Anonymous Referee #1

For final publication, the manuscript should be:
  accepted as is

Suggestions and responses:

The manuscript has been nicely improved after the revision and most of the suggestions I did have been taken into consideration.
Reading in your answer that you are considering the possibility of improving the model by including the size of the tree in a future study reinforces my belief that it could have been done in this one as well, and that it would have been great and would have given more value to your study.
I do not understand in your answer why it makes sense to write that a radius of 5 m is a hypothesis dictated by biology. But fortunately, this does not appear in the revised manuscript and the new figure 5 is more convincing than the previous one

  Thank you for your comments and we appreciate the feedback.

Anonymous Referee #3

For final publication, the manuscript should be:
  accepted subject to technical corrections

Suggestions and responses:

Pennington et al. have provided a nice revision of their original manuscript, which successfully addresses my concerns except for these relatively minor issues:

- I was sorry to see that some version of the figure from the authors' "OVERALL RESPONSE TO R3" did not make it into the revised draft. I found it helpful for understanding how BA5 might impact Rs via T without having a statistically significant effect on Rs directly. However, if the authors and editor do not feel the need for such a figure, then I do not object.

  We believe that while the figure we provided in the response provided a valuable explanation for the Rs:T:BA significance result, it did not add enough information to the whole "story" of localized basal area effect but think it will be a good addition to the supplemental information section.

- lines 57 ff.: The phrasing of this paragraph (particularly the first and last sentences) portrays the influence of vegetation distribution on Rs as a hypothesis to be tested, whereas the citations show that root respiration has already been shown to contribute substantially to Rs, and Rs has already been shown to be higher near tree stems — i.e. the spatial distribution of vegetation has already been shown to affect the spatial distribution of Rs. Additionally, the logic of the first sentence is flawed: the fact that "[a]t large scales, Rs differs between vegetation types and biomes" does not even imply that vegetation type affects Rs (it could be that soil type or other factors affect both

vegetation type and Rs), let alone "that the spatial distribution of vegetation might strongly affect Rs via plant root respiration" (which involves logical leaps to spatial distribution and root respiration). This is the paragraph that needs to clearly lay out what's already known about the influence of vegetation on Rs and state the knowledge gap that this study is going to fill, which is not really about the spatial distribution of Rs, but rather about how vegetation affects the response of Rs to environmental drivers (especially temperature).

This is a good point, and we have reworked this paragraph for logical consistency, and to emphasize that while it's clearly established that Rs varies spatially due to vegetation, much less is understood about the implications of this for the overall Rs sensitivity to environmental conditions.

- lines 111-114: Soil moisture and temperature measurements don't belong in a section called "Soil respiration measurements".

We have changed the title of this subsection to "Soil respiration and ancillary measurements".

- line 116: Strictly speaking, these are not "tree proximity measurements", but rather local basal area measurements.

We have changed the title of this subsection to "Local basal area measurements" to match the wording in the title and study description.

- line 132: I acknowledge the author's response regarding heteroscedasticity and the logarithm, but I think "ensure homoscedasticity" is too strong language here. I would say that taking the logarithm dampens heteroscedasticity.

We have changed the wording here.

- line 134: I think this "equation" requires a bit of justification/derivation. E.g. why SM and SM^2? Does this notation mean that log(Rs) is taken to be linearly related to T5*BA5 and to T20*BA5 but quadratically related to SM? Why?

We have clarified this point in the methods. Basically, over the course of a year, we expect that Rs might be limited by both too little SM and too much SM–i.e., it would respond both positively and negatively to SM changes depending on the degree of soil anoxia. For this reason a quadratic SM term is included in the model. In this forested temperate ecosystem we don't expect to ever see a decline of Rs with temperature increase, however.

- line 297 ff.: The conclusion is awkward. Mention of Rs is left out of the first sentence, about the key finding. In the second sentence, "these sites" is ambiguous as to whether you're referring to all your study sites or just the sites with high BA5. In the third sentence, the fact that "soil respiration at this site [is] highly dynamic and variable" is a direct observation, not something suggested by the findings.

We have revised the wording in this paragraph to better highlight Rs findings and to clarify that Rs variability (a direct observation) may contribute to the localized basal area influence we found and high sample requirements at our study sites (suggested by findings).

- Fig. 3: The inset adds no information and should be removed. The color scale could be put in its place instead, reduce the overall space required by the figure.

Thank you for the feedback, we've replaced the inset graph with the color scale.

-------- END OF RESPONSES -------

Localized basal area affects soil respiration temperature sensitivity in a coastal deciduous forest

Biogeosciences

Stephanie C. Pennington*[1], Nate G. McDowell[2], J. Patrick Megonigal[3], James C. Stegen[2], and Ben Bond-Lamberty[1]

*Corresponding author, stephanie.pennington@pnnl.gov

1. Joint Global Change Research Institute, Pacific Northwest National Laboratory, 5825 University Research Ct. #3500, College Park, MD 20740 USA

2. Pacific Northwest National Laboratory, Biological Sciences Division, Richland, WA, USA

3. Smithsonian Environmental Research Center, Edgewater, MD, USA

Abstract

Soil respiration (R_s), the flow of CO_2 from the soil surface to the atmosphere, is one of the largest carbon fluxes in the terrestrial biosphere. The spatial variability of R_s is both large and poorly understood, limiting our ability to robustly scale it in space. One factor in R_s spatial variability is the autotrophic contribution from plant roots, but it is uncertain how the presence of plants affects the magnitude and temperature sensitivity of R_S. This study used one year of R_s measurements to examine the effect of localized basal area on R_S in the growing and dormant

3

seasons, as well as during moisture-limited times, in a temperate, coastal, deciduous forest in eastern Maryland, USA. In a linear mixed-effects model, tree basal area within a 5 m radius (BA_5) exerted a significant positive effect on the temperature sensitivity of soil respiration. Soil moisture was the dominant control on R_S during the dry portions of the year while soil moisture, temperature, and BA_5 all exerted significant effects on R_S in wetter periods. Our results suggest that autotrophic respiration is more sensitive to temperature than heterotrophic respiration at these sites, although we did not measure these source fluxes directly, and that soil respiration is highly moisture-sensitive, even in a record-rainfall year. The R_S flux magnitudes (0.35-15.3 μmol m^{-2} s^{-1}) and variability (coefficient of variability 10%-23% across plots) observed in this study were comparable to values observed in similar forests. Six R_S observations would be required in order to estimate the mean across all study sites to within 50%, and 516 would be required in order to estimate it to within 5%, with 90% confidence. A better understanding of the spatial interactions between plants and microbes, as well as the strength and speed of above- and belowground coupling, is necessary to link these processes with large scale soil-to-atmosphere C fluxes.

Introduction

Soil respiration (R_s), the flow of CO_2 from the soil to the atmosphere, is among the largest C fluxes in the terrestrial biosphere (Granier et al., 2000, Bond-Lamberty, 2018; Le Quéré et al., 2018), but remains poorly constrained both temporally and spatially at all scales. Unlike other large C fluxes such as net primary production, net ecosystem exchange, and gross primary production, Rs cannot be measured, even indirectly, at scales larger than a few square meters (Bond-Lamberty et al., 2016). Though global-scale Rs varies between vegetation types and

biomes (Raich et al., 2002; Raich and Schlesinger, 1992), and responds to disturbances such as land use and climate changes (Hursh et al., 2017; Schlesinger and Andrews, 2000), it is uncertain how these patterns arise from local-scale variability, limiting our ability to robustly scale the process.

~~is an important carbon (C) flux at ecosystem (Granier et al., 2000) to global scales. R~~s~~ is among the largest C fluxes in the terrestrial biosphere (Bond-Lamberty, 2018; Le Quéré et al., 2018),~~ and is known to vary ~~but poorly constrained at large scales, although at large scales Rs clearly differs At the global scale Rs is known to vary between vegetation types and biomes (Raich et al., 2002; Raich and Schlesinger, 1992), and. It is~~and thus it is important to understand its variability and sensitivity to vary with ~~disturbances~~processes ~~such as land use and climate changes (Hursh et al., 2017; Schlesinger and Andrews, 2000). Unlike other large C fluxes such as net primary production, net ecosystem exchange, and gross primary production, R~~s~~ cannot be measured, even indirectly, at scales larger than a few square meters (Bond-Lamberty et al., 2016), limiting our ability to robustly scale the process~~it in space~~.~~

One obstacle to robust measurements of R$_s$ is that the spatial and temporal variability ~~of R$_s$~~is both large and poorly understood. This high variability has consequences for the sampling strategy required to accurately measure R$_s$ at the stand scale (Rodeghiero and Cescatti, 2008; Saiz et al., 2006) and limits our ability to upscale R$_s$ measurements to eddy covariance tower scales (Barba et al., 2018). Controls on the spatiotemporal~~spatial~~ variability of R$_s$ differ among sites and ecosystems and include plant species, ecosystem productivity (Reichstein et al., 2003), soil temperature (Fang et al. 1998), moisture, spatial variability of vegetation, management, and soil compaction (Epron et al., 2004). ~~This high variability has consequences for the sampling strategy required to accurately measure R~~s~~ at the stand scale (Rodeghiero and Cescatti, 2008;~~

~~Saiz et al., 2006) and limits our ability to upscale R_s measurements to eddy covariance tower~~ ~~scales (Barba et al., 2018).~~ The collective responses of plants and microbes to these factors determine the sensitivity of ecosystems to changes in temperature, precipitation, and other global change factors.

~~At large scales, R_s differs between vegetation types and biomes (Raich et al., 2002; Raich and Schlesinger, 1992), implying that the spatial distribution of vegetation might strongly affect R_s via p~~Plant root respiration, ~~which~~ constitutes ~50% of R_s in many ecosystems (Subke et al., 2006). At ecosystem scales, a number of studies have examined how the spatial distribution of R_s is affected by vegetation. R_s is typically higher closer to tree stems (Epron et al., 2004; Tang and Baldocchi, 2005), and with higher nearby stem density (Schwendenmann and Macinnis-Ng, 2016; Stegen et al., 2017). Photosynthesis is also a driver of the rhizospheric component of soil respiration (Hopkins et al., 2013), and influences seasonal trends in root contribution to total soil respiration (Brændholt et al., 2018; Högberg et al., 2001). Any spatial influences of plants on the magnitude and environmental sensitivities of R_s might thus be expected to be strong in temperate, deciduous forests, as such forests tend to be highly productive (Gillman et al., 2015; Luyssaert et al., 2007).

This study examines the effect of tree proximity on measured R_s in a mid-Atlantic, deciduous forest in the Chesapeake Bay, USA region. We hypothesized that:

(i) the amount of basal area close to R_s measurement locations would exert a significant and positive effect on measured R_s after taking into account the effects of abiotic drivers;

(ii) this effect would occur in the growing (leaf on) season, but not in the dormant (leaf off) season, because root respiration is much higher during the growing season; and

(iii) this effect would be stronger during drier times of year, because trees might maintain access to deep soil moisture (Burgess et al., 1998) and thus continue respiring even when the surface soil is dry.

To test these hypotheses we performed a spatially explicit analysis of one year of frequent R_s measurements in a temperate coastal deciduous forest in eastern Maryland, USA. Our study was conducted in the Chesapeake Bay watershed, an area subject to rapid rates of sea level rise (Ezer and Corlett, 2012; Sallenger et al., 2012) that may exert significant effects on the carbon cycling of coastal ecosystems (Rogers et al., 2019).

Methods

Site characteristics

This study was conducted in a mid-Atlantic, temperate, deciduous forest at the Smithsonian Environmental Research Center (SERC) in Edgewater, MD, USA. Three sites were chosen along Muddy Creek, a stream draining into an arm of Chesapeake Bay. Each site was separated by ~1 km (**Figure 1a**). These sites were comprised of both lowland and upland forest with a mean annual precipitation of 1001 mm and mean annual temperature of 12.9°C (Pitz and Megonigal, 2017). Dominant tree species include *Liriodendron tulipifera*, *Fagus grandifolia*, and *Quercus spp.*; soil types vary between Collington, Wist, and Annapolis soil. (**Table 1**). At each

site, three 20 m x 40 m plots were installed, separated by ~25 m and oriented perpendicular to the creek. The total elevation change between plots at each site was ~2 m. Within each plot, we installed 4, 20-cm diameter PVC collars, randomly separated from each other by 2–15 m, for a total of 36 measurement collars. Collars were installed ~1 week prior to the first sampling and left in place for the duration of the study.

Soil respiration and ancillary measurements

Soil respiration measurements were taken using an infrared gas analyzer (LI-8100A, LI-COR Inc., Lincoln, NE) with a 20 cm diameter soil chamber attached. Measurements were taken every 10-14 days from April 2018 to April 2019. The IRGA measures concentrations every second over a one minute period and calculates the CO_2 flux as the exponential regression of CO_2 accumulation in the closed chamber system over unit area and time; two successive measurements were taken at each collar and averaged. Vegetation was removed from inside the collar, and new vegetation was re-clipped as necessary, to remove any aboveground autotrophic flux, so that the IRGA was measuring only soil-to-atmosphere CO_2. Soil moisture and temperature (T_5) were also recorded at 5 cm depth, using auxiliary sensors attached to the LI-8100A, at the same time as soil respiration measurements. Temperature at 20 cm depth (T_{20}) was also recorded using a hand-held thermometer at the time of measurement.

Local basal area~~Tree proximity~~ measurements

We recorded distance from the soil collar, diameter at breast height (1.37 m), and species of each tree within a 15 meter radius of each soil respiration measurement point (**Figure 1b**). Dead trees were included in the dataset but only account for < 1% of total forest basal area.

Cumulative basal area was calculated at each 1 m radial distance from the collar, summing the cross-sectional areas of all trees within each distance. Tree root extent can be highly variable, but generally roots extend at least to the edge of the tree canopy (Stone and Kalisz, 1991). Mature tree canopies at SERC are ~5 m in radius (S. Pennington, personal observation), and we adopted this distance as an *a priori* assumption to test for the effect of basal area at 5 meters (BA_5) on R_s.

Statistical analysis

Respiration data were checked visually for artifacts or unusual outliers, but we did not exclude any data *a priori*. Data were then combined with the proximity measurements described above based on collar number. We used a linear mixed-effects model to test for the influence of BA_5 on R_s, treating temperature, soil moisture (SM), and BA_5 as fixed effects, and site as a random effect (Equation 1). R_s frequently follows a nonlinear response in relation to SM, so a quadratic SM term (Sierra et al., 2015) was included in the model. To ensure homoscedasticity of model residuals, the dependent variable R_s was transformed by taking its natural logarithm to minimize heteroscedasticity, and thus the full linear model was specified as:

$$\log(R_s) \sim T_5 * BA_5 + T_{20} * BA_5 + SM + SM^2 \qquad \text{(Equation 1)}$$

We used restricted maximum likelihood estimation using the *lme4* package (Bates et al., 2015) in R version 3.5.3 (R Development Core Team, 2019). All models were examined for influential outliers and deviations from normality. Non-significant terms were then eliminated using a forward-and-back stepwise algorithm (using the R package *MASS* version 7.3-47) based on the Akaike Information Criterion. Residuals from all fitted models were plotted and checked for trends or heteroscedasticity.

Our secondary hypotheses, that effect of BA_5 varies with growing season and soil moisture, were tested by subsetting the R_s data. We treated April 15-October 14 as the growing season, based on 2018 leaf-out and senescence, and October 15-April 14 as the dormant season. Soil moisture data were split up into equal thirds (low, <0.188 m^3 m^{-3}; medium, 0.188-0.368 m^3 m^{-3}; and high, >0.368 m^3 m^{-3}; all values volumetric). We then applied the statistical model described above to each subset of the data to test for BA_5 significance in the model. The 'relaimpo' package version 2.2-3 was used to calculate relative importance metrics for all terms in each model, in particular its 'lmg' metric that averages sequential sums of squares over all orders of regressors (Lindeman et al., 1980), providing a robust decomposition of model R^2.

We used the spatial variability between collars within individual plots to estimate the number of samples required for a robust estimate of the R_s 'population mean', i.e., a spatially-representative mean. Specifically, we used a Student's t-test to calculate this based on the standard deviation of hourly R_s, the desired power of the test, and the allowable delta (difference from the true mean value), following Davidson et al. (2002).

Results

We measured R_s, soil temperature, and soil moisture on 31 different days across the one-year period (Figure 2). Soil temperatures ranged from 0.1 to 27.5 °C (at 5 cm) and 1.7 to 24.4 °C (at 20 cm); volumetric soil moisture values were 0.01-0.56. R_s fluxes ranged from 0.35 µmol m^{-2} s^{-1} (in January 2019) to 15.3 µmol m^{-2} s^{-1} (in July 2018). The coefficient of variability (CV) between collars within plots, a measure of spatial variability, was 16.7% ± 4.0. This implied that a large number of samples was required to estimate soil respiration accurately (Table 2).

There was large variability in the basal area and number of trees close to the measurement collars (Figure 3). The mean number of trees within 1 m, 5 m, and 10 m distance were one, six, and 21 trees (with respective nearby basal areas of 0.0002 m^2, 0.24 m^2, and 0.88 m^2). Within our maximum radius of measurement, 15 m, there were on average 43 trees and 1.64 m^2 of cumulative basal area, ranging from a minimum of 0.43 m^2 to a maximum of 3.55 m^2. The forest was thus highly spatially variable in its distribution of trees relative to the R_s measurement collars.

Effect of BA on R_s

The linear mixed-effects model using temperature, soil moisture, and basal area within five meters (BA_5) predicted 37% of the R_s variability (conditional $R^2 = 0.37$). BA_5 was not significant by itself in a Type III ANOVA using this model ($\chi^2 = 0.081$, $P = 0.776$), but exhibited strong and significant interactions with T_5 and T_{20} (**Table 3**). In addition, the residuals of a model fit without BA_5 had a significant trend with BA_5 (**Figure 4**). Separating the data into growing- and dormant-season subsets provided contrasting results. In the growing season, model outputs were similar to those of the full year model, with BA_5 having significant interactions with T_5 and T_{20} (data not shown). The dormant season model, however, was quite different: only T_{20} ($P \leq 0.000$) and soil moisture ($P = 0.0377$) were significant terms. In addition, the dormant season model explained more of the R_s variability (AIC = 119.80, marginal $R^2 = 0.48$). In summary, collars with higher basal area within 5 m had significantly higher temperature sensitivity of soil respiration, while basal area within 5 m of sampling points was not correlated with R_s during the dormant season.

There were strong differences between the driest and wettest thirds of the data, but our hypothesis that any basal area affect would be strongest in the driest time of year was not supported. In the driest third of the data, neither BA_5 nor its interaction with T_5 was significant ($P = 0.096$ and 0.054 respectively); T_{20} was never significant; and the dominant control was instead soil moisture ($\chi^2 = 15.23$, $P < 0.001$). In contrast, the wettest-third model resembled the full-year model, with BA_5 interacting with temperature, and soil moisture also significant.

Sensitivity test

Our *a priori* choice of 5 m for the basal area test was one of many possible choices, and could potentially bias the results, as the actual extent of tree roots at these sites is unknown. Re-fitting the main statistical model and calculating variable importance metrics across a wide range of distances, however, showed that basal area and its interactions with T_5 and T_{20} were almost always statistically significant (**Figure 5**). Generally the BA effects were not significant at short (< 3 m) distances; this is expected, given that few collars were that close to trees. Interestingly, the BA effects remained significant all the way to our maximum measured distance of 15 m. In summary, our *a priori* analytical choice of a 5 m radius did not appear to bias our results.

Discussion

The R_s fluxes observed in this study, 0.35-15.3 μmol m^{-2} s^{-1}, were comparable to values in similar forests (Giasson et al., 2013) as well as those from the Soil Respiration Database (SRDB; Bond-Lamberty and Thomson, 2010), a synthesis of annual R_s studies (0 to 14.7 μmol m^{-2} s^{-1}, n = 1281 temperate deciduous studies). We observed a R_s CV of 10-22% between plots, a

value also comparable to previous studies. In a study of R_s in conifer forests and grasslands, Rodeghiero (2008) reported 28.9-41.5% variability, Davidson et al. (2000) about 30% in forest ecosystems, and a much broader range (0.11-84.5%) for temperature, deciduous forests from the SRDB.

Sample size requirements to estimate annual R_s were high at SERC compared to previous studies. For example, to be within 10% of the mean R_s flux at 95% confidence required from 41 (Davidson et al., 2002) in Harvard Forest, to 72 (Adachi et al., 2005) in a secondary forest, to 129 sample points in this study. Within forest biomes, topography and stand structure (Søe and Buchmann, 2005) can be dominant controls. Significant spatial variation in stand structure and topography across the study domain may have resulted in high variability seen in this study. In particular, the measurement points at our study sites ranged from 3-15 meters in elevation (Table 1), as at all sites the land rises quickly away from Muddy Creek. These elevation gradients mean that some measurement points drain more quickly than others, creating strong differences in soil water content (CV 16.7% ± 4.0 within plots) and thus Rs. This is consistent with the idea that topographic complexity can be an important and complex factor in R_s variation across sites (Riveros-Iregui et al. 2012) .

Interactions between basal area and temperature sensitivity on R_s

Many studies have examined whether autotrophic respiration (R_a) or heterotrophic respiration (R_h) is more temperature-sensitive, and reached varying conclusions (Aguilos et al., 2011; Boone et al., 1998; Wei et al., 2010). In this study, the Type III SS interaction between BA5 and temperature was highly significant, meaning that collars with higher basal area within 5 m exhibited significantly higher temperature sensitivity of soil respiration. This suggests that R_a

might be more sensitive to temperature than R_h at these sites. However, it is important to note that we did not directly measure the autotrophic and heterotrophic source fluxes contributing to the overall R_s flux. Instead, we assume that collars closer to trees have a larger fraction of R_s contributed by Ra, an assumption also made in previous studies such as Tang and Baldocchi (2005).

Mechanistically, these findings could be explained by a number of processes. When substrate supply from root exudates is higher during the growing season, R_s tends to be more sensitive to temperature (Luo and Zhou, 2006), presumably because under these conditions R_s is tightly coupled with photosynthesis (Ekblad and Högberg, 2001), as roots access photosynthate before microbes, and thus can respond more strongly to temperature changes. Leaf phenology likely also plays a role in a deciduous forest such as the one studied here, where the growth of photosynthetically active foliage in the spring can promote carbon allocation belowground and hence R_a. Input of leaf material in the fall may also stimulate R_h (Curiel Yuste et al., 2004; Epron et al., 2001; Ruehr et al., 2010) and is dependent on tree size and distribution (Bréchet et al., 2011).

There is also abundant evidence that soil moisture influences temperature sensitivity: Suseela et al. (2012), for example, found that R_s is less sensitive to temperature during water-limited times. If trees' roots have access to water consistently, their respiratory flux R_a measured at the soil surface as part of R_s will be more temperature-sensitive on average, because R_a will be limited by soil moisture less frequently (Misson et al., 2006). It is important to note that these various mechanisms are not mutually exclusive.

Soil moisture controls on BA significance

We hypothesized that any BA_5 effect would be particularly strong during the driest third of the year, but instead found that only soil moisture controlled R_s during these periods, while neither temperature nor tree proximity (BA_5) was significant. This demonstrates that R_s is highly moisture-sensitive at these sites, but does not support our idea that trees might have access to deeper or different water sources than surface soil microbes. Soil moisture is considered to be a primary R_s control in Mediterranean and desert ecosystems (Cable et al., 2010), but interestingly even this deciduous forest, in a year with record rainfall (National Weather Service, 2019), experienced significant moisture restrictions on R_s.

Dormant season R_s controls

Tree basal area within 5 m of our R_s sampling points was not significant in the dormant season model, supporting our hypothesis that total R_a contribution is often lower during the dormant reason than the growing season (Hanson et al., 2000), which suggests that R_a contributes less to R_s during the dormant season. This is expected, given the physiological link between photosynthesis and root respiration (Sprugel et al., 1995). Interestingly, T_5 was not significant in the dormant season model, but rather T_{20} was the dominant control. The study site is in a mid-Atlantic, temperate location with cold air temperatures during the winter. Deeper soils are more insulated from cold air temperatures, allowing more favorable conditions for respiration and thus making T_{20} a dominant control on R_s during these times.

Limitations of this study

A number of limitations should be noted in our study design and execution. First, this was not a fully spatially-explicit analysis; we did not map the collars relative to each other, nor

construct a full spatial map of the forest stands (Atkins et al., 2018). Such mapping can be useful to examine the R_s spatial structure in more detail, as for example in Stegen et al. (2017), but our approach to mapping relative distances to trees provides an alternative spatial study construct. In a similar vein, Tang and Baldocchi (2005) measured R_s within a transect of two oak trees to draw inferences on the spatially variable contribution of R_h and R_a. Our study design still provides useful spatial information, however: the 15 m max distance in **Figure 5** implies that the range of a semivariogram, i.e. the distance of maximum autocorrelation, would be at least this far. This means that BA remained significant all the way to our maximum measured distance of 15 m, implying that the spatial influence of large trees persisted at least this far (Högberg et al., 2001).

Second, this study tested the effect of basal area on R_s, based on the assumption that BA is proportional to fine root biomass, the respiration of which is driven (with some time lag) by photosynthesis and this in turn drives root respiration dynamics (Vose and Ryan, 2002). Stems with a diameter below 2 cm and understory were not inventoried or, as a result, included in the hypothesis-testing statistical models. If root respiration is instead correlated with number of stems, which are disproportionately small due to forest demographics, this would bias our results. There are not many understory/saplings at these sites (**Table 1**), however.

Conclusion

We found that measurement collars with higher tree basal area within 5 m had significantly higher temperature sensitivity of R_s. R_s was also highly moisture-sensitive at all of our study sites~~these sites~~, with large differences among R_s in low- versus high-moisture times. These findings, in conjunction with large sample size requirements, suggests that the highly

dynamic and variable nature of soil respiration at this site lends itself to localized basal area effects on R_s to be highly dynamic and variable. This could have implications for measurement requirements in sites with particular stand structures. A better understanding of the spatial interactions between plants and microbes through R_h and R_a partitioning, as well as the speed and coupling between above- and belowground processes, is necessary to link these processes with collar- and ecosystem-scale soil-to-atmosphere C fluxes.

Author contributions

This study was designed by B.B.-L. and S.C.P. All fieldwork and data analysis was performed by S.C.P., except for the statistical analysis, which was written by B.B.-L. N.M., J.P.M., and J.C.S. provided feedback on the study design, analysis, and interpretation of results. S.C.P. wrote the manuscript in close collaboration with all authors.

Competing Interests

There are no competing interests reported by the authors that might have influenced the work described in this manuscript.

Code/Data Availability

All code and data necessary to reproduce our results are available in our online GitHub repository (https://github.com/PNNL-PREMIS/PREMIS-ghg) and permanently archived at Figshare (DOI if accepted).

References

Adachi, M., Bekku, Y. S., Konuma, A., Kadir, W. R., Okuda, T. and Koizumi, H.: Required sample size for estimating soil respiration rates in large areas of two tropical forests and of two types of plantation in Malaysia, For. Ecol. Manage., 210(1), 455–459, 2005.

Aguilos, M., Takagi, K., Liang, N., Watanabe, Y., Goto, S., Takahashi, Y., Mukai, H. and Sasa, K.: Soil warming in a cool-temperate mixed forest with peat soil enhanced heterotrophic and basal respiration rates but Q10 remained unchanged, Biogeosciences, 2011.

Atkins, J. W., Bohrer, G., Fahey, R. T., Hardiman, B. S., Morin, T. H., Stovall, A. E. L., Zimmerman, N. and Gough, C. M.: Quantifying vegetation and canopy structural complexity from terrestrial LiDAR data using the forestr r package, edited by S. Goslee, Methods Ecol. Evol., 9(10), 2057–2066, 2018.

Barba, J., Cueva, A., Bahn, M., Barron-Gafford, G. A., Bond-Lamberty, B., Hanson, P. J., Jaimes, A., Kulmala, L., Pumpanen, J., Scott, R. L., Wohlfahrt, G. and Vargas, R.: Comparing ecosystem and soil respiration: Review and key challenges of tower-based and soil measurements, Agric. For. Meteorol., 249(Supplement C), 434–443, 2018.

Bates, D., Mächler, M., Bolker, B. and Walker, S.: Fitting Linear Mixed-Effects Models Using lme4, Journal of Statistical Software, Articles, 67(1), 1–48, 2015.

Bond-Lamberty, B.: New techniques and data for understanding the global soil respiration flux, Earth's Future, doi:10.1029/2018EF000866, 2018.

Bond-Lamberty, B. and Thomson, A. M.: A global database of soil respiration data, Biogeosciences, 7, 1915–1926, 2010.

Bond-Lamberty, B., Epron, D., Harden, J. W., Harmon, M. E., Hoffman, F. M., Kumar, J., McGuire, A. D. and Vargas, R.: Estimating heterotrophic respiration at large scales: challenges, approaches, and next steps, Ecosphere, 7(6), d01380, 2016.

Boone, R. D., Nadelhoffer, K. J., Canary, J. D. and Kaye, J. P.: Roots exert a strong influence on the

temperature sensitivity of soil respiration, Nature, 396(6711), 570–572, 1998.

Brændholt, A., Ibrom, A., Larsen, K. S. and Pilegaard, K.: Partitioning of ecosystem respiration in a beech forest, Agric. For. Meteorol., 252, 88–98, 2018.

Bréchet, L., Ponton, S., Alméras, T., Bonal, D. and Epron, D.: Does spatial distribution of tree size account for spatial variation in soil respiration in a tropical forest?, Plant Soil, 347(1), 293, 2011.

Burgess, S. S. O., Adams, M. A., Turner, N. C. and Ong, C. K.: The redistribution of soil water by tree root systems, Oecologia, 115(3), 306–311, 1998.

Cable, J. M., Ogle, K., Lucas, R. W., Huxman, T. E., Loik, M. E., Smith, S. D., Tissue, D. T., Ewers, B. E., Pendall, E. G., Welker, J. M., Charlet, T. N., Cleary, M., Griffith, A., Nowak, R. S., Rogers, M., Steltzer, H., Sullivan, P. F. and van Gestel, N. C.: The temperature responses of soil respiration in deserts: a seven desert synthesis, Biogeochemistry, 103(1-3), 71–90, 2010.

Curiel Yuste, J., Janssens, I. A., Carrara, A. and Ceulemans, R.: Annual Q10 of soil respiration reflects plant phenological patterns as well as temperature sensitivity, Glob. Chang. Biol., 10(2), 161–169, 2004.

Davidson, E. A., Verchot, L. V., Cattânio, J. H., Ackerman, I. L. and Carvalho, J. E. M.: Effects of soil water content on soil respiration in forests and cattle pastures of eastern Amazonia, Biogeochemistry, 48(1), 53–69, 2000.

Davidson, E. A., Savage, K. E., Verchot, L. V. and Navarro, R.: Minimizing artifacts and biases in chamber-based measurements of soil respiration, Agric. For. Meteorol., 113, 21–37, 2002.

Ekblad, A. and Högberg, P.: Natural abundance of 13C in CO2 respired from forest soils reveals speed of link between tree photosynthesis and root respiration, Oecologia, 127, 305–308, 2001.

Epron, D., Le Dantec, V., Dufrene, E. and Granier, A.: Seasonal dynamics of soil carbon dioxide efflux and simulated rhizosphere respiration in a beech forest, Tree Physiol., 21(2/3), 145–152, 2001.

Epron, D., Nouvellon, Y., Roupsard, O., Mouvondy, W., Mabiala, A., Saint-André, L., Joffre, R., Jourdan, C., Bonnefond, J.-M., Berbigier, P. and Hamel, O.: Spatial and temporal variations of soil respiration in a Eucalyptus plantation in Congo, For. Ecol. Manage., 202(1), 149–160, 2004.

Ezer, T. and Corlett, W. B.: Is sea level rise accelerating in the Chesapeake Bay? A demonstration of a novel new approach for analyzing sea level data, Geophys. Res. Lett., 39(19), doi:10.1029/2012GL053435, 2012.

Giasson, M.-A., Ellison, A. M., Bowden, R. D., Crill, P. M., Davidson, E. A., Drake, J. E., Frey, S. D., Hadley, J. L., Lavine, M., Melillo, J. M., Munger, J. W., Nadelhoffer, K. J., Nicoll, L., Ollinger, S. V., Savage, K. E., Steudler, P. A., Tang, J., Varner, R. K., Wofsy, S. C., Foster, D. R. and Finzi, A. C.: Soil respiration in a northeastern US temperate forest: a 22-year synthesis, Ecosphere, 4, art. 140, 2013.

Gillman, L. N., Wright, S. D., Cusens, J., McBride, P. D., Malhi, Y. and Whittaker, R. J.: Latitude, productivity and species richness: Latitude and productivity, Glob. Ecol. Biogeogr., 24(1), 107–117, 2015.

Granier, A., Biron, P. and Lemoine, D.: Water balance, transpiration and canopy conductance in two beech stands, Agric. For. Meteorol., 100(4), 291–308, 2000.

Hanson, P. J., Edwards, N. T., Garten, C. T. and Andrews, J. A.: Separating root and soil microbial

contributions to soil respiration: A review of methods and observations, Biogeochemistry, 48(1), 115–146, 2000.

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A. F. S., Ekblad, A., Högberg, M. N., Nyberg, G., Ottosson-Löfvenius, M. and Read, D. J.: Large-scale forest girdling shows that current photosynthesis drives soil respiration, Nature, 411(6839), 789–792, 2001.

Hopkins, F. M., Gonzalez-Meler, M. A., Flower, C. E., Lynch, D. J., Czimczik, C. I., Tang, J. and Subke, J.-A.: Ecosystem-level controls on root-rhizosphere respiration, New Phytol., 199(2), 339–351, 2013.

Hursh, A., Ballantyne, A., Cooper, L., Maneta, M., Kimball, J. and Watts, J.: The sensitivity of soil respiration to soil temperature, moisture, and carbon supply at the global scale, Glob. Chang. Biol., 23(5), 2090–2103, 2017.

Le Quéré, C., Andrew, R. M., Friedlingstein, P., Sitch, S., Pongratz, J., Manning, A. C., Korsbakken, J. I., Peters, G. P., Canadell, J. G., Jackson, R. B., Boden, T. A., Tans, P. P., Andrews, O. D., Arora, V. K., Bakker, D. C. E., Barbero, L., Becker, M., Betts, R. A., Bopp, L., Chevallier, F., Chini, L. P., Ciais, P., Cosca, C. E., Cross, J., Currie, K., Gasser, T., Harris, I., Hauck, J., Haverd, V., Houghton, R. A., Hunt, C. W., Hurtt, G., Ilyina, T., Jain, A. K., Kato, E., Kautz, M., Keeling, R. F., Klein Goldewijk, K., Körtzinger, A., Landschützer, P., Lefèvre, N., Lenton, A., Lienert, S., Lima, I., Lombardozzi, D., Metzl, N., Millero, F., Monteiro, P. M. S., Munro, D. R., Nabel, J. E. M. S., Nakaoka, S.-I., Nojiri, Y., Padin, X. A., Peregon, A., Pfeil, B., Pierrot, D., Poulter, B., Rehder, G., Reimer, J., Rödenbeck, C., Schwinger, J., Séférian, R., Skjelvan, I., Stocker, B. D., Tian, H., Tilbrook, B., Tubiello, F. N., Laan-Luijkx, I. T. van der, Werf, G. R. van der, van Heuven, S., Viovy, N., Vuichard, N., Walker, A. P., Watson, A. J., Wiltshire, A. J., Zaehle, S. and Zhu, D.: Global Carbon Budget 2017, Earth System Science Data, 10(1), 405–448, 2018.

Lindeman, R. H., Merenda, P. F. and Gold, R. Z.: Introduction to bivariate and multivariate analysis, Glenview, IL, Scott: Foresman and company, 1980.

Luo, Y. and Zhou, X.: Soil Respiration and the Environment, Elsevier/Academic Press, Amsterdam., 2006.

Luyssaert, S., Inglima, I., Jung, M., Richardson, A. D., Reichstein, M., Papale, D., Piao, S., Schulze, E.-D., Wingate, L., Matteucci, G., Aragão, L. E. O. C., Aubinet, M., Beer, C., Bernhofer, C., Black, K. G., Bonal, D., Bonnefond, J.-M., Chambers, J. L., Ciais, P., Cook, B. D., Davis, K. J., Dolman, A. J., Gielen, B., Goulden, M. L., Grace, J., Granier, A., Grelle, A., Griffis, T. J., Grünwald, T., Guidolotti, G., Hanson, P. J., Harding, R. B., Hollinger, D. Y., Hutyra, L. R., Kolari, P., Kruijt, B., Kutsch, W. L., Lagergren, F., Laurila, T., Law, B. E., Le Maire, G., Lindroth, A., Loustau, D., Malhi, Y., Mateus, J., Migliavacca, M., Misson, L., Montagnani, L., Moncrieff, J. B., Moors, E. J., Munger, J. W., Nikinmaa, E., Ollinger, S. V., Pita, G., Rebmann, C., Roupsard, O., Saigusa, N., Sanz, M. J., Seufert, G., Sierra, C. A., Smith, M.-L., Tang, J., Valentini, R., Vesala, T. and Janssens, I. A.: CO2 balance of boreal, temperate, and tropical forests derived from a global database, Glob. Chang. Biol., 13(12), 2509–2537, 2007.

Misson, L., Gershenson, A., Tang, J., McKay, M., Cheng, W. and Goldstein, A. H.: Influences of canopy photosynthesis and summer rain pulses on root dynamics and soil respiration in a young ponderosa pine forest, Tree Physiol., 26(7), 833–844, 2006.

National Weather Service: Record Rain and Flooding of 2018, [online] Available from: https://www.weather.gov/lwx/2018floods (Accessed 30 May 2019), 2019.

Pitz, S. and Megonigal, J. P.: Temperate forest methane sink diminished by tree emissions, New Phytol., 214(4), 1432–1439, 2017.

Raich, J. W. and Schlesinger, W. H.: The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate, Tellus B Chem. Phys. Meteorol., 44(2), 81–99, 1992.

Raich, J. W., Potter, C. S. and Bhagawati, D.: Interannual variability in global soil respiration, 1980-94, Glob. Chang. Biol., 8, 800–812, 2002.

R Development Core Team: R: A language and environment for statistical computing. Version 3.5.3. [online] Available from: http://www.R-project.org/, 2019.

Reichstein, M., Rey, A., Freibauer, A., Tenhunen, J. D., Valentini, R., Banza, J., Casals, P., Cheng, Y., Grünzweig, J. M., Irvine, J., Joffre, R., Law, B. E., Loustau, D., Miglietta, F., Oechel, W. C., Ourcival, J.-M., Pereira, J., Peressotti, A., Ponti, F., Qi, Y., Rambal, S., Rayment, M. B., Romanya, J., Rossi, F., Tedeschi, V., Tirone, G., Xu, M. and Yakir, D.: Modeling temporal and large-scale spatial variability of soil respiration from soil water availability, temperature and vegetation productivity indices, Global Biochemical Cycles, 17(4), art. no. 1104 (15 p.), 2003.

Rodeghiero, M. and Cescatti, A.: Spatial variability and optimal sampling strategy of soil respiration, For. Ecol. Manage., 255, 106–112, 2008.

Rogers, K., Kelleway, J. J., Saintilan, N., Megonigal, J. P., Adams, J. B., Holmquist, J. R., Lu, M., Schile-Beers, L., Zawadzki, A., Mazumder, D. and Woodroffe, C. D.: Wetland carbon storage controlled by millennial-scale variation in relative sea-level rise, Nature, 567(7746), 91–95, 2019.

Ruehr, N. K., Knohl, A. and Buchmann, N.: Environmental variables controlling soil respiration on diurnal, seasonal and annual time-scales in a mixed mountain forest in Switzerland, Biogeochemistry, 98(1), 153–170, 2010.

Saiz, G., Green, C., Butterbach-Bahl, K., Kiese, R., Avitabile, V. and Farrell, E. P.: Seasonal and spatial variability of soil respiration in four Sitka spruce stands, Plant Soil, 287(1-2), 161–176, 2006.

Sallenger, A. H., Jr, Doran, K. S. and Howd, P. A.: Hotspot of accelerated sea-level rise on the Atlantic coast of North America, Nat. Clim. Chang., 2, 884, 2012.

Schlesinger, W. H. and Andrews, J. A.: Soil respiration and the global carbon cycle, Biogeochemistry, 48(1), 7–20, 2000.

Schwendenmann, L. and Macinnis-Ng, C.: Soil CO_2 efflux in an old-growth southern conifer forest (Agathis australis) – magnitude, components and controls, , doi:10.5194/soil-2-403-2016, 2016.

Sierra, C. A., Trumbore, S. E., Davidson, E. A., Vicca, S. and Janssens, I. A.: Sensitivity of decomposition rates of soil organic matter with respect to simultaneous changes in temperature and moisture, Journal of Advances in Modeling Earth Systems, 7(1), 335–356, 2015.[1]

Søe, A. R. B. and Buchmann, N.: Spatial and temporal variations in soil respiration in relation to stand structure and soil parameters in an unmanaged beech forest, Tree Physiol., 25, 1427–1436, 2005.

Sprugel, D. G., Ryan, M. G., Brooks, J. R., Vogt, K. A. and Martin, T. A.: Respiration from the organ level to the stand, in Resource Physiology of Conifers, edited by W. K. Smith and T. M. Hinckley, pp. 255–299, Academic Press, San Diego., 1995.

Stegen, J. C., Anderson, C. G., Bond-Lamberty, B., Crump, A. R., Chen, X. and Hess, N.: Soil respiration across a permafrost transition zone: spatial structure and environmental correlates, Biogeosciences,

14(18), 4341–4354, 2017.

Stone, E. L. and Kalisz, P. J.: On the maximum extent of tree roots, For. Ecol. Manage., 46(1), 59–102, 1991.

Subke, J.-A., Inglima, I. and Cotrufo, M. F.: Trends and methodological impacts in soil CO2 efflux partitioning: A metaanalytical review, Glob. Chang. Biol., 12(2), 921–943, 2006.

Suseela, V., Conant, R. T., Wallenstein, M. D. and Dukes, J. S.: Effects of soil moisture on the temperature sensitivity of heterotrophic respiration vary seasonally in an old-field climate change experiment, Glob. Chang. Biol., 18(1), 336–348, 2012.

Tang, J. and Baldocchi, D. D.: Spatial-temporal variation in soil respiration in an oak-grass savanna ecosystem in California and its partitioning into autotrophic and heterotrophic components, Biogeochemistry, 73(1), 183–207, 2005.

Vose, J. M. and Ryan, M. G.: Seasonal respiration of foliage, fine roots, and woody tissues in relation to growth, tissue N, and photosynthesis, Glob. Chang. Biol., 8, 182–193, 2002.

Wei, W., Weile, C. and Shaopeng, W.: Forest soil respiration and its heterotrophic and autotrophic components: Global patterns and responses to temperature and precipitation, Soil Biol. Biochem., 42(8), 1236–1244, 2010.

Table 1 | Study site characteristics of each site along Muddy Creek, including trees per hectare, cumulative basal area, main soil types, and dominant tree species by percent of basal area. Values are mean ± standard deviation of N=3, 800 m² plots.

Site	Trees (ha⁻¹)	BA (m² ha⁻¹)	Dominant Soil Type	Dominant Tree Species (by BA %)	Altitude (m)
GCReW (38.876 °N, 76.553 °W)	637.5 ± 57.3	45.2 ± 7.344.6 ± 4	Collington-Wist complex; Collington and Annapolis soils	28% *Liriodendron tulipifera* 11% *Quercus spp.* 11% *Fagus grandifolia*	3-10
Canoe Shed (38.884 °N, 76.557 °W)	529.2 ± 93.8	40.4 ± 6	Annapolis fine sandy loam	26% *Quercus spp.*, 23% *L. tulipifera* 20% *F. grandifolia*	7-10
North Branch (38.887 °N, 76.563 °W)	806.9 ± 180.7	34.5 ± 7.8	Collington and Annapolis soils; Collington, Wist, and Westphalia soils	42% *F. grandifolia* 26% *Quercus spp.* 12% *Liquidambar styraciflua*	8-20

Table 2. Sample size required to estimate soil respiration with a particular error (delta, left column, fraction of mean flux), for different statistical power values. Values are mean (standard deviation) between plots. "Power" is the probability that the test rejects the null hypothesis when a specific alternative hypothesis is true, and informally connotes the degree of confidence that the measurement within some delta value of the true mean.

		Power (1 - β)				
delta	0.5	0.6	0.7	0.8	0.9	0.95
0.05	61 (24)	95 (37)	143 (55)	219 (84)	362 (138)	516 (196)
0.10	16 (6)	24 (10)	36 (14)	55 (21)	91 (35)	129 (49)
0.25	3 (1)	4 (2)	6 (3)	9 (4)	15 (6)	21 (8)
0.50	1 (1)	1 (1)	2 (1)	3 (1)	4 (2)	6 (2)

Table 3. Summary of linear mixed-effects model testing main hypothesis of the effect of nearby tree basal area on soil respiration (the dependent variable). Terms tested include soil temperature at 5 and 20 cm (T_5 and T_{20} respectively), basal area (BA), and soil moisture (SM). Model AIC = 381.6, marginal R^2 = 0.36.

	value	degrees of freedom	t-value	p-value
(Intercept)	-0.767 ± 0.148	440	-5.199	0.000
T_5	0.010 ± 0.009	440	1.055	0.292
BA_5	0.022 ± 0.219	440	0.098	0.922
T_{20}	0.095 ± 0.011	440	8.397	0.000
SM	2.505 ± 0.699	440	3.581	0.004
$I(SM^2)$	-3.542 ± 1.144	440	-3.095	0.002
T_5:BA_5	0.079 ± 0.036	440	2.181	0.030
BA_5:T_{20}	-0.069 ± 0.041	440	-1.689	0.092

Figure 1 | a) Tree proximity measurement schematic. Distance to each tree was recorded within a 15 meter radius of each soil respiration measurement point, along with DBH and species. b) Map of the Smithsonian Environmental Research Center with the three sites labeled in black.
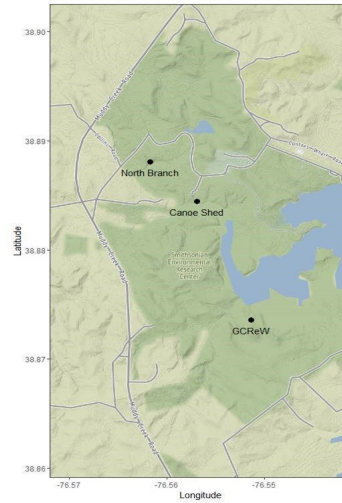
a)
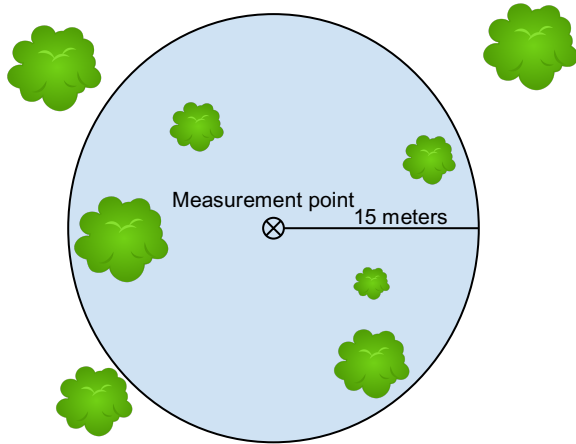
b)

Figure 2 | CO$_2$ flux over time from April 2018 to April 2019 for 36 measurement points across three sites; red line shows the seasonal trend using a loess smoother.
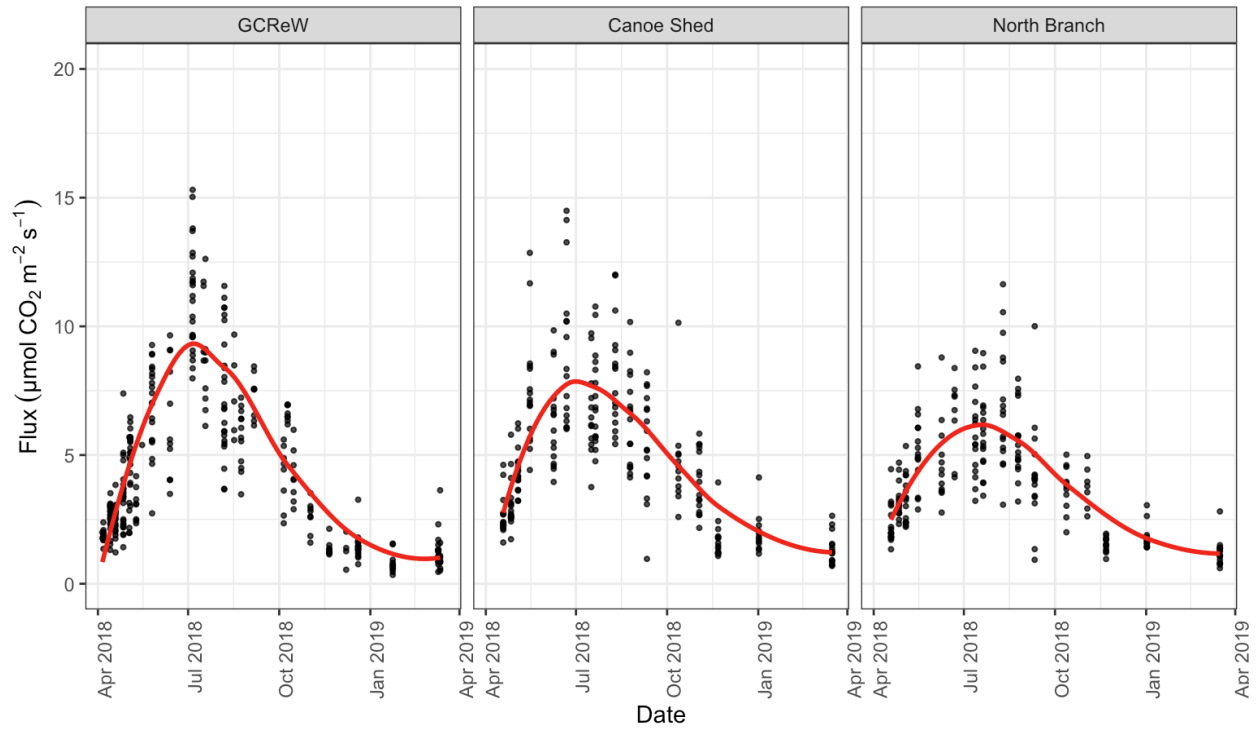
Figure 3 | Cumulative basal area for each collar (N = 36) up to 15 meters; color indicates number of trees at each distance. Inset graph shows a close up of 0 to 5 meters for more detail.
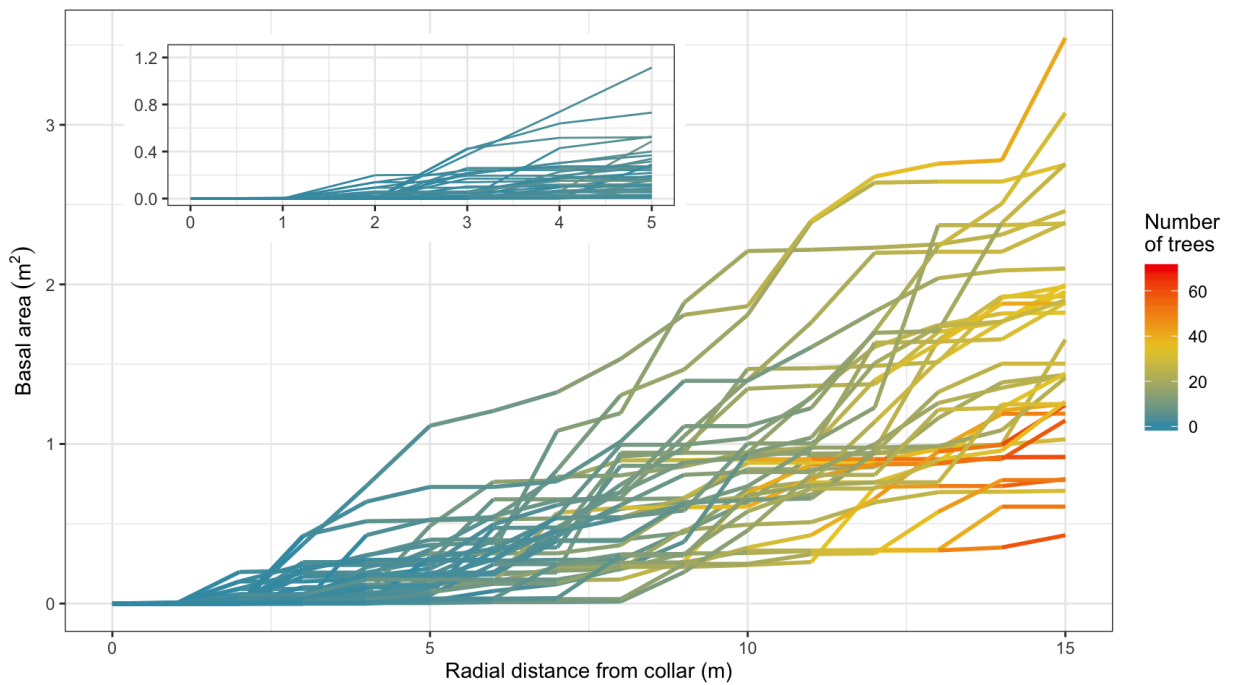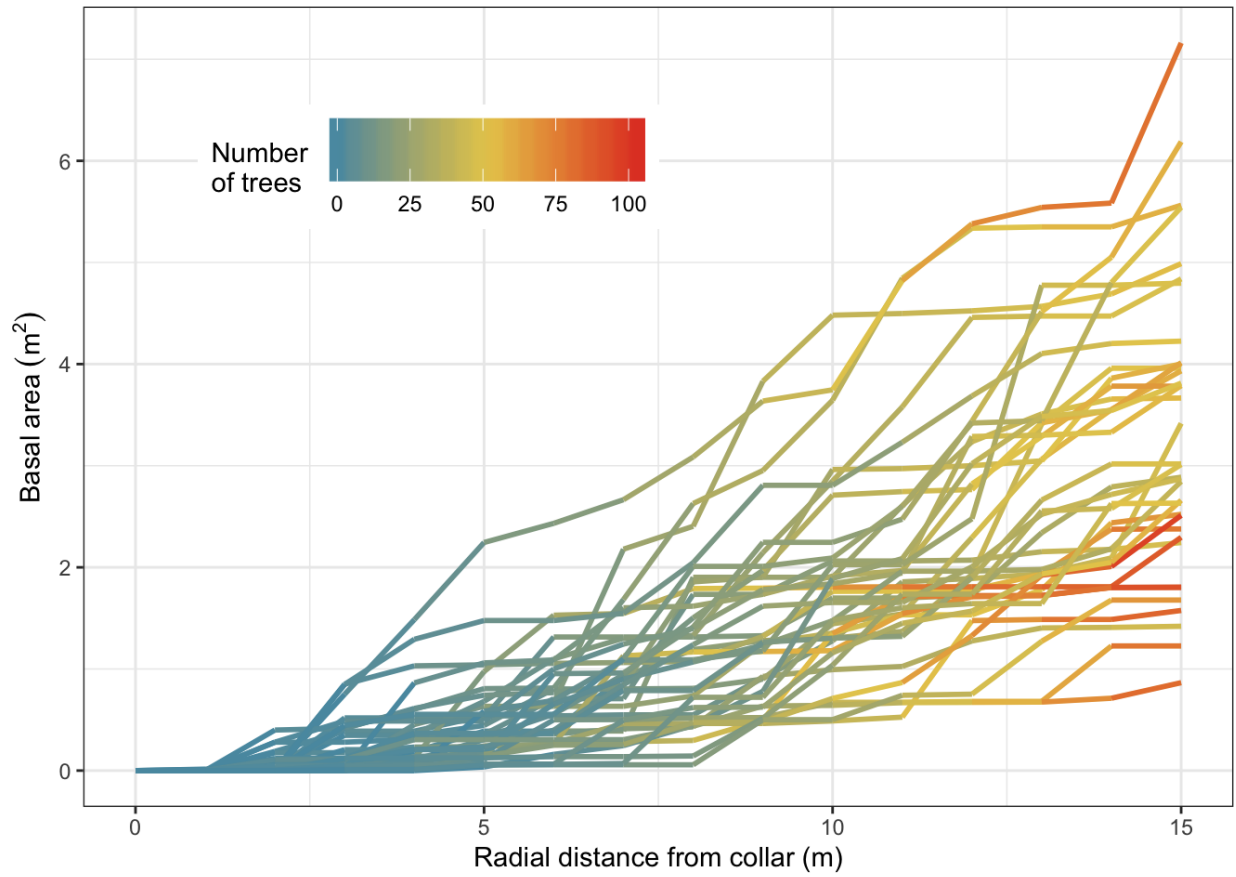
Figure 4. Residuals of a soil respiration model, incorporating temperature and soil moisture as independent variables, versus cumulative tree basal area within 5 m, by site. Each point is an individual observation (cf. **Figure 2**). Regression lines are shown for each site; black line is the overall trend. Note that 5 extreme points are out of the plot but are accounted for in the regression lines.
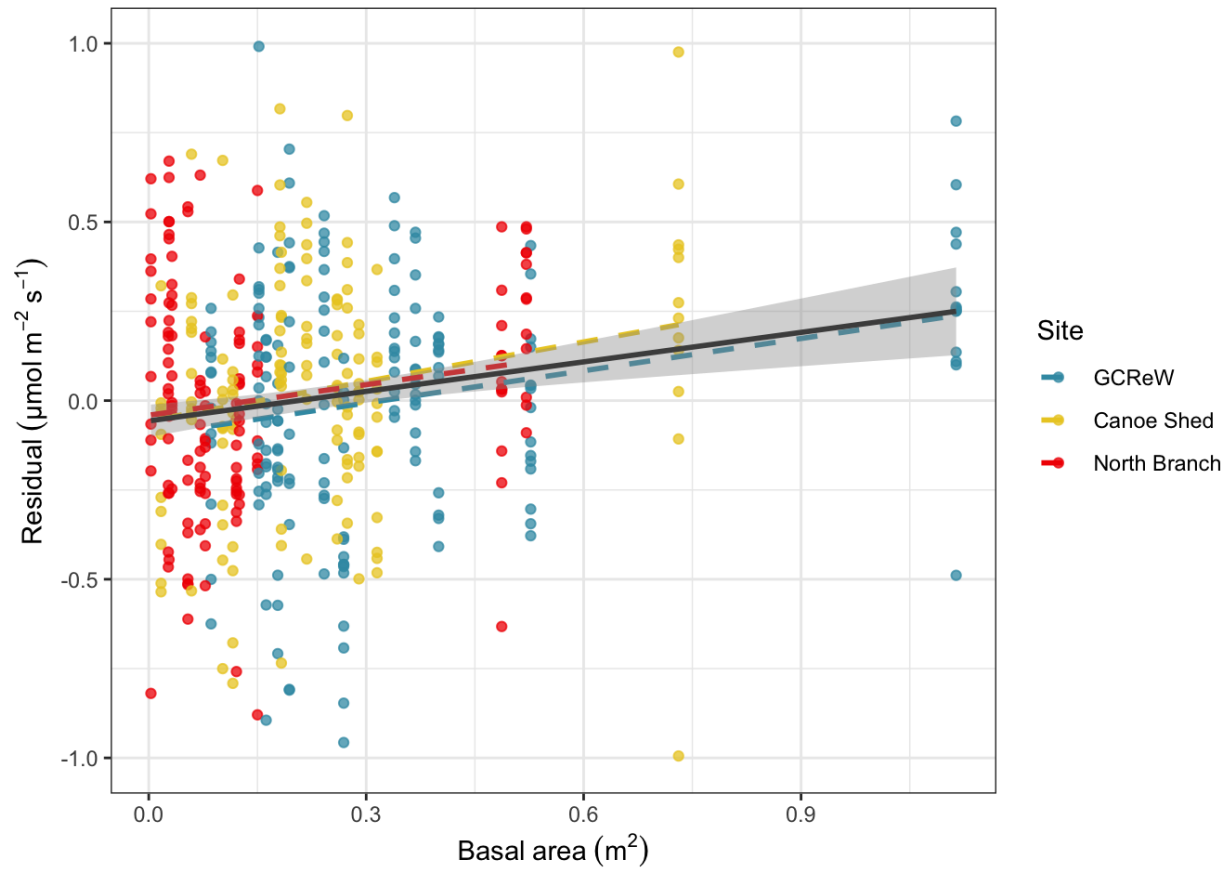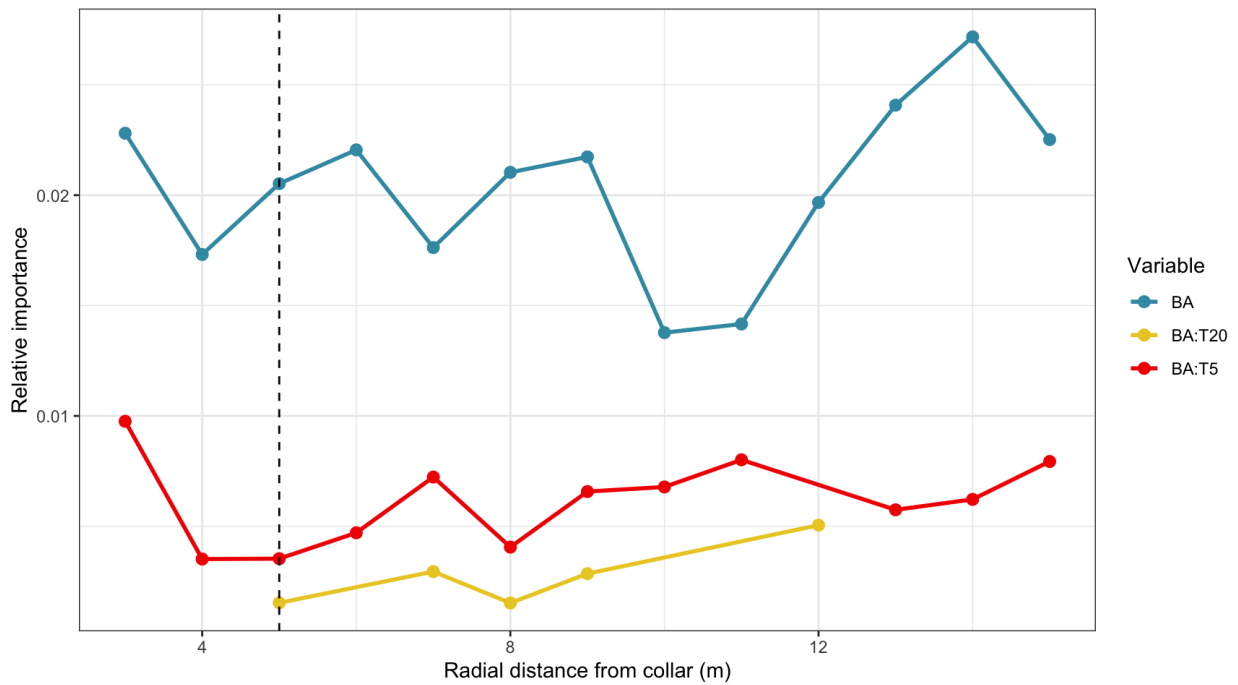
Figure 5. Test of robustness of results, run at various distances from soil respiration measurement collars (x axis). Lines show the variable importance (calculated as R^2 partitioned by averaging over orders; see Methods) of basal area (BA), as well as the interaction of BA and temperatures at 5 and 20 cm (T_5 and T_{20} respectively). Vertical dashed line shows the 5 m radius used in **Table 3** and **Figure 4** results. Note that 'missing' BA:T20 (in yellow) dots at distances < 5 m and >12 m mean that the terms were dropped from the model and are thus not significant.

--- END OF MANUSCRIPT ---