^{18}O^{12}C^{16}O$ by eddy covariance, extending our isotopic flux dataset into its fifth full year. This dataset is unprecedented in its duration and precision.

Our first major finding is that ecosystem respiration (and in particular, aboveground respiration) was lower during the day than at night during the first half of the growing season (Fig. 1), which is the time of year when nighttime leaf respiration is substantial. This finding is the first robust evidence of the inhibition of leaf respiration by light (the "Kok effect") (Heskel et al., 2013) at the ecosystem scale. In contrast, standard partitioning of NEE (Reichstein et al., 2005)—which has underlain the design and evaluation of terrestrial biosphere models used on climate prediction (Parazoo et al., 2014; Stöckli et al., 2008) and of remote sensing indices of global biosphere productivity (Parazoo et al., 2014; Turner et al., 2003)—neglects the Kok effect and assumes that respiration is higher during the day than at night (Fig. 1).

Our second major finding is that because it neglects the Kok effect, standard partitioning of NEE substantially overestimates ecosystem photosynthesis (by ~25%) and daytime respiration (by ~100%) in the first half of the growing season at our site.

Our third major finding is that the response of ecosystem photosynthesis to light is stable until autumnal senescence (Fig. 2), whereas standard NEE partitioning portrays that light-response as gradually declining through the growing season both at our site and in temperate forests more generally (Falge et al., 2002). Similar declines have been attributed to leaf ageing or water stress in other forests (Constable & Rawson, 1980; Grassi et al., 2005); the influence of such effects on photosynthesis is exaggerated by standard partitioning.

By comparing the isotopic signatures of photosynthesis and belowground respiration, we also found that the effective age of the substrate for belowground respiration was 2 weeks or

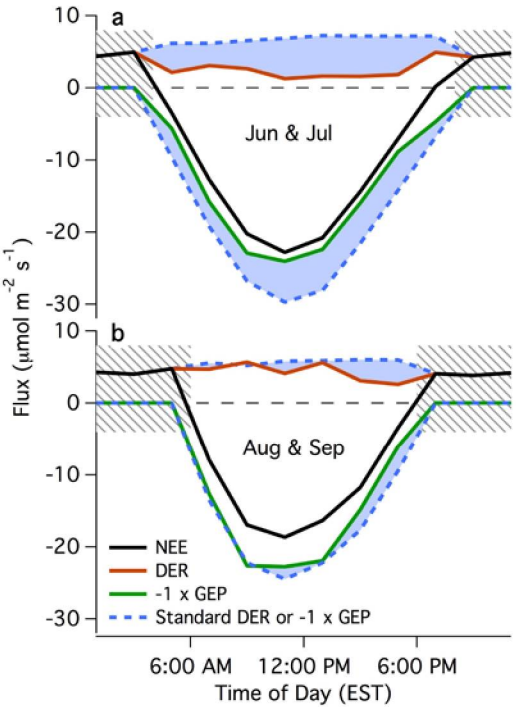

Figure 1 Composite diel cycles show that photosynthesis and daytime respiration are less than predicted in the first half of the growing season. Fluxes in the flux tower's relatively homogeneous and well-sampled southwest quadrant averaged across the three years, 2011-2013, for (**a**) June-July and (**b**) August-September. Differences from standard partitioning results for the same data are shaded in blue. Lines connect means for each 2-hour bin. Partitioning is only done for daylight periods; GEP is set to zero in the dark (hatched areas). From Wehr et al., 2015, *in review*.

less, and that the isotopic disequilibrium (i.e. the isotopic difference between the ecosystem's photosynthetic carbon input and respiratory carbon output) was stable at about -1 ‰ between the completion of leaf expansion and the onset of senescence (Fig. 3). The sign of the disequilibrium is opposite to that predicted based on the Suess effect but is in agreement with observations in a subalpine conifer forest (Bowling et al., 2014), suggesting that post-photosynthetic fractionation leads to sequestration of isotopically enriched carbon in wood or soil (Bowling et al., 2014).

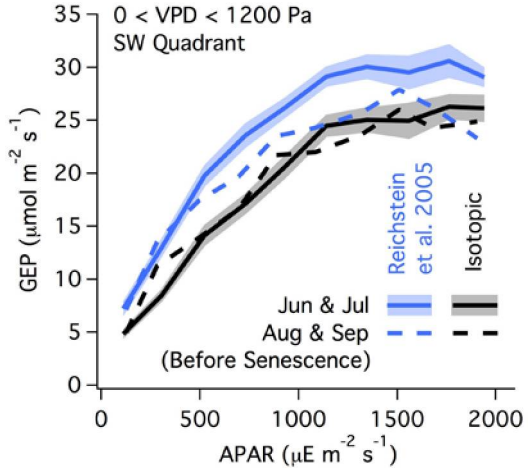

Figure 2 The ecosystem-scale light-response curve is invariant over the season. Responses of GEP from isotopic and standard partitioning to Absorbed Photosynthetically Active Radiation (APAR), in June-July and August-September, for Vapor Pressure Deficits (VPD) between 0 Pa and 1200 Pa, in the flux tower's southwest quadrant. Lines connect means for each APAR bin, and pale bands show standard errors in the means calculated from variability within each bin. Bands are omitted from the August-September curves for clarity. From Wehr et al., 2015, *in review*.

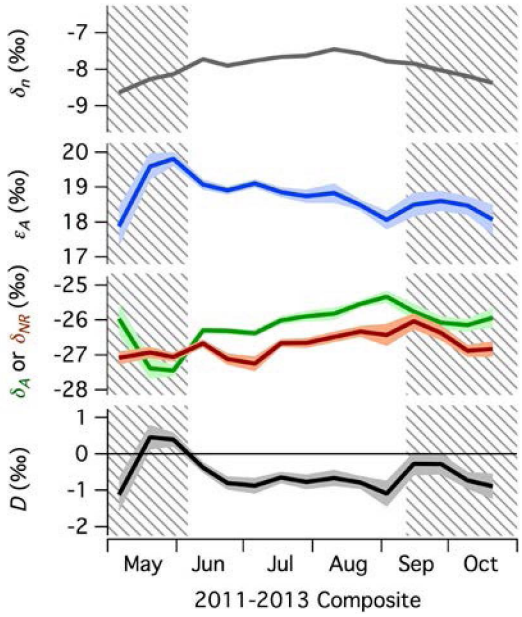

Figure 3 Composite seasonal cycles of the isotopic composition, discrimination, and disequilibrium. Shown are the isotopic composition of CO_2 in the canopy airspace, δ_n; the apparent fractionation by net photosynthetic assimilation (also called discrimination), ε_A; the isotopic signatures of net photosynthetic assimilation, δ_A, and non-foliar respiration, δ_{NR}; and the isotopic disequilibrium, $D = \delta_{NR} - \delta_A$. Dark lines connect flux-weighted means including all daylight hours for each 12-day bin, except in the case of δ_{NR}, where the lines connect simple means for each bin including all nighttime hours (because δ_{NR} is derived from nighttime Keeling plots rather than daytime flux measurements). Light shaded bands show standard errors in the flux-weighted means (or just standard errors in the means for δ_{NR}), based on variability within each bin. Hatched areas indicate leaf expansion and abscission.

3.2. Soil Surface CO_2 Efflux

The soil surface chamber system was successfully integrated with the isotopic quantum cascade laser spectrometer in 2012, and the isotopic composition of belowground respiration was thereby measured during three growing seasons (2012, pre-trenching, and 2013-2014, post-trenching). Unfortunately, comparison of the isotopic composition of belowground respiration between the trenched and control plots revealed that the isotopic signatures of autotrophic and heterotrophic respiration were indistinguishable and so could not be used to partition belowground respiration into these two components at our site. This finding is in keeping with the very recent findings of Bowling et al. (2015) in a subalpine conifer forest. We therefore relied on the trenching manipulation to assess auto- and heterotrophic respiration.

In the late fall of 2012, a trench was dug (to 1m depth) around a 5x5m area, severing all roots leading into the treatment plot. Plastic tarp was placed along the walls of the trenched plot to prevent new root in-growth and then backfilled. Four automated soil respiration chambers were placed within the trenched plot and four in an adjacent control plot area. Measurements from the non-trenched plot (control) represent the combined *Rh(heterotrophic)* and *Ra* (autotrophic) components of *Rt (total soil respiration).* Fluxes from the trenched plot represent, *Rh* and the difference (*Rt-Rh*) represents *Ra*.

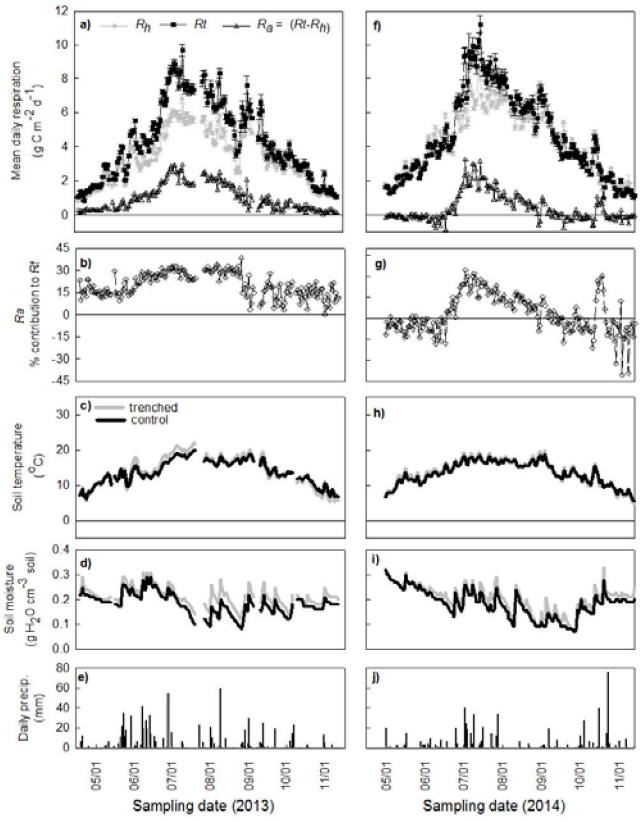

Figure 4: 2013 and 2014 mean daily summary of respiration (a,f), % *Ra* contribution to *Rt* (b,g), mean daily soil temperature at 10cm (c,h), mean daily soil water content at 10cm (d,i) and daily precipitation (e,j). % *Ra* contribution to *Rt* is %*Ra*=((*Rt-Rh*)/*Ra*)*100.

Prior to trenching in 2012, *Rt* measured from control chambers and those measured from pre- trenched chambers were not significantly different (t-test, 95% confidence, p value = 0.20). Following trenching, the mean daily *Rt*, *Rh* and *Ra* showed a seasonal pattern in both 2013 and 2014 (Figure 4a, f). The % contribution of *Ra* to *Rt* (Figure 4 b,g) peaked in the mid-summer months, when soil temperatures were warmest and trees were most active, but *Ra* showed a smaller contribution to *Rt* in the spring and fall, likely due to decreased aboveground activity.

Seasonal estimates of *Rt*, *Rh* and *Ra* for 2013 and 2014 were determined by summing respiration rates over each sampling season. Any missing data were linearly interpolated between sample dates. On a daily time-step, the % contribution of *Ra* to *Rt* varied considerable (Figure 4b, g), with greatest rates occurring during the mid-summer months. Maximum %*Ra* in 2013 was 38% and 34% in 2014. Seasonal estimates of *Rt* were similar in both 2013 and 2014 (Table 1) however the seasonal average % contribution of *Ra* to *Rt* was higher in 2013 (23%) and much lower in 2014 (9%).

Quantifying the artifacts of the trenching method

The trenching method can cause disturbance to the soil-plant-atmospheric ecosystem resulting in some potential limitations and induced artifacts. Reduced water uptake in trenched plots, via loss of evapotranspiration, could change soil water content, which is one of the environmental controllers of *Rt* in many ecosystems. Since soil moisture is an important driver of *Rh*, this artifact may have important implications for *Rh* measured in the trenched plots. Further, the recently severed dead roots may temporarily increase available carbon substrate for microbial decomposition resulting in higher observed *Rh*.

Table 1

Year	Total Control *Rt*- Flux	Total Trenched *Rh*-Flux	Uncorrected *Ra* (*Rt*-*Rh*)	Corrected *Rh_rw* for root and water artifacts	Corrected *Ra*	% corrected *Ra* contribution to *Rt*
2013	916	707	209 *(23%)*	603 *(553-656)*	313 *(260-363)*	34% *(28%-37%)*
2014	935	852	83 *(9%)*	735 *(700-770)*	200 *(165-235)*	21% *(17%-25%)*

Total seasonal fluxes (2013 was 209 days and 2014 was 201 days) with corrected estimates from the trenched plot, corrected for both root decomposition and temperature and water content artifacts. All units are g C m^{-2} $season^{-1}$. % in brackets are % *Ra* contribution to *Rt*. These models passed the Gelman-Rubin test for convergence. *Rh_rw* is the trenched plot heterotrophic respiration corrected for the artifacts of recently severed roots and moisture differences.

Data from root decomposition bags were used to determine a root decay constant. Along with prior estimates of root biomass (Abramoff) used Epron *et al.* (1999) C-loss equation to estimate C-loss due to recently severed roots on a daily time-step from 2013 through 2014. This daily time step of C loss was subtracted from observed *Rh* to "correct" observed *Rh* values for artifacts due to recently severed roots. The carbon loss was 6% for 2013 and 4% for 2014.

The trenched plot showed higher soil temperature and moisture compared to the control plot, which is assumed to be the "true" unaltered conditions (Figure 4). *Rh* was modelled using a soil temperature and moisture measured within the trenched plot. We then applied the modelled parameters from and predicted respiration from the trenched plot using the soil moisture and temperature from the control plot. Using the % difference between these modelled values, we subtracted these estimate from observed *Rh* to "correct" *Rh* for the artifacts of soil moisture and temperature. For 2013, the modeled soil temperature and water content function had an overall effect of reducing measured estimates of *Rh* by 8% and for 2014 reduced *Rh* by 10%

Table 1 shows the final "corrected" annual estimates of *Ra* and *Rh* for 2013 and 2014. The artifacts from the trenching method have a significant effect on partitioned estimates of *Ra* and *Rh* and need to be accounted for when using this method.

3.3. Root Phenology

We measured the phenology and partitioning of C allocated belowground across the growing season in three dominant stand types at the Harvard Forest. Northern red oak (*Quercus rubra*) and eastern hemlock (*Tsuga canadensis*) dominate the tower footprint. White ash (*Fraxinus americana*) is present in the tower footprint and is associated with arbuscular-mycorrhizal fungi, providing a useful contrast to ectomycorrhizal-associated oak and hemlock.

Root production

Root production and turnover were measured April–December 2012, March–November 2013, and April–November 2014 using a BTC-100x high magnification minirhizotron camera system (Bartz Technology Company, Carpenteria, CA). Root growth was positively correlated with soil temperature in each stand ($P < 0.001$), but was not correlated with precipitation. As a result root growth was concentrated in mid-summer, but with distinct stand-level differences (Figure 5, a-f). Red oak root growth occurred in 1–3 flushes over the growing season, with highest mortality in mid-to-late-summer (Figure 5, a-c). Root mortality was not correlated either with soil temperature ($P = 0.13$) or precipitation ($P = 0.76$).

In red oak and white ash stands the peak in maximum canopy greenness occurred ~20 days earlier than in the eastern hemlock stand, but the peak in root growth occurred ~50 days earlier. As a result, the deciduous stands had a smaller offset between maximum canopy greenness and peak root growth than did the hemlock stand. In 2012 hemlock fine root NPP was positive but in 2013 and 2014 there was no net production of fine roots.

Fine root growth was initiated earlier in the growing season in the hardwood stands compared to the hemlock stand (Figure 5, a-c). The deciduous hardwood stands also had more synchronous above- and belowground phenology than the evergreen hemlock stand, consistent with a recent meta-analysis (Abramoff and Finzi 2015). The offset between maximum canopy greenness, a proxy for aboveground phenology, and root growth was about 30 days shorter in the deciduous stands (Figure 5).

Root respiration

CO_2 efflux was measured directly on recently severed fine roots using an infrared gas analyzer (LI6400, LiCor Biosciences, Lincoln, NE). The activation energy, a proxy for apparent temperature sensitivity, of mass-specific root respiration was lowest in the ash stands and highest in the red oak stand, reflecting the high rate of respiration in ash that was maintained throughout the growing season. In red oak and eastern hemlock, respiration rates more closely followed the seasonal cycle of temperature. The concentration of nitrogen was significantly higher in the fine root tissue from the ash stand compared to root tissue from the other two stands. Given the correlation between tissue N concentration and root respiration (Burton et al. 2002), root chemistry may be a proximate cause for the consistently high mass-specific respiration rates observed in the ash stand.

When incubated at a common temperature the rate of root respiration increased across the growing season in all three species (Figure 6). This suggests an increase in photosynthate allocation to roots through time and that the decline of respiration rates in the fall is related to temperature rather than acclimation of root respiration or substrate limitation. This observation is qualitatively similar to that of Burton & Pregitzer (2003) who did not find acclimation of root respiration across the growing season in sugar maple stands in Michigan. Both studies contrast with the apparent acclimation of root respiration in response to experimental soil warming at the Harvard Forest (Burton et al. 2008).

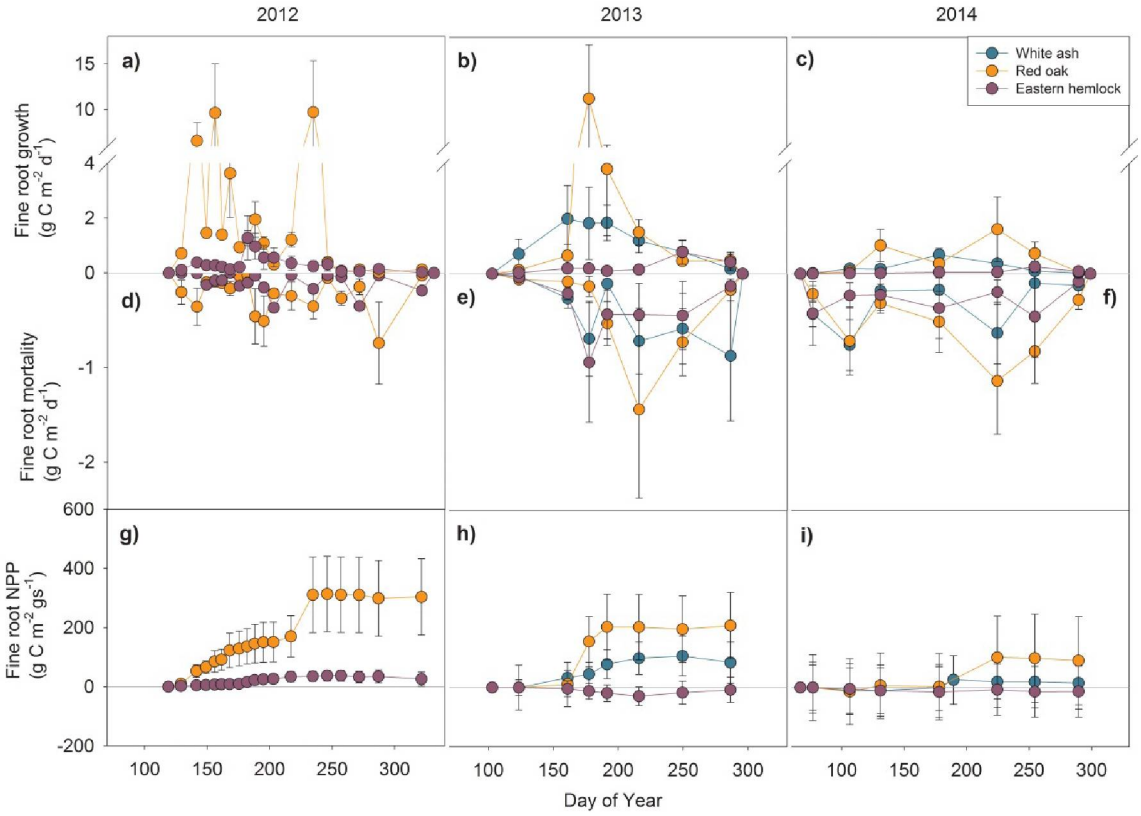

Figure 5. Growth (a-c), mortality (d-f), and net primary production (NPP, g-i) of fine roots. In 2012, n = 4 and n = 9 for red oak and eastern hemlock, respectively. In subsequent years, n = 10 for each stand. Error bars are standard error of the mean. There was a significant effect of year and stand on both growth and mortality. The eastern hemlock stand had significantly less growth and mortality than red oak and white ash stands ($P < 0.001$), but red oak and white ash stands were not different from each other (growth was only marginally different, $P < 0.1$).

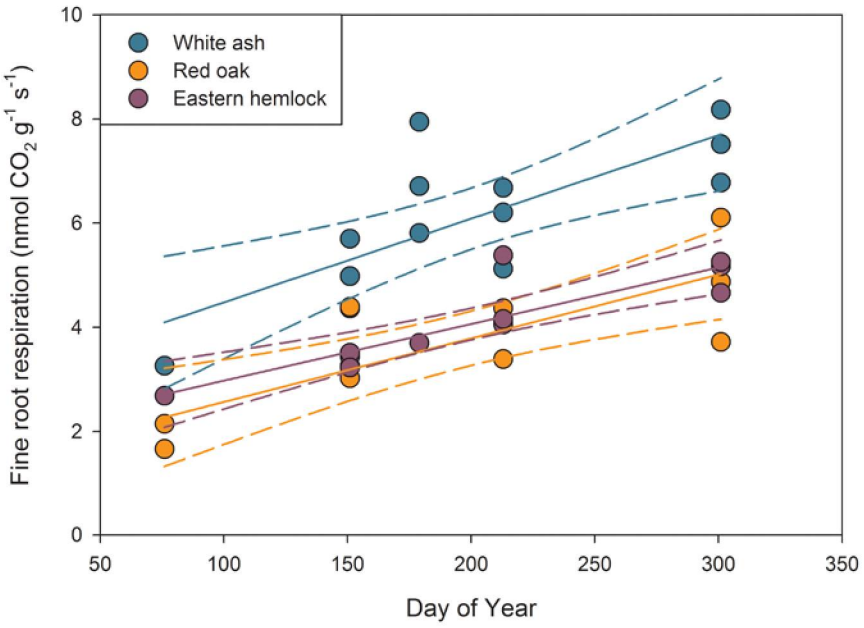

Figure 6. Fine root respiration measured in the lab at temperatures ranging between 16ºC and 25ºC, with a mean of 22.8 ± 0.5ºC. There was a significant positive relationship between fine root respiration and sample date for white ash ($\beta = 0.016$, $F_{1,11} = 15.3$, $P < 0.01$, $R^2_{adj} = 0.54$), red oak ($\beta = 0.012$, $F_{1,9} = 17.6$, $P < 0.01$, $R^2_{adj} = 0.62$), and eastern hemlock ($\beta = 0.011$, $F_{1,9} = 32.1$, $P < 0.001$, $R^2_{adj} = 0.76$). Dash lines are 95% confidence intervals around the regression fit.

Nonstructural carbohydrates

The pool of NSC was estimated monthly from May to November 2011 and four times from March to November 2012 using the method of Chow & Landhausser (2004). There was large inter-annual variability in the concentration of NSC in fine roots. In 2012, there was a decline in NSC concentration mid-summer relative to the spring and fall. In 2011, there was a slight but significant increase in NSC concentration across the growing season ($P < 0.001$).

Root exudates

Root exudates were collected from six fine root systems per stand in June and August 2012, and April, May, July and October 2013 following the method of Phillips *et al.* (2008, 2011). Exudation rate was highly variable and there was no clear stand-level difference or seasonal pattern, although there were significantly lower exudation rates in early spring compared to summer and fall. This pattern of exudation mirrors the seasonal increase in mass-specific rates of root respiration and supports the idea of a progressive increase in C allocation belowground across the growing season (Figure 6).

Total Belowground Carbon Flux

We estimated total belowground C flux ($TBCF_{top}$, g C m^{-2} gs^{-1}) using two different approaches. First, a top-down estimate is defined as:

$$TBCF_{top} = F_{efflux} + F_{leaching} - F_{litter} + \Delta(C_{roots} + C_{soil}) \quad [1]$$

where F_{efflux} is the growing season rate of soil respiration, $F_{leaching}$ is the flux of dissolved organic C into streamwater, F_{litter} is litterfall, and $\Delta(C_{roots} + C_{soil})$ is the growing season change in the C pool associated with fine roots and soil.

Second, a bottom-up estimate ($TBCF_{bottom}$, g C m^{-2} gs^{-1}) is defined as:

$$TBCF_{bottom} = F_{roots} + F_{resp} + F_{exudates} \quad [2]$$

where F_{roots} is gross fine root production, F_{resp} is fine and coarse root respiration, and $F_{exudates}$ is root exudation. F_{roots}, $F_{exudates}$, and fine root respiration are estimated using measurements from this study. All other fluxes were estimated by synthesizing recent and archived site data.

Estimates of TBCF were largest in the red oak stand and smallest in the eastern hemlock stand. $TBCF_{top}$ was larger than $TBCF_{bottom}$ in all of the stands.

The phenology of TBCF differed between stands. Red oak stands allocated more C belowground earlier in the growing season compared to white ash and eastern hemlock. The peak in $TBCF_{bottom}$ in red oak was coincident with the spring ramp-up of GPP. In eastern hemlock stands, the phenology of $TBCF_{bottom}$ is not pronounced. In both oak and hemlock stands, soil respiration peaks later in the season than either GPP or $TBCF_{bottom}$ (Figure 7).

TBCF varied between 28 and 35% of GPP. Consistent with our third hypothesis, red oak allocated C belowground earlier in the growing season compared to eastern hemlock. Furthermore, red oak had the highest rate of TBCF and the greatest proportional allocation to fine root production (Figure 8). The phenology of TBCF mirrored the phenology of root growth in the red oak stand, as a result of the high proportional allocation of root growth to TBCF.

The modest C investment in root production in the hemlock stand contrasts with evergreen trees in general, which tend to allocate a substantial fraction of C to roots and ectomycorrhizal symbionts (Clemmensen et al. 2013) Recently, this stand became infested by the invasive pest hemlock woolly adelgid (HWA), which became widespread at the Harvard Forest in 2012. The HWA feeds on phloem sap at the base of needles and progressively kills adult trees within 3-10 years of infestation (Orwig 2002, Orwig et al. 2008). Since 2012 there have been visible signs of crown thinning and HWA-induced tree mortality. Compared to 2012, the allocation of C to root production in the hemlock stand dropped by 11% in 2013 and 72% in 2014. Thus it seems that low TBCF in this stand reflects the negative effect of the HWA on photosynthesis and tree health. Surveys of hemlock roots in infested stands in Connecticut found that ectomycorrhizal colonization, bacterial abundance in the adjacent rhizosphere, and root C:N all declined (Vendettuoli et al. 2015).

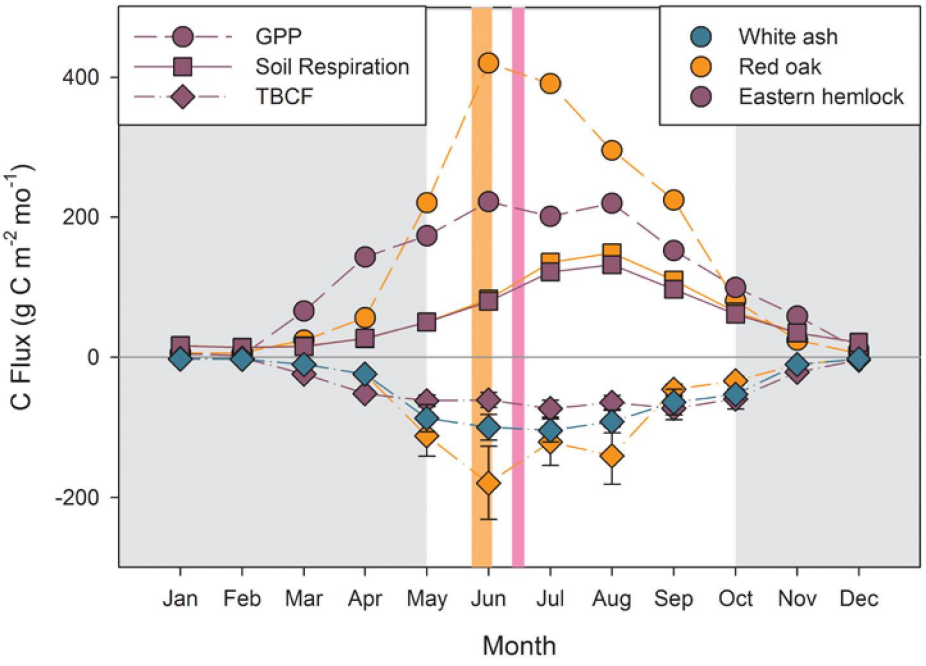

Figure 7. Time series of gross primary production estimated using data from the Harvard Forest Environmental Measurement Site flux tower and the hemlock tower in 2012 (circles). Soil respiration for the eastern hemlock and red oak stands (squares) and $TBCF_{bottom}$ (diamonds) for red oak, eastern hemlock and white ash stands calculated using root GPP, respiration and exudation from May to October. For Jan–Apr and Nov–Dec when data were not available (shaded areas), I used the median ratio of TBCF:GPP to extrapolate TBCF from GPP data. This ratio (5^{th}, 95^{th} percentiles) was 0.42 (0.23, 0.5) for hardwoods and 0.36 (0.28, 0.57) for hemlock. The orange and pink colored bars show the maximum canopy greenness in red oak and eastern hemlock stands, respectively, for this study period determined using PhenoCam data (http://phenocam.sr.unh.edu/).

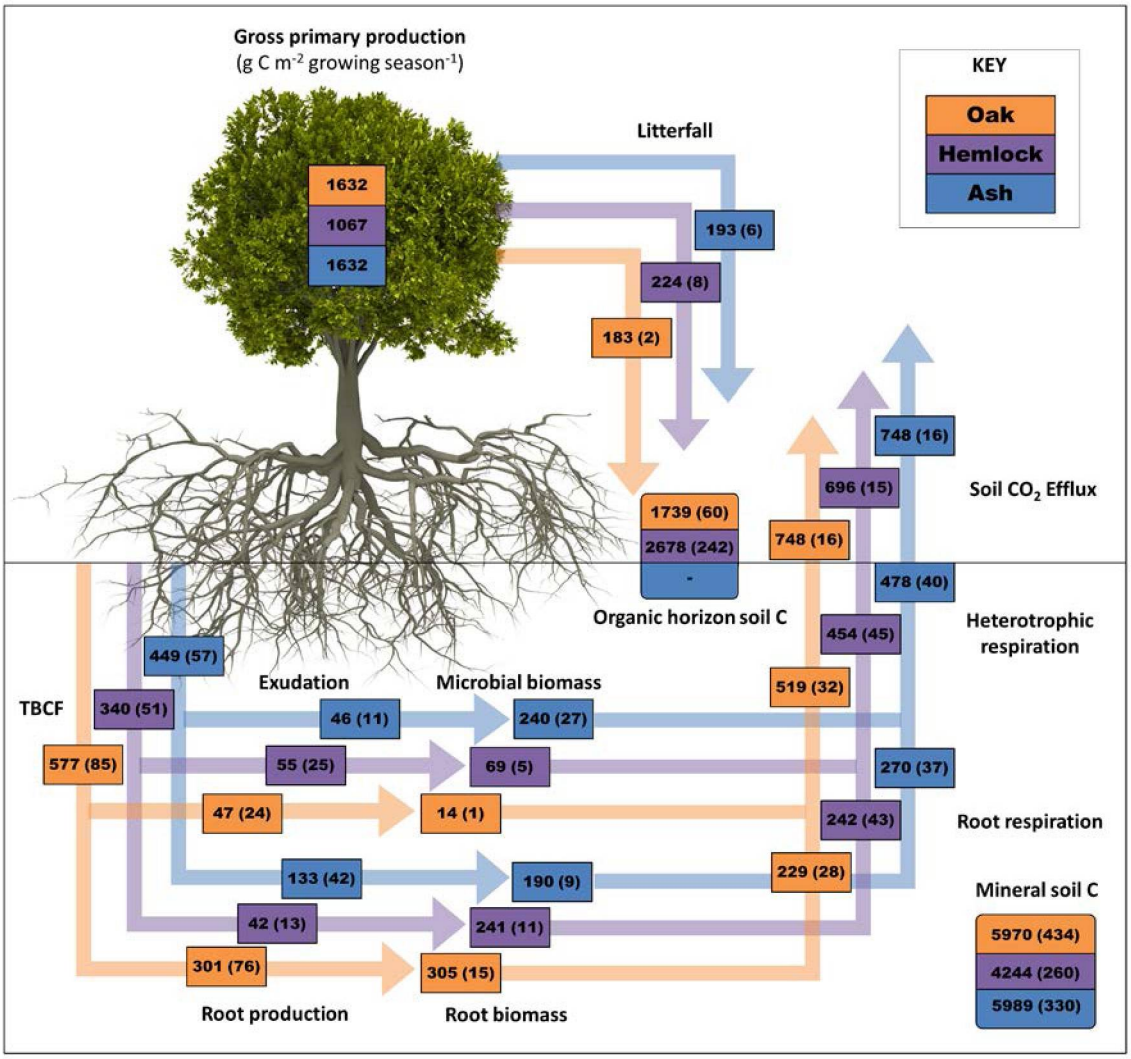

Figure 8. Gross primary production and belowground fluxes (litterfall, $TBCF_{bottom}$ [fine root production, root respiration, exudation], heterotrophic respiration, soil CO_2 efflux) and pools (microbial biomass, root biomass, organic horizon soil C, mineral horizon soil C) for each stand. Heterotrophic respiration is estimated as the difference between soil CO_2 efflux and root respiration. Standard error is reported in parentheses next to each pool or flux value, and all units are g C m^{-2} gs^{-1}. The growing season is defined as the six month period from May-October.

Modeling

We developed a numerical model to simulate the effect of root exudate quantity and stoichiometry on heterotrophic respiration. This model was developed by merging the Dual Arrhenius Michaelis-Menten model of Davidson *et al.* (2012) with the Microbial Carbon and Nitrogen Physiology Model of Finzi *et al.* (2015). The combined model, DAMM-MCNiP, reproduced measured rates of heterotrophic respiration over the growing season, and performed particularly well compared to each model alone when confronted with wet-up events. We found that the stoichiometry of root exudates influences both the amount and the mechanism by which priming occurs. At low C:N, SOC loss is caused by an increase in microbial efficiency (Figure9 a,b). At high C:N, SOC loss is caused by an increase in microbial biomass (Figure 9c).

Spatially Resolved Measurements of Enzyme Activity

We developed a quantitative framework for estimating the spatial extent of the rhizosphere using image analysis of 2-D zymographs that may be able to improve estimates of the exudate diffusion distance. We designed root boxes with hinged panels that allow access to a vertical, rooted soil surface. We incubated substrates for four C-, N- and P- degrading enzymes against N=24 soil surfaces after Dong *et al.* (2007). We then developed an image analysis protocol to measure enzyme activity using darkness of staining, automatically detect roots in a digital image of the soil surface, overlay the two datasets (Figure 10) and calculate relationships between extracellular enzyme activity and distance to the nearest root. This method is still under development, but we can detect significant negative linear relationships between extracellular enzyme activity and distance to a root.

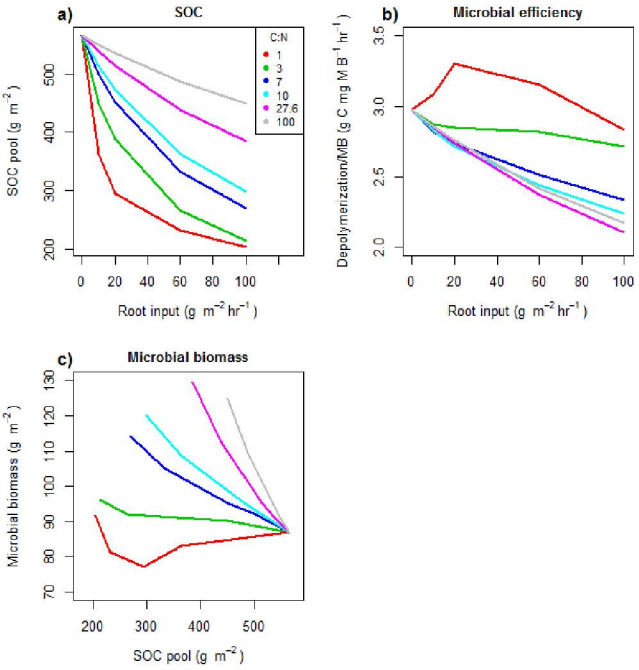

Figure 9. (a) SOC as a function of root inputs. (b) Microbial efficiency (depolymerization per unit microbial biomass) as a function of root inputs. (c) Microbial biomass as a function of the SOC pool. In panels a-c, root input C:N varies between 1 and 100 (colored lines).

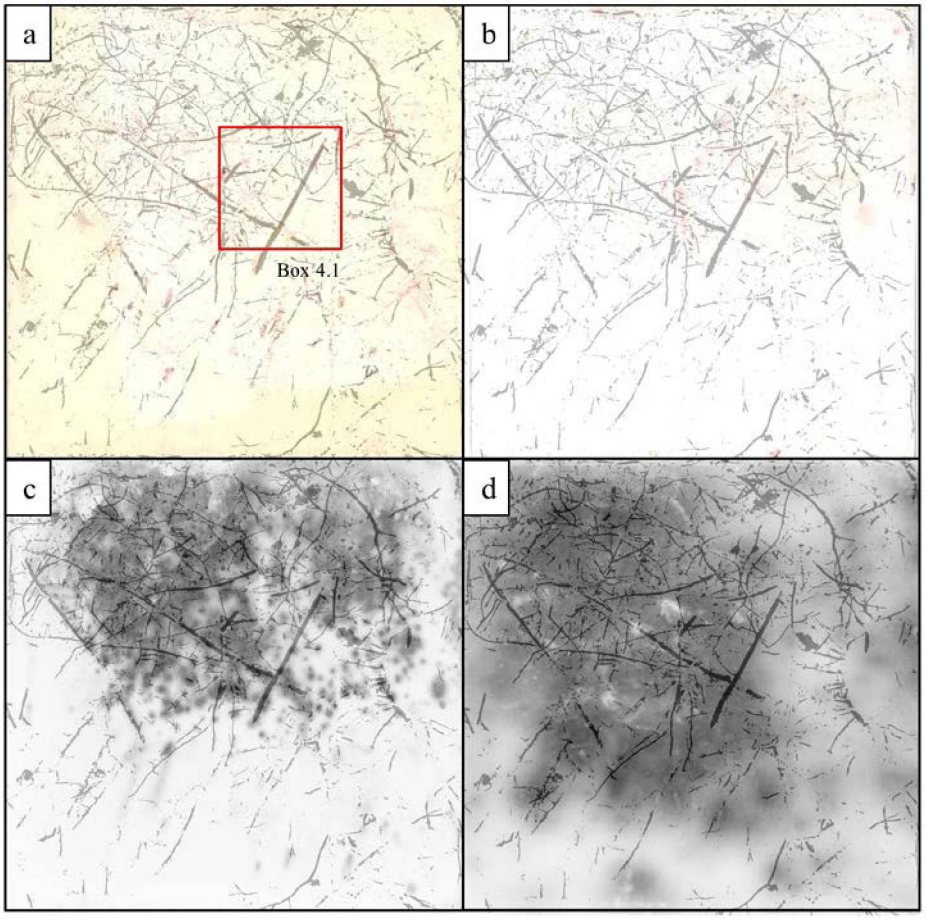

Figure 10. Zymograms measuring the activity of (a) acid phosphatase, (b) aminopeptidase, (c) beta-glucosidase, and (d) N-acetyl-beta-D-glucosaminidase on the same hemlock stand root box panel. Thresholded mask of roots is overlaid on each zymogram at 70% transparency. Box 4.1 is the image subset used in very-fine-resolution analysis.

3.4. Ecosystem Modeling

A ^{13}C tracking sub-system of the Ecosystem Demography 2 (ED2) model is now fully implemented (Figure 11). In the new model formulation, the isotopic composition of photosynthetic carbon flux is modeled by integrating the Farquhar et al. (1982) photosynthetic isotope discrimination model with ED2's existing carbon assimilation system. The isotopic compositions of ecosystem pools and fluxes are then derived by propagating the signal from this input mechanistically throughout carbon pools in plants, soil, and canopy airspace. In addition, we have developed and implemented a generalized framework for optimizing the ED2 model's predictions and parameters. We have used this framework to improve the model's predictions of above- and below-ground carbon fluxes.

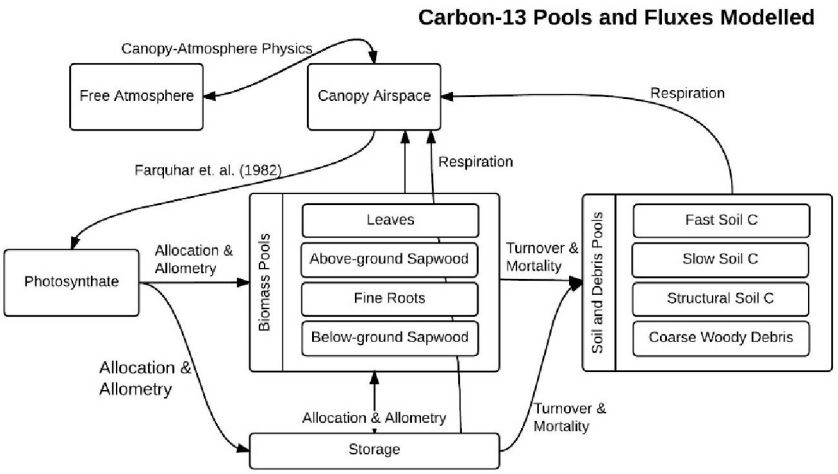

Figure 11: Simplified schematic of carbon-13 integration into the ED2 model. Arrows denote carbon fluxes, boxes denote carbon pools. Each of these pools and fluxes has a total-carbon content and carbon-13 content assigned.

Changes to the ED2 Model Formulation

To make the isotopic accounting as realistic and accurate as possible, we made two changes to the underlying ED2 systems, explicitly partitioning ecosystem growth and maintenance fluxes across the model's carbon pools and reducing the timescale of plant carbon allocation. The partitioning of growth and maintenance fluxes was motivated by a mis-match between model predictions and the total soil respiration data that had been overlooked in previous studies (e.g. Medvigy et al. 2009). As illustrated in Figure 12a, the previous version of ED2 substantially under-predicted the fraction of soil respiration attributable to autotrophs. Associated with this was an under-prediction of total soil respiration since the model's predictions of heterotrophic respiration rates were near their measured values. Explicit partitioning growth and maintenance respiration between the model's different carbon pools allowed attribution of these fluxes to different plant tissues (above-ground: leaves and stem; below-ground coarse and fine roots). The results of this on soil respiration also illustrated in Figure 12b.

Regarding the time-scale change, ED2 previously calculated most fluxes on sub-daily time-step during which there were no explicit plant-internal sources of respiration and newly assimilated carbon was not associated with any particular plant tissue. At the end of each day an accounting was undertaken and tissues or stored carbon were incremented or decremented to preserve carbon balance. This system thereby used carbon for respiration prior to deciding its source and it accumulated (and used) carbon without deciding what tissue it was located in. It was therefore incompatible with a fully mechanistic 13C sub-model, so the model was revised to explicitly track the sources and sinks of each modeled flux, while maintaining the reproducibility of previous results.

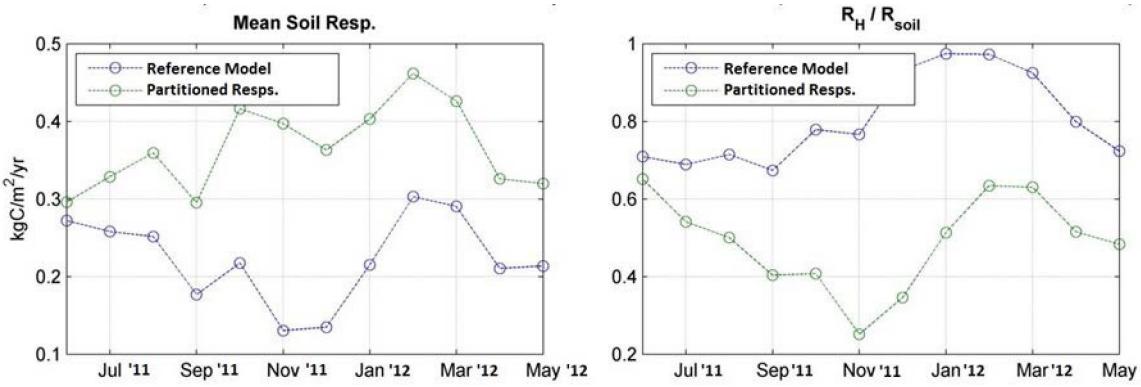

Figure 12: Representative values of soil respiration (panel a, left) and the fraction of soil respiration (panel b, right) attributable to heterotrophs, under the previous model formulation (blue lines) and the updated version with partitioned growth and maintenance respiration terms (green lines). The predictions of the fraction of soil respiration attributable to heterotrophs from the updated model plotted in panel b correspond are considerably closer to field measurements of this ratio (e.g. Hanson et al. 2000, Tang et al. 2015).

Optimization Framework

While working to develop novel model parameter estimates we overhauled our optimization software. The new software remedies drawbacks of the old by being modular, extensible, and maintainable, and it provides entirely new features such as support for a range of optimization algorithms and parallelization. It has already been used with the model for other grant-supported work in addition to that discussed here, and is publicly available via GitHub.

Optimization Results

We used the model optimization framework to develop improved model predictions and establish new parameter estimates using a combination of conventional measurements of carbon fluxes and stocks, and using the measured ^{13}C isotopic composition of Net Ecosystem Exchange (NEE).

Model optimization using conventional measurements of carbon stocks and fluxes: we first conducted optimizations using measurements of NEE (on hourly, daily, monthly and yearly timescales) in conjunction with measurements of soil respiration and above-ground woody carbon growth and mortality, to develop new and improved parameter estimates for the ED2 model. These estimates also allowed us to quantify changes in parameter uncertainty and the predictive accuracy of the model attributable exclusively to the subsequent incorporation of the ^{13}C isotopic data.

The results of the optimization are summarized in Figure 13. Key changes to the model predictions are an increase both photosynthesis and plant carbon-loss rates, resulting in improved night time and soil respiration rates as well as slightly reduced storage reserves.

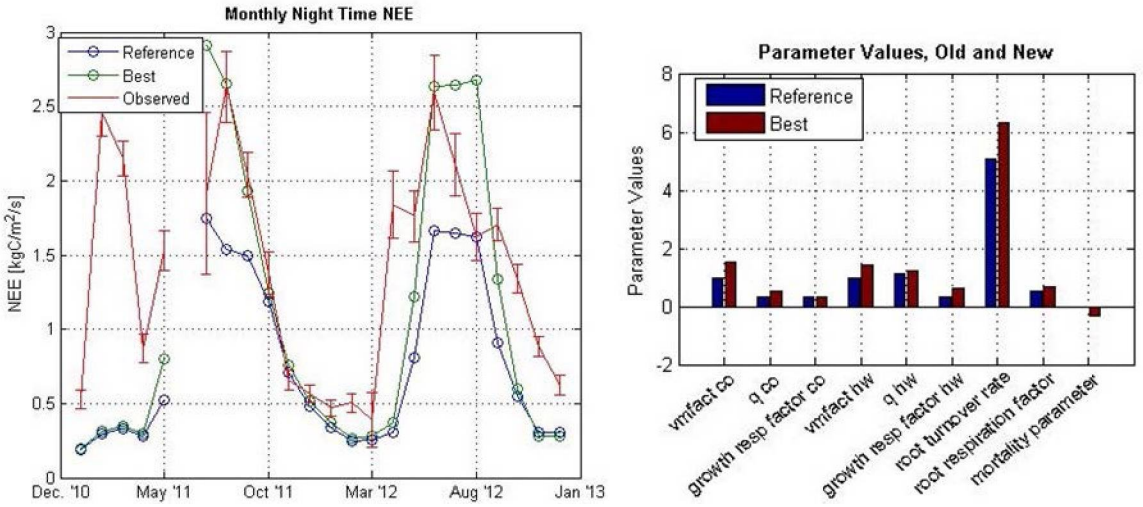

Figure 13 Panel a: Improved predictions of night-time Net Ecosystem Exchange (NEE) following model optimization. Panel b: Parameter values before and after performing baseline model fitting. Abbreviations are as follows: vmfact is a multiplicative factor of total photosynthetic rate, q is the allometric ratio of leaves to roots, growth resp factor and root respiration factors are multiplicative factors in total root and growth-respiration, and root turnover rate is fine-root biomass per year. "co" and "hw" are abbreviations for conifer and broad-leaf hardwood plant functional types respectively. The values of the parameters are less important than their changes, virtually all of which reflect increased assimilation as well as increased respiration and turnover loss.

Model optimization using ^{13}C *Isotope Measurements*: As noted above, we have finalized the structure of ED2's new internal isotopic accounting system and have finished building it into the model. This system takes static or dynamically prescribed atmospheric CO_2 isotope ratios and propagates them into the modeled canopy airspace using ED2's existing biophysics subroutines, computes the ratio of heavy to total assimilated carbon using the Farquhar et al. (1982) photosynthetic isotope discrimination model, then determines carbon-13 content of plant tissues, losses to respiration and losses to soil such that each ecosystem pool or flux has a content strictly reflecting its inputs and outputs. The results of this system, as illustrated below, fall within the range of values for ecosystem pools and fluxes found within the literature.

Preliminary optimizations of model parameters using the newly measured isotopic composition of NEE have yielded increases in estimates of photosynthetic and turnover rates similar to those observed in our baseline optimizations (Figure 14). This is notable in part because, whereas the parameter changes observed in the baseline optimizations primarily improve the fit of night time NEE, soil efflux, and basal area growth and mortality rates, our initial isotopic optimizations were designed to examine the interplay between photosynthetic rate and respiration associated with growth and maintenance. Fitting these three parameters in hardwoods to either total NEE exclusively or to total NEE and its isotopic composition together has allowed us to start quantifying the effects of using the isotopic data in a simpler to understand system than the approximately 10-parameter and 20 data-set system.

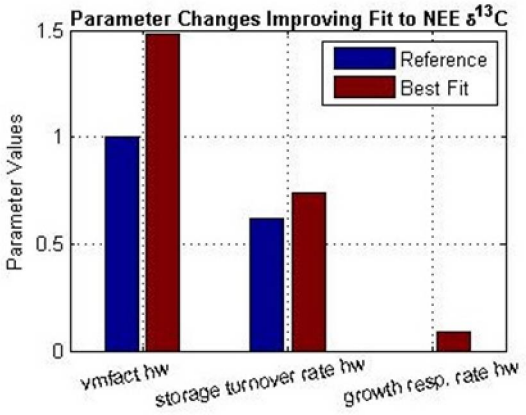

Figure 14: Changes in model parameters responsible for increased NEE isotopic content fit. As in Figure 13b, Vmfact is photosynthetic rate and 'hw' indicates parameters apply to broadleaf-deciduous plant functional types.

The parameter changes we've observed as a result of the fitting to ^{13}C NEE measurement exert countervailing influences on total NEE, resulting in no net change in total flux magnitude or seasonality (which is sensible because the model fits new flux data well), but they improve the prediction of the ^{13}C isotopic flux composition and have begun to highlight the types of dependencies between above and below-ground systems that it is possible to explore using the isotopic data in conjunction with the new model formulation. The results of this fitting on the ^{13}C isotopic composition of NEE and of soil respiration are shown below in Figures 15 and 16 respectively.

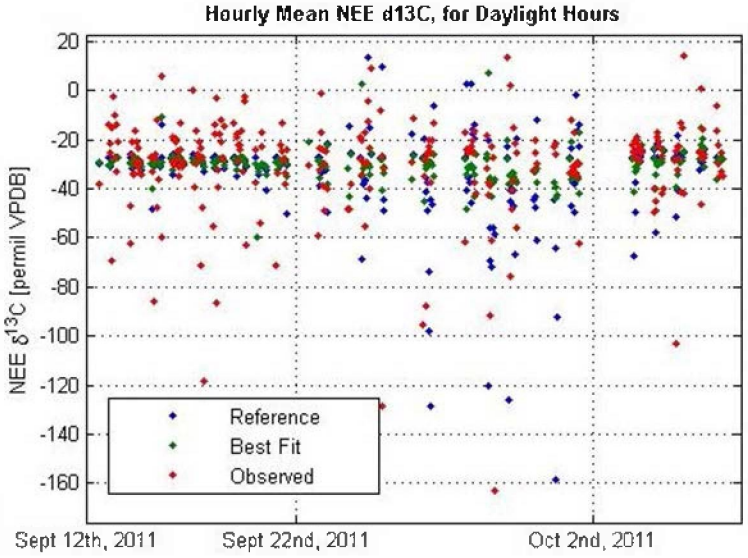

Figure 15: Predicted NEE composition shows less variability than observations but traces the average values. Although difficult to see, the hourly best-fit predictions have been shifted upward relative to the reference.

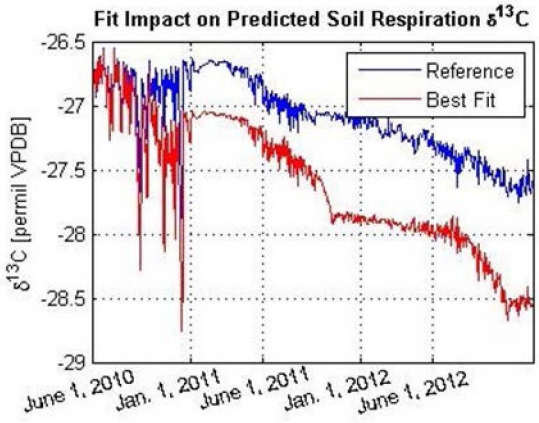

Figure 16: Illustration of the impact of increased photosynthetic and respiratory rates on soil respiration isotopic composition. These curves likely reflect differing equilibria in soil and plant isotope compositions given the different model parameterizations.

Ongoing Modeling Work

We are currently completing a final set of optimizations to assess the impact of the new isotopic data on model's predictions and parameter values. These optimizations expand the set of model parameters being optimized from three to ten, and will assess the uncertainty and general validity in these new parameters, and their impact on simulations of short-term and long term forest carbon dynamics. The findings of our analysis will then be written up for publication.

4. Publications and Presentations Supported by this Grant

4.1. Published Articles

Abramoff, RZ, Finzi AC. 2015. Are above-and below-ground phenology in sync? *New Phytologist,* 205:1054-1061.

Drake JE, Darby BA, Giasson MA, Kramer MA, Phillips RP, Finzi AC. 2013. Stoichiometry constrains microbial responses to root exudation: insights from a model and experiment in a temperate forest. *Biogeosciences* 10:821-838.

Finzi AF, Abramoff RZ, Darby BA, Spiller KS, Brzostek ER, Phillips RP. 2014. Rhizosphere processes are quantitatively important components of terrestrial carbon and nutrient cycles. *Global Change Biology.* 21:2082-2094.

Wehr R and S. R. Saleska (2015). An improved isotopic method for partitioning net ecosystem-atmosphere CO_2 exchange. *Agricultural and Forest Meteorology* 214-215, 515–531.

Wehr R, J. W. Munger, D. D. Nelson, J. B. McManus, M. S. Zahniser, S. C. Wofsy, and S. R. Saleska (2013). Long-term eddy covariance measurements of the isotopic composition of the ecosystem-atmosphere exchange of CO_2 in a temperate forest. *Agricultural and Forest Meteorology* 181, 69-84.

4.2. Articles in Press

Abramoff RZ, Finzi AF. Seasonality and partitioning of root allocation to rhizosphere soils in a mid-latitude forest. *Ecosphere.* (*in review*)

4.3. Articles in Preparation

Abramoff RZ, Finzi AF. Where does the rhizosphere end? Spatially resolved measurements of in situ soil extracellular enzyme activity (*in prep*)

Abramoff RZ, Davidson EA, Finzi AF. A parsimonious modular approach to building a mechanistic belowground carbon and nitrogen model (*in prep*)

Savage K, E Davidson, R Abramoff, A Finzi, M-A Giasson (2015). Partitioning Soil Respiration: Quantifying the Artifacts of the Trenching Method.

Wehr R, J. W. Munger, J. B. McManus, D. D. Nelson, M. S. Zahniser, E. A. Davidson, S. C. Wofsy, and S. R. Saleska (2015). The seasonality of temperate forest photosynthesis and respiration. In review at *Nature*.

4.4. Presentations

Abramoff RZ, Toomey M, Klosterman S, Finzi AC. 2012. Root phenology in a mid-latitude forest. [poster] *American Geophysical Union Fall Meeting*, San Francisco, CA.

Abramoff RZ, Finzi AC. 2012. Root phenology in a mid-latitude forest. [talk] *Boston University Ecology, Behavior, and Evolution Departmental Chalk Talk*, Boston, MA.

Abramoff, RZ, Knapp SP, Klosterman S, Finzi AC. 2012. Phenology of belowground carbon allocation in a mid-latitude forest. [poster] *Long Term Ecological Research All Scientists' Meeting*, Estes Park, CO.

Abramoff, RZ, Knapp SP, Klosterman S, Finzi AC. 2012. Phenology of belowground carbon allocation in a mid-latitude forest. [poster] *Ecological Society of America Annual Meeting*, Portland, OR.

Abramoff RZ and Finzi AC. 2015. The secret lives of roots: Seasonality and partitioning of belowground C to three Harvard Forest stands. [talk] *Harvard Forest Ecology Symposium*, Petersham, MA.

Abramoff RZ, Davidson EA, Finzi AC. 2015. A parsimonious modular approach to building a mechanistic belowground C and N model. [talk] *American Geophysical Union Fall Meeting*, San Francisco, CA.

Abramoff, RZ and Finzi AC. 2014. What is the relationship between aboveground and belowground phenology? A meta-analysis and case study. [talk] *Ecological Society of America Annual Meeting*, Sacramento, CA.

Abramoff RZ and Finzi AC. 2014. Linking above and belowground phenology at Harvard Forest and beyond. [talk] *Northeast Natural History Conference*, Springfield, MA.

Abramoff RZ, Finzi AC. 2013. Root phenology at Harvard Forest and Beyond. [presentation] *American Geophysical Union Fall Meeting*, San Francisco, CA.

Abramoff RZ and Finzi AC. 2013. Root phenology of red oak and eastern hemlock-dominated stands at Harvard Forest. [poster] *Harvard Forest Ecology Symposium*, Petersham, MA.

Knapp SP, Abramoff RZ, Finzi AC. Seasonality of Fine Root Growth, Mortality, and Carbon Allocation in Temperate Forest Trees. [talk] *Harvard Forest REU Symposium*, Petersham, MA.

Savage K, E Davidson, R Abramoff, A Finzi, M-A Giasson. Partitioning Soil Respiration: Quantifying the Artifacts of the Trenching Method. *2014 American Geophysical Union Fall Meeting*. San Francisco, U.S.A.

Savage K, E. Davidson, D. Hollinger and S. Saleska. 2014. Autotrophic and heterotrophic contributions to soil respiration: A tale of two trenches. TES/SBR Joint Investigator Meeting, Potomac MD.

Scott D, Moorcroft PR. Isotopes and Forest Dynamics in an Optimized Model (ED2) at Howland and Harvard Forests. Poster presented at: Harvard Forest Symposium; 2015 March; Petersham MA.

Scott D, Moorcroft PR. Carbon-13 in the Ecosystem Demography Model (ED2). Poster session presented at: Harvard Forest Symposium; 2014 March; Petersham MA.

Scott D. Soil Dynamics in the ED Model. Presented at ED+ Meeting: Harvard University; 2013 January; Cambridge MA.

Wehr R, J. W. Munger, S. C. Wofsy, J. B. McManus, D. D. Nelson, M. S. Zahniser, and S. R. Saleska (2014). Seasonal and Inter-Annual Patterns in Ecosystem-Scale Photosynthesis and Respiration in a Temperate Forest Revealed by Isotopic Partitioning of NEE. *2014 American Geophysical Union Fall Meeting*. San Francisco, U.S.A.

Wehr R, S. R Saleska, J. W. Munger, M. S Zahniser, J. B. McManus, and D. D Nelson (2014). New Insights on Canopy Photosynthesis from novel Isotopic Flux Partitioning in a temperate forest. *EGU General Assembly 2014.* Vienna, Austria.

Wehr R, J. W. Munger, J. B. McManus, D. D Nelson, M. S Zahniser, and S. R Saleska (2013). Diel and Seasonal Behavior of Canopy Photosynthesis Revealed by Novel Isotopic Flux Partitioning in a Temperate Forest. *2013 American Geophysical Union Fall Meeting.* San Francisco, U.S.A.

Wehr R, Abramoff R, Davidson EA, Finzi, AC, Giasson M-A, McManus JB, Moorcroft PR, Munger JW, Nelson DD, Saleska SR, Savage K, Scott D, Wofsy SC, Zahniser M. 2013. Partitioning CO_2 fluxes with isotopologue measurements and modeling to understand mechanisms of forest carbon sequestration. [poster] *2013 Harvard Forest Ecology Symposium*, Petersham, MA.

Wehr R, Abramoff R, Davidson EA, Finzi, AC, Giasson M-A, McManus JB, Moorcroft PR, Munger JW, Nelson DD, Saleska SR, Savage K, Scott D, Wofsy SC, Zahniser M. 2013. Partitioning CO_2 fluxes with isotopologue measurements and modeling to understand mechanisms of forest carbon sequestration. [poster] *2013 Harvard Forest Ecology Symposium*, Petersham, MA.

5. Awards

Boston University Biogeosciences Fellowship Research Award (2013)
Boston University Teaching Fellowship (2010, 2012, 2013)
American Geophysical Union Outstanding Student Paper Award (2013)
Department of Energy Student Travel Fellowship (2014)
George R. Bernard, Jr. Travel Award (2012, 2013, 2014)
American Association of University Women Dissertation Fellowship (2014)
American Geophysical Union Student Travel Grant Award (2012, 2014)
Boston University Biogeoscience Symposium Outstanding Oral Presentation Award (2015)

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